User Guide

Kitalysis™ High-Throughput Screening Platform

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

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KitAlysis™ High-Throughput Screening Platform Overview

In the high-pressure environment of discovery chemistry, time is everything. While general catalytic methods exist, substrate dependency makes it difficult to predict optimal conditions in practice. A thoughtful screening process is the best way to cover a large amount of chemical space quickly while using small amounts of often precious intermediates.

KitAlysis™ High-Throughput Screening Kits provide the optimal solution to quickly and efficiently identify or optimize suitable catalytic reaction conditions.

Features include:

- Microscale format requires only ~100 mg total of each substrate to run 24 unique chemical reactions (3–4 mg per reaction)
- Reaction array (catalysts, solvents, bases, and temperature) is designed and validated by practicing medicinal/process chemists in some of the largest pharma companies in the world
- All components are included. End-user supplies only substrate and commonly used lab essentials (pipettes and syringes)
- KitAlysis™ Labware eliminates the need to set up reactions in a glove box

KitAlysis™ technology showcases the most widely used catalytic methods of synthetic chemists in both academia and industry, translated into an off-the-shelf screening system. Now, any bench chemist can rapidly run 24 unique microscale reactions in parallel with conditions tailored to ensure the best possible chance of success.

KitAlysis™ Benchtop Inertion Box Set-up

Materials Included

KitAlysis™ Benchtop Inertion Box with Lid

Additional Materials Needed

1. Tygon tubing (I.D. x O.D. 14 in. x 3/8 in.)
2. 3-way (Y) plastic or glass tubing connector

Description

The *KitAlysis*™ Benchtop Inertion Box provides an inert environment to run oxygen sensitive cross-coupling reactions easily and robustly in a laboratory fume hood.

General Set Up

1. Place the Inertion Box as close to your inert gas source as possible while allowing you good access to the box for kit set up.
2. Cut 3 pieces of tubing minimizing any excess where possible.
   a. 1 piece to be attached from your inert gas source to the three way valve.
   b. Two identical pieces to be attached first to 3-way valve and then to either side of the KitAlysis™ Benchtop Inertion Box
3. Once tubing is in place, turn nitrogen flow on until you can hear a steady flow of nitrogen coming through the box. This is roughly 1-2 full revolutions of the nitrogen inlet knob on the typical fume hood. In general: you should be able to hear the flow of nitrogen over the normal noise of your operating hood while carrying on a conversation at an “inside voice” level.
4. **NEVER TURN ON THE HEAT under the Inertion Box. The Reaction Block must be removed for heating and should never be heated directly inside in the Inertion Box.**

For Best Results

1. Place the KitAlysis™ Benchtop Inertion Box in a reasonable spot as far back from the sash as possible while still allowing good access to the box for reaction set up. This helps to eliminate the increased air flow towards the front of the hood from disrupting the nitrogen flow coming into the Inertion Box.
2. Lower the sash as much as possible to reduce extraneous air flow while still allowing easy access to the Inertion Box.
3. Do not run hood on “emergency flow” while you are using your Inertion Box.
4. Always place your substrate reaction vials (or empty dummy vials) in the center holes on either side of the reaction block (see diagram below). This helps to maintain an even and consistent flow.
5. Purge the box with the reaction block and lid in place for 5 minutes before adding chemicals. Purge an additional 5 minutes after placement of chemicals into the Inertion Box and before making substrate solutions/ dosing to reaction wells.
6. **Always keep the flow of nitrogen on** at the same level during the entire set up. The term “purge” means to simply let the Inertion Box sit under the regular nitrogen flow without any movement within the box.

To be used with: KitAlysis™ 24-Well Reaction Block and Screwdriver Set

For best result, the block and the lid should be placed in the Inertion Box prior to the first degassing.

KitAlysis™ High-Throughput Base Reaction Screening Kit

Kit Design

The base kit was designed to provide the best possible chance of finding good reaction conditions for cross-coupling reactions:

- pre-weighed base or base solution (provided)
- 10–20 mmol coupling partner (user supplied)
- 10 mmol aryl halide substrate (user supplied)
- catalyst and ligand (user supplied).
- 4 solvents (DMF, MeOH, THF, and Toluene) (provided)

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

Designed for the first-time user and should be read completely before getting started. Best if used in conjunction with the **video** as not all steps are outlined in the video in detail. This guide includes trouble-shooting tips, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Excel Sheet:

Each sheet is designed to be used with the specific experimental design chosen by you depending upon the attributes of your substrates. The downloadable excel files are specific to the kit being run and can be found within each **Step-by-Step User Guide**.

They have the following features:

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included In Your KitAlysis™ base-2pk High-Throughput Screening Kit

Contents in each of the 2 individually sealed Mylar (foil bags):

- 22 (11 x 2) pre-weighed bases in glass vials loaded with stir bars, topped with cap mat.
- 4 empty 4 mL reaction vials

The screening sets come pre-loaded with 30 µmol of base in each vial according to the following design.

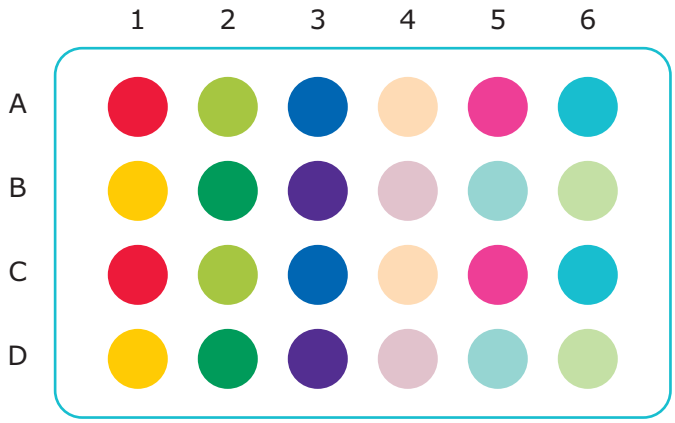


Figure 1. KitAlysis™ Well Plate Map

Description	Cat. No.	Vial
K_3PO_4	**RDD019**	A1, C1
K_2CO_3	**900501**	A2, C2
KOAc	**791733**	A3, C3
$KHCO_3$	**237205**	A4, C4
KOH	**757551**	A5, C5
NaOH	**795429**	A6, C6
Cs_2CO_3	**441902**	B1, D1
LiHMDS 1.0 M in THF	**225770**	B2, D2
NaOtBu 2.0 M in THF	**702706**	B3, D3
DBU	**139009**	B4, D4
Et_3N	**900632**	B5, D5
Chemist's Choice	n/a	B6, D6

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes:

- 2 (2 mL) ampule each of the following: DBU, Et_3N, LiHMDS, NaOtBu
- 4 (2 mL) ampule each of the following: DMF, MeOH, THF, Toluene

KitAlysis™ 24-well Reaction Block Replacement Films-2EA

- Pack of 2 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

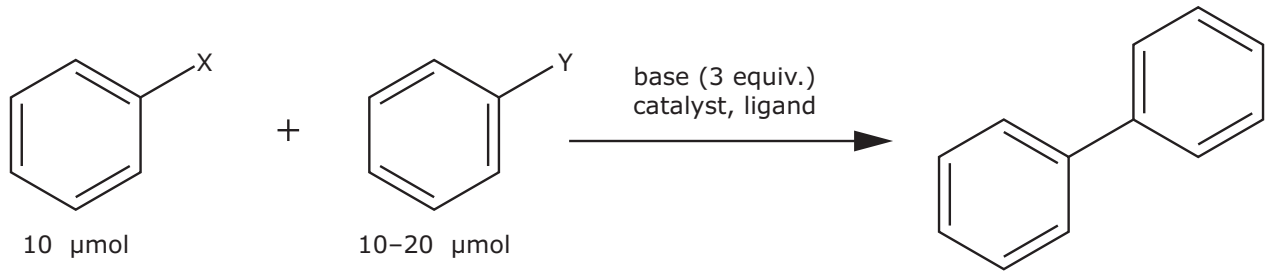
Stir Bars-8 Individually Packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures.

Additional Recommended Materials (Sold Separately)

- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

KitAlysis™ Base Step-by-Step Guide



Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ High-throughput Base Screening Kit and you will use the following components
  - Plate of pre-weighed bases in glass vials loaded with stir bars and topped with cap mat
- 1 ampule each of 2 of the following: MeOH, Toluene, DMF, THF
- 1 ampule each of the following: LiHMDS (1.0 M in THF), NaOtBu, DBU, Et_3N
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 4 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 2 (1 mL) syringes with long needles
- Chemist's Choice base
- Your substrates, ligands, catalysts
- Nitrogen (or Argon): from the hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC autosampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

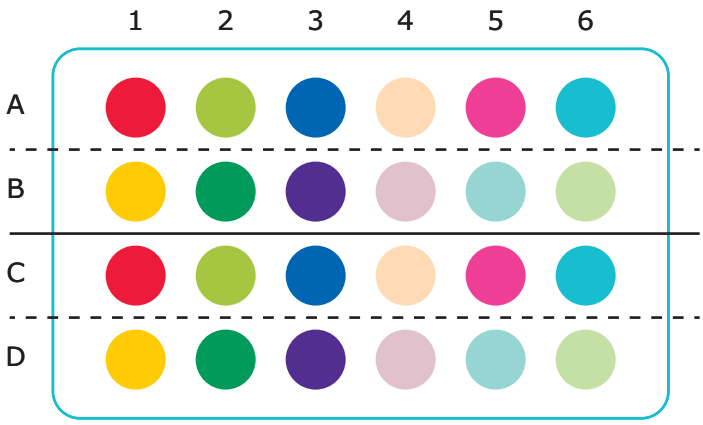
Set-Up Procedure:

- Preheat a hot plate to desired temperature if heating required (use oil bath or second reaction block to hold temperature and avoid spiking).
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid (make sure all holes, including the temperature probe hole, line up with the corresponding holes on the film).
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block **with lid** into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Weigh desired substrates, ligands, catalysts directly into two empty 4 mL reaction vials according to recipe (provided in the downloadable excel file) **omitting solvent. Add one stir bar to each vial mixture.** Label as “Solvent 1 Substrate Mixture A” and “Solvent 2 Substrate Mixture B”).
- Weigh Chemist’s Choice base (30 µmol) into remaining two empty 4 mL reaction vials.
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the “Solvent 1 Substrate Mixture A” in the center hole on the left hand side of the Inertion Box diffuser tray. Place the “Solvent 2 Substrate Mixture B” in the center hole on the right hand side of the plate. Place Chemist’s Choice base vials in open holes next to Reaction Block. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the mat on.

  Note: B6 and D6 should be empty and can be used to check orientation.

- Using an ampule cracker, open 1 ampule each of the 2 selected solvents and 4 base solutions and quickly place into the holes located along the bottom and side of the Inertion Box, below and next to the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials and Chemist’s Choice base vials.
- Stir mixtures until in solution (1–2 min). For slurries, see “additional tips” below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded bases in the reaction block.
- Using a pipette, dose 4 base solutions and Chemist’s Choice solution into appropriate vials according to scheme below.
- Dose stock solutions
  - Dose 100 µL of “Solvent 1 Substrate Mixture A” to vials A1–A6 and B1–B6 according to scheme below.
  - Dose 100 µL of “Solvent 2 Substrate Mixture B” into vials C1–C6 and D1–D6 according to scheme below.

    You may have a very small amount of excess solution remaining for each mixture. Save it as a reaction standard for HPLC/TLC later.



Base	Vial
K_3PO_4	A1, C1
K_2CO_3	A2, C2
KOAc	A3, C3
$KHCO_3$	A4, C4
KOH	A5, C5
NaOH	A6, C6
Cs_2CO_3	B1, D1
LiHMDS	B2, D2
NaOtBu	B3, D3
DBU	B4, D4
Et_3N	B5, D5
Chemist's Choice	B6, D6

After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**

- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check as you go along to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate (if necessary) with probe through the lid and inserted into the block. If necessary, heat at 60 °C for desired reaction time stirring at or near 300 rpm.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.
- At reaction completion, follow the below Work-Up Procedure

Quench Solution Recipe

- 24.5 mL CH_3CN
- 0.5 mL AcOH

Note: This recipe makes 25 mL which is enough stock solution for both screening sets in the KitAlysis™ High-Throughput Base Screening Kit.

Work-Up Procedure and Analysis

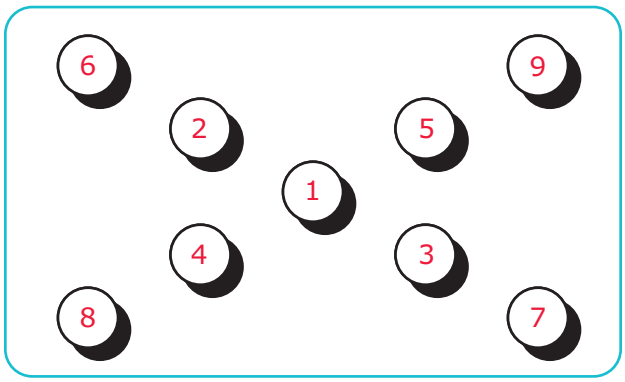
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of acetonitrile to each 24 individually labeled (A1, A2 etc,) HPLC vials or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (see "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block . **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.

Additional Tips:

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light even pressure to the lid of the block to keep the cover flat while sealing.

7. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
8. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
9. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



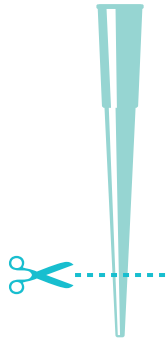
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole:

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions:

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: Base Reaction

Important Note

To best enable scale-up success, the use of glove box is highly recommended. However, modified bench top techniques are provided below.

General Guidelines for Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental

All solids (base, substrates, catalyst) were weighed on a bench top balance and added to a cooled, oven dried flask[1,2] equipped with an oven dried stir bar. The reaction was capped and then purged and backfilled with nitrogen (3x) with a nitrogen-fed manifold needle. Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. If a liquid bases was required, it was added as the final component via syringe. When all constituents were added, the nitrogen line was removed[3] and the reaction allowed to stir at the required temperature for the duration of the reaction.

1. *See the* **Cross-Coupling Reaction Guide** *for more detailed additional guidance and troubleshooting tips.*
2. *Reaction vials capable of being sealed with a microwave cap are very useful as well. By design, the caps do not need to be replaced once punctured.*
3. *For higher temperatures, a reflux condenser operated under nitrogen can be used*

KitAlysis™ C-N (Buchwald-Hartwig) High-Throughput Screening Kit

Kit Design

C-N (Buchwald-Hartwig) cross-coupling kit was designed to provide the best possible chance of success and is run with:

- 10 µmol aryl halide
- 15–20 µmol amine (primary, secondary), use 20 µmol if the material is not too precious
- 1 µmol catalyst per vial (1:1 Pd:Ligand); 12 in this system
- 30 µmol base (NaOt-Bu or Cs_2Co_3)
- 0.1 M solvent concentration (Dioxane or DMAc)

The C-N (Buchwald-Hartwig) cross-coupling kit allows the end user ultimate control and flexibility based upon their specific chemical system. For each kit, there are four possible screens that can be run based upon the substrates you provide:

- Two Solvents, Weak Base (Cs_2CO_3)
- Two Solvents, Strong Base (NaOt-Bu)
- One Solvent (Dioxane), Two Bases
- One Solvent (DMAc), Two Bases

The following guidelines can be used to determine your starting point:

Substrate characteristics:	Screen Type:	Attempt:
base sensitive	**C-N: Two Solvents, Weak Base**	first
base tolerant	**C-N: Two Solvents, Strong Base**	first
base tolerant	**C-N: Dioxane, Two Bases**	second: based upon substrate solubility
base tolerant	**C-N: DMAc, Two Bases**	second: based upon substrate solubility
Not Sure	**C-N: Two Solvents, Strong Base**	first
Not Sure	**C-N: Two Solvents, Weak Base**	second

The kit was purposefully designed to be modular, meaning that you can run 4 different kits, 4 identical kits, one of each, or any combination. All of the supplies can be mixed and matched to meet your needs.

Step-by-step user guides and an excel sheet for stock solution recipes that can be downloaded within each step-by-step user guide hyperlinked above.

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all of the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

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- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contain all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included in Your KITALYSIS-CN-1KT High-Throughput Screening Kit

Contents in each of the 4 individually sealed Mylar (foil bags):

- 24 (12 x 2) pre-weighed catalysts in glass vials loaded with stir bars, topped with cap mat.

The screening sets come pre-loaded with 1 µmol of catalyst in each vial according to the following design.

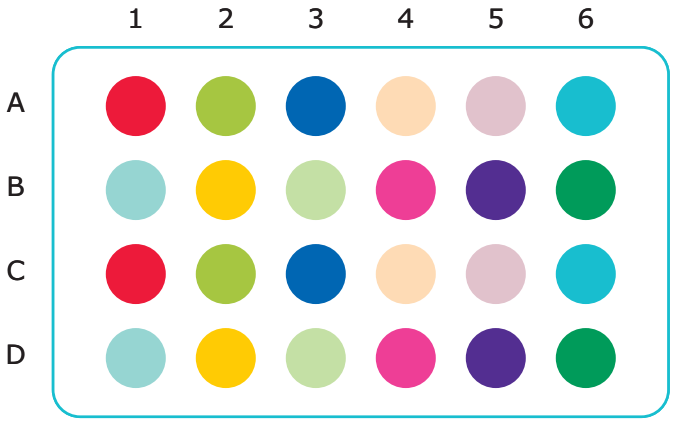


Figure 1. KitAlysis™ Well Plate Map

Description	Cat. No.	Vial
BrettPhos Pd G3	**761605**	A1, C1
CPhos Pd G3	**763004**	A2, C2
DavePhos Pd G3	**804959**	A3, C3
$P(t\text{-}Bu)_3$ Pd G2	**756482**	A4, C4
Josiphos SL-J009-1 Pd G3	**747130**	A5, C5
MorDalPhos Pd G3	**792357**	A6, C6
BINAP Pd G3	**804967**	B1, D1
RuPhos Pd G2	**753246**	B2, D2
SPhos Pd G2	**753009**	B3, D3
tBuXPhos Pd G3	**762229**	B4, D4
XantPhos Pd G3	**763039**	B5, D5
XPhos Pd G3	**763381**	B6, D6

- Cesium Carbonate: ground and sieved in 2 pre-weighed vials with product labels and lids
- 2 empty reaction vials with lids

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes:

- Dioxane: 10 x 2 mL of degassed, anhydrous Sure/Seal™
- DMAc: 10 x 2 mL of degassed, anhydrous Sure/Seal™
- NaOt-Bu Solution: 4 x 0.5 mL degassed anhydrous Sure/Seal™ 2 M THF solution

Internal Standard:

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the hyperlinked screen types in the "Kit Design" section above.

KitAlysis™ 24-well Reaction Block Replacement Films-4EA

- Pack of 4 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

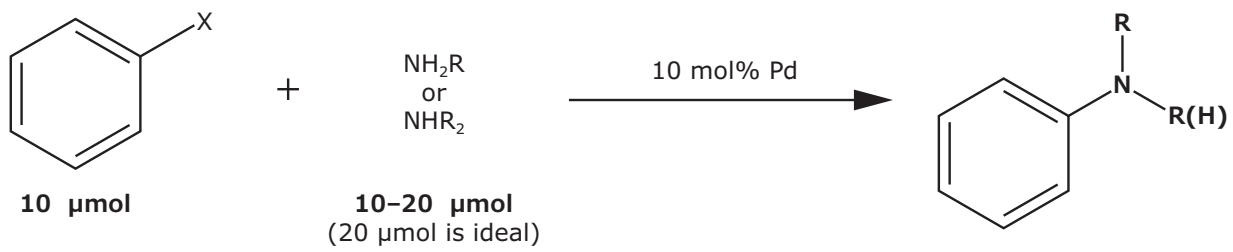
Stir Bars-8 individually packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures.

Additional Recommended Materials (Sold Separately)

- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Two solvents (Dioxane, DMAc), weak base (Cs_2CO_3) Step-by-Step Guide for Buchwald-Hartwig Amination Screening Kit



10 µmol

10–20 µmol
(20 µmol is ideal)

Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit and you will use the following components
  - 12 x 2 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 2 reaction vials containing preweighed Cs_2CO_3
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 1 (2 mL) ampule each of 1,4-Dioxane and DMAc (in ampule boxes)
- 2 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 2 (1 mL) syringes with long needles
- Your aryl halide and amine
- Nitrogen (or Argon): from the hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC autosampler block, or TLC plates.

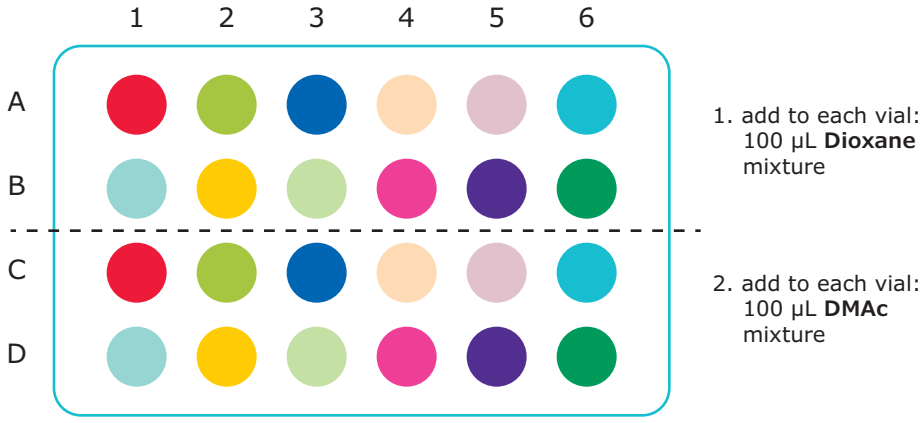
Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

Preheat a hot plate to 100 °C (use oil bath or second reaction block to hold temperature and avoid spiking).

- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid and verify all holes, including the temperature probe hole, line up with the corresponding holes on the film.
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Make substrate mixtures directly into provided preweighed Cs_2CO_3 vial according to recipe **omitting solvent**. Label as "Dioxane Substrate Mixture A" and "DMAc Substrate Mixture B"). **If amine is volatile, add it last (after solvent mixtures have been added) directly to requisite reaction vials via syringe from a separate vial that has been quickly purged and then capped before placing into Inertion Box.**
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the "Dioxane Substrate Mixture A" in the center hole on the left hand side of the Inertion Box diffuser tray. Place the "DMAc Substrate Mixture B" in the center hole on the right hand side of the plate. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the cap mat on.
- Using an ampule cracker, open 1 ampule each of 1,4-Dioxane and DMAc and quickly place into ampule holes located along the bottom of Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials.
- Stir mixtures until in solution (1–2 min). For slurries, see "additional tips" below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose 100 µL of Dioxane solution mixture to vials A1–A6 and vials B1–B6 according to scheme below. You may have a very small amount of excess solution remaining. Save it as a reaction standard for HPLC/TLC later.
- Dose 100 µL DMAc solution mixtures to vials C1–C6 and vials D1–D6 according to scheme below.



3. add **15 µL** NaOtBu 2M THF solution to all 24 vials using pipette

- After all substrate mixtures have been dosed according to the recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. Ensure the temperature probe holes line up on both the lid and the block.
- The screw-on lid according to directions and patterns are shown in the “additional tips” section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on a preheated hot plate with a probe through the lid and inserted into the block. Heat at 100 °C overnight stirring at or near 300 rpm. If the substrate is sensitive to base, lower the temperature to between 60 °C–80 °C. Turn off nitrogen flow to box and dispose of any unused chemicals.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.

Quench Solution Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit)

  Note: This recipe makes 50 mL which is enough stock solution for all four screening set in the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit
- Follow the below Work-up Procedure

Work-Up Procedure and Analysis

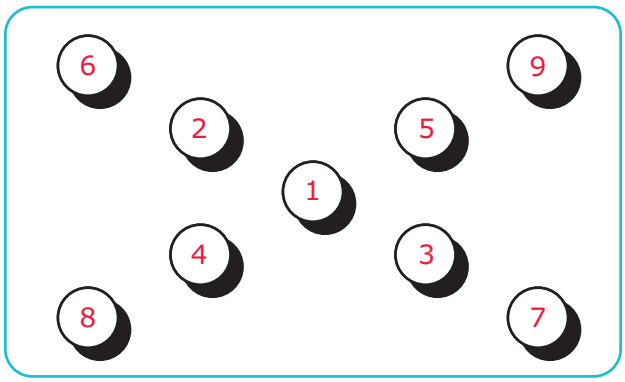
- Cool Reaction Block. Remove lid using a small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten the middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 5 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution.
- While the plate is resting, add 700 µL of quench solution to each 24 individually labeled (A1, A2, etc,) HPLC vial or to each of 24 wells of a 96-well HPLC/UPLC autosampler block.
- Remove the lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run-on HPLC autosampler

Additional Tips:

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



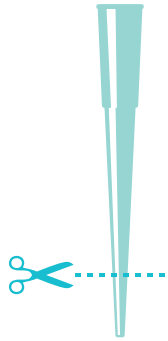
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole:

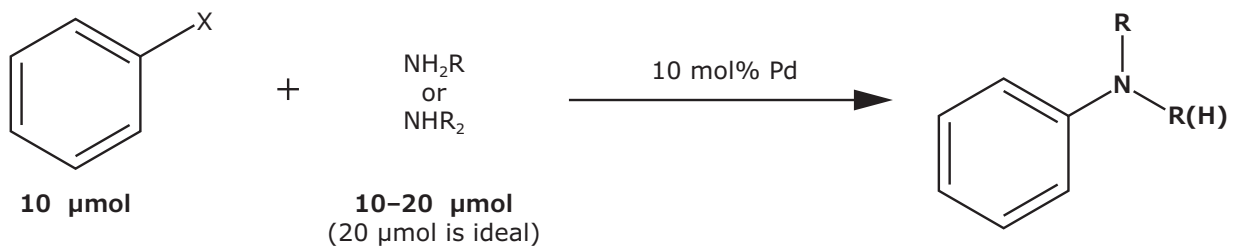
Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions:

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring while you draw aliquots for dosing into the corresponding reaction vial.



Two solvents (Dioxane, DMAc), Strong Base (NaOt-Bu) Step-by-Step Guide for Buchwald-Hartwig Amination Reaction Screening Kit



10 µmol

10–20 µmol
(20 µmol is ideal)

Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit and you will use the following components
  - 12 x 2 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 2 empty 4 mL reaction vials
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 1 (2 mL) ampule each of 1,4-Dioxane and DMAc (in ampule boxes)
- 2 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

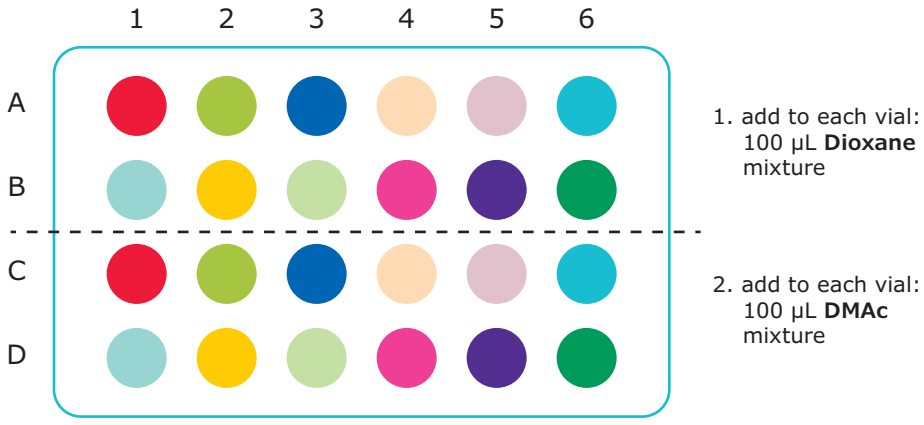
- Pipette (0–100 µL) and tips
- 2 (1 mL) syringes with long needles
- Aryl halide and amine
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 100 °C. Use an oil bath or second reaction block to hold temperature and avoid spiking.
- If base sensitive substrate, reduce temperature to between 60 °C and 80 °C.
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid and verify all holes, including the temperature probe hole, line up with the corresponding holes on the film
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details)
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and purge 5 minutes. Leave nitrogen flowing for remainder of set up.
- Make substrate mixtures directly into the empty 4 mL reaction vials according to recipe (provided in the downloadable excel file) **omitting solvent and NaOt-Bu solution**. **Add one stir bar to each vial mixture.** Label as "Dioxane Substrate Mixture A" and "DMAc Substrate Mixture B"). **If amine is volatile, add it last (after solvent mixtures have been added) directly to requisite reaction vials via syringe from a separate vial that has been quickly purged and then capped before placing into Inertion Box.**
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the "Dioxane Substrate Mixture A" in the center hole on the left hand side of the Inertion Box diffuser tray. Place the "DMAc Substrate Mixture B" in the center hole on the right hand side of the plate. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the mat on.
- Using an ampule cracker, open 1 ampule each of Dioxane, DMAc, and NaOt-Bu solution and quickly place into ampule holes located along the bottom of Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole two times by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials. **DO NOT add NaOt-Bu solution to either substrate mixture yet—it is added separately last.**
- Stir mixtures until in solution (1–2 min). For slurries, see "additional tips" below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose 100 µL of Dioxane solution mixture to vials A1–A6 and vials B1–B6 according to scheme below. You may have a very small amount of excess solution remaining. Save it as a reaction standard for HPLC/TLC later.
- Dose 100 µL DMAc solution mixtures to vials C1–C6 and vials D1–D6 according to scheme below.



3. add **15 µL** NaOtBu 2M THF solution to all 24 vials using pipette

- After solvents mixtures are added, dose 15 µL of the NaOt-Bu solution to each of the 24 vials.
- After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 100 °C overnight stirring at or near 300 rpm. If substrate is sensitive to base, lower the temperature to between 60 °C and 80 °C. Turn off nitrogen flow to box and dispose of any unused chemicals.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.

Quench Solution Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit)

  Note: This recipe makes 50 mL which is enough stock solution for all four screening set in the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit
- Follow the below Work-up Procedure

Work-Up Procedure and Analysis

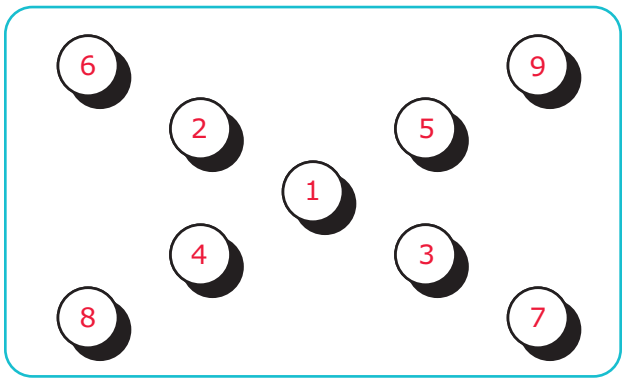
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 5 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution.
- While plate is resting, add 700 µL acetonitrile to each 24 individually labeled (A1, A2 etc,) HPLC vial or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block . **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. May need to optimize ratio of sample to dilution factor for your unique system.

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



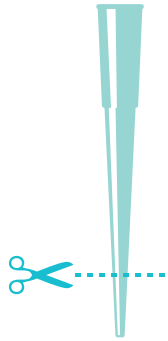
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

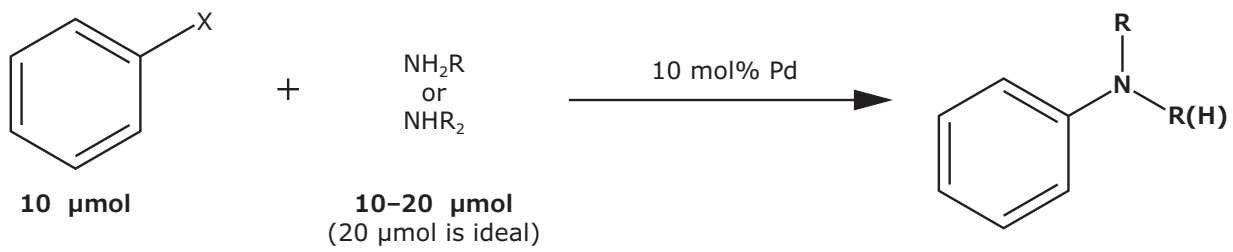
Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring while you draw aliquots for dosing into the corresponding reaction vial.



One Solvent (Dioxane), two bases (NaOtBu, Cs_2CO_3) Step-by-Step Guide for Buchwald-Hartwig Amination Reaction Screening Kit



10 µmol

10–20 µmol
(20 µmol is ideal)

Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit and you will use the following components
  - 12 x 2 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 1 reaction vial containing preweighed Cs_2CO_3
  - 1 empty 4 mL reaction vial
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 2 (2 mL) ampule each of 1,4-Dioxane (in ampule boxes)
- 2 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

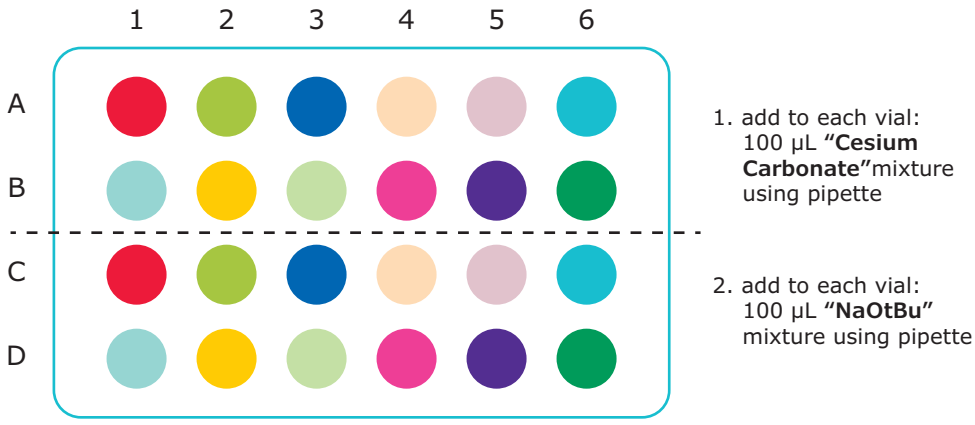
- Pipette (0–100 µL) & tips
- 1 (1 mL) syringes with long needles
- Your aryl halide and amine
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 100 °C (use oil bath or second reaction block to hold temperature and avoid spiking).
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid (make sure all holes, including the temperature probe hole, line up with the corresponding holes on the film)
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Make substrate mixtures directly into provided empty 4 mL reaction vial and preweighed Cs_2CO_3 vial according to recipe **omitting solvent and NaOt-Bu solution**. Add one stir bar to each vial mixture. Label as "Cs_2CO_3 Substrate Mixture A" and "NaOt-Bu Substrate Mixture B". **If amine is volatile, add it last (after solvent mixtures have been added) directly to requisite reaction vials via syringe from a separate vial that has been quickly purged and then capped before placing into Inertion Box.**
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the "Cs_2CO_3 Substrate Mixture A" in the center hole on the left hand side of the Inertion Box diffuser tray. Place the "NaOt-Bu Substrate Mixture B" in the center hole on the right hand side of the plate. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the mat on.
- Using an ampule cracker, open 2 ampules of Dioxane and 1 of NaOt-Bu solution and quickly place into ampule holes located along the bottom of Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Bechtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials. **DO NOT add NaOt-Bu solution to substrate mixture B yet—it is added separately last, directly to the reaction vials.**
- Stir mixtures until in solution (1–2 min). For slurries, see "additional tips" below.
- While mixtures are stirring, carefully remove the cap mat covering the 24-vial, preloaded catalysts in the reaction block.
- Dose 100 µL of "Cs_2CO_3 Substrate Mixture A" solution to vials A1–A6 and vials B1–B6 according to scheme below. You may have a very small amount of excess solution remaining. Save it as a reaction standard for HPLC/TLC later.
- Dose 100 µL "NaOt-Bu Substrate Mixture B" solution to vials C1–C6 and vials D1–D6 according to scheme below.
- After solvent mixtures are added, dose 15 µL of the NaOt-Bu solution to each vial in vials C1–C6 and vials D1–D6 according to scheme below.



3. add **15 µL** NaOtBu 2M THF solution to vials C1–D6 using pipette

- After all substrate mixtures have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 100 °C overnight stirring at or near 300 rpm. If substrate is sensitive to base, lower the temperature to between 60 °C–80 °C. Turn off nitrogen flow to box and dispose of any unused chemicals.
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Quench Solution Recipe

- 49 mL CH_3CN
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  Note: This recipe makes 50 mL which is enough stock solution for all four screening set in the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit
- Follow the below Work-up Procedure

Work-Up Procedure and Analysis

- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
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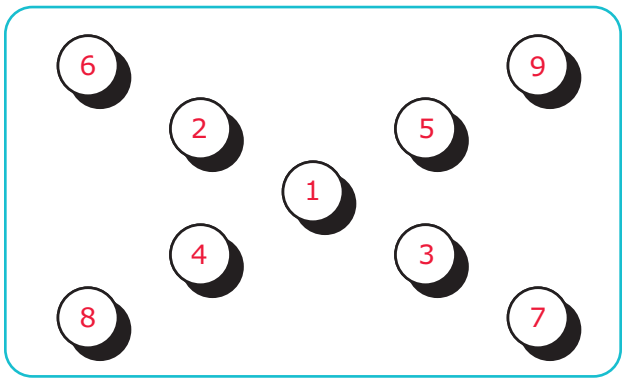
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler.

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



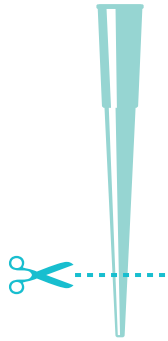
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

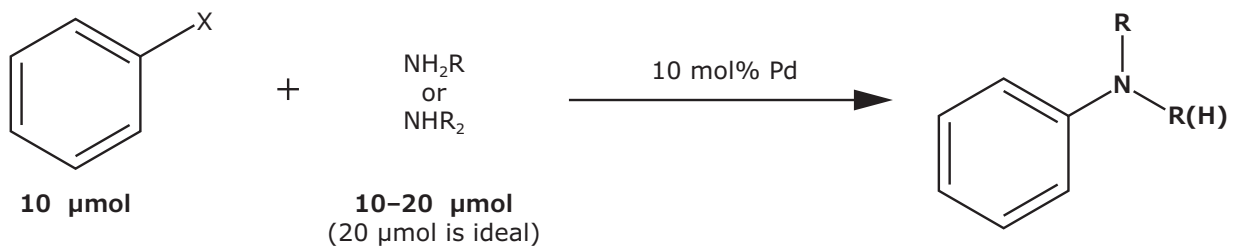
Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring while you draw aliquots for dosing into the corresponding reaction vial.



One Solvent (DMAc), two bases (NaOtBu, Cs_2CO_3) Step-by-Step Guide for Buchwald-Hartwig Amination Reaction Screening Kit



10 µmol

10–20 µmol
(20 µmol is ideal)

Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit and you will use the following components
  - 12 x 2 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 1 reaction vial containing pre-weighed Cs_2CO_3
  - 1 empty 4 mL reaction vial
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 2 (2 mL) ampule each of DMAc (in ampule boxes)
- 1 ampule of NaOt-Bu 2M solution
- 2 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately). Provided with the KitAlysis™ 24-Well Reaction Block

Additional (not included) items needed:

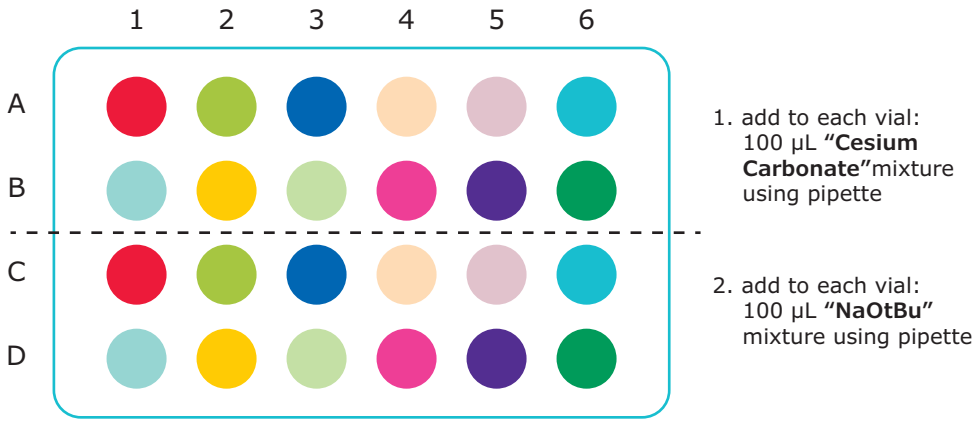
- Pipette (0–100 µL) & tips
- 1 (1 mL) syringes with long needles
- Your aryl halide and amine
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 100 °C (use oil bath or second reaction block to hold temperature and avoid spiking).
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid (make sure all holes, including the temperature probe hole, line up with the corresponding holes on the film)
- Check all screws to ensure they are not stripped. Replace any stripped screws with supplied replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Make substrate mixtures directly into supplied the empty 4 mL reaction vial and the vial containing pre-weighed Cs_2CO_3 according to recipe (provided in the downloadable excel file) **omitting solvent and NaOt-Bu solution**. Add one stir bar to each vial mixture. Label as "Cs_2CO_3 Substrate Mixture A" and "NaOt-Bu Substrate Mixture B". **If amine is volatile, add it last (after solvent mixtures have been added) directly to requisite reaction vials via syringe from a separate vial that has been quickly purged and then capped before placing into Inertion Box.**
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the "Cs_2CO_3 Substrate Mixture A" in the center hole on the left hand side of the Inertion Box diffuser tray. Place the "NaOt-Bu Substrate Mixture B" in the center hole on the right hand side of the plate. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the mat on.
- Using an ampule cracker, open 2 ampules of DMAc and 1 of NaOt-Bu solution and quickly place into ampule holes located along the bottom of Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials. **DO NOT add NaOt-Bu solution to substrate mixture B yet—it is added separately last, directly to the reaction vials.**
- Stir mixtures until in solution (1–2 min). For slurries, see "additional tips" below.
- While mixtures are stirring, carefully remove the cap mat covering the 24-vial, preloaded catalysts in the reaction block.
- Dose 100 µL of "Cs_2CO_3 Substrate Mixture A" solution to vials A1–A6 and vials B1–B6 according to scheme below. You may have a very small amount of excess solution remaining. Save it as a reaction standard for HPLC/TLC later.
- Dose 100 µL "NaOt-Bu Substrate Mixture B" solution to vials C1–C6 and vials D1–D6 according to scheme below.
- After solvent mixtures are added, dose 15 µL of the NaOt-Bu solution to vial in vials C1–C6 and vials D1–D6 according to scheme below.



3. add **15 µL** NaOtBu 2M THF solution to vials C1–D6 using pipette

- After all substrate mixtures have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the “additional tips” section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 100 °C overnight stirring at or near 300 rpm. If substrate is sensitive to base, lower the temperature to between 60 °C–80 °C. Turn off nitrogen flow to box and dispose of any unused chemicals.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.

Quench Solution Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit)

  Note: This recipe makes 50 mL which is enough stock solution for all four screening set in the KitAlysis™ Buchwald-Hartwig Amination Reaction Screening Kit
- Follow the below Work-up Procedure

Work-Up Procedure and Analysis

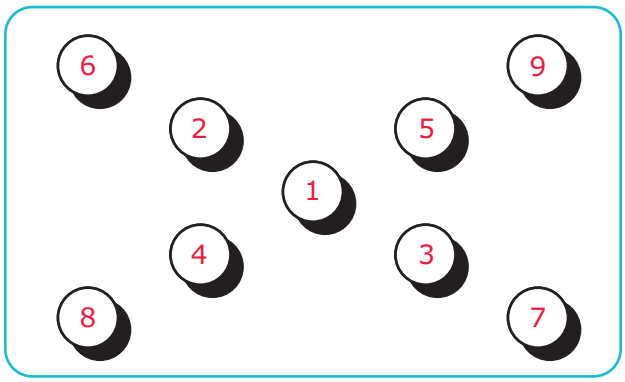
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 5 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution.
- While plate is resting, add 700 µL of quench solution to each 24 individually labeled (A1, A2 etc.,) HPLC vial or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block . **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern supplied below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



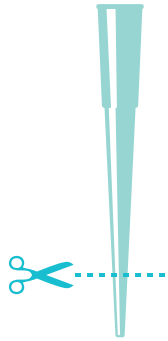
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole:

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions:

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: C-N (Buchwald-Hartwig) Amination Reaction

Kit to Hit Scale-Up Guide

Important Note: All of the preformed catalysts used in the kit are air and moisture stable complexes in their commercially available form. Once activated by base under the reaction conditions they become sensitive to air. To best enable scale-up success, the use of standard Schlenk technique is recommended.

General Guidelines for Catalytic Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)[1]
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)[1]
- Keep reactions under an atmosphere of nitrogen

Example Experimental

All solids (base, substrates, catalyst) were weighed on a bench top balance and added to a cooled, oven dried flask[1] equipped with an oven dried stir bar. The reaction was capped and then purged and backfilled with nitrogen (3x) with a nitrogen-fed manifold needle. Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. If a liquid bases was required, it was added as the final component via syringe. When all constituents were added, the nitrogen line was removed[2] and the reaction allowed to stir at the required temperature for the duration of the reaction.

1. *Reaction vials capable of being sealed with a microwave cap are very useful as well. By design, the caps do not need to be replaced once punctured.*
2. *For higher temps, a reflux condenser operated under nitrogen can be used.*

KitAlysis™ High-Throughput *Medium* (5, 6, 7) Ring Closing Metathesis Reaction Screening Kit

Kit Design

The Medium Ring Closing Metathesis kit was designed to provide the best possible chance of finding good reaction conditions to close 5, 6, and 7-membered rings and is run with:

- 20 µmol of your RCM substrate
- 1 µmol catalyst per vial; 6 in this system. 5 mol% per reaction.
- 0.2M solvent concentration (ClPh, Toluene, *i*-PrOAc, and Me-THF)
- TFA neat. Optional additive. See kit step-by-step user guide for details and guide on when to use.
- 60 °C (Recommended). Temperatures not to exceed 80 °C. Lower temperatures may also be optional but 60 °C is an excellent overall starting point.

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

The step-by-step user guide is designed for the first-time user and should be read completely before getting started; and used in conjunction with the **video**. This guide includes trouble-shooting tips, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Excel Sheet:

The downloadable excel files are specific to the kit being run and can be found within each **Step-by-Step User Guide**. They have the following features:

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included in Your KitAlysis-RCM-2PAK High-Throughput Screening Kit

Contents in each of the 2 individually sealed Mylar (foil bags):

- 24 (6 x 4) pre-weighed catalysts in glass vials loaded with stir bars, topped with cap mat.
- 4 empty reaction vials with lids (to make stock solutions)

The screening sets come pre-loaded with 1 µmol of catalyst in each vial according to the following design.

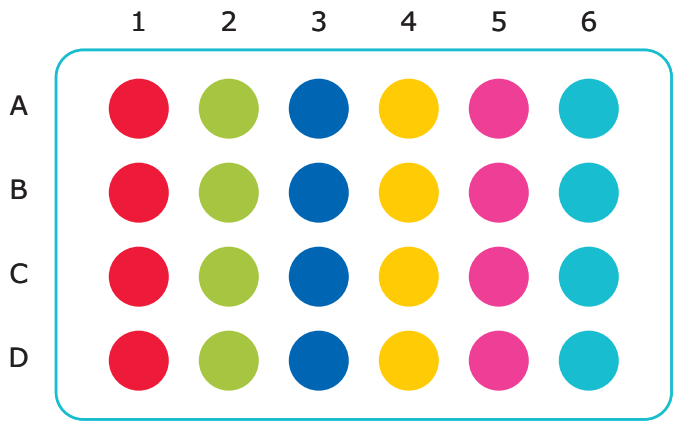


Figure 1. KitAlysis™ Ring Closing Metathesis Well Plate.

Description	Cat. No.	Vial
Grubbs Catalyst™ 2nd Generation	**569747**	A1, B1, C1, D1
Hoveyda-Grubbs Catalyst™ 2nd Generation	**569755**	A2, B2, C2, D2
Grubbs Catalyst™ C571	**682373**	A3, B3, C3, D3
Grubbs Catalyst™ C711	**729345**	A4, B4, C4, D4
Grubbs Catalyst™ 1st Generation	**579726**	A5, B5, C5, D5
Grubbs Catalyst™ C827	**682365**	A6, B6, C6, D6

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes:

- Chlorobenzene (ClPh): 4 x 2 mL of degassed, anhydrous Sure/Seal™
- Toluene: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- Isopropyl acetate (*i*-PrOAc): 4 x 2 mL of degassed, anhydrous Sure/Seal™
- Methyl THF (Me-THF): 4 x 2 mL of degassed, anhydrous Sure/Seal™
- TFA (neat): 2 x 2 mL degassed, anhydrous Sure/Seal™

Internal Standard:

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the hyperlinked screen types in the "Kit Design" section above.
- KitAlysis™ 24-well Reaction Block Replacement Films-2EA
- Pack of 2 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

Stir Bars-8 Individually Packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures.

Additional Recommended Materials (Sold Separately)

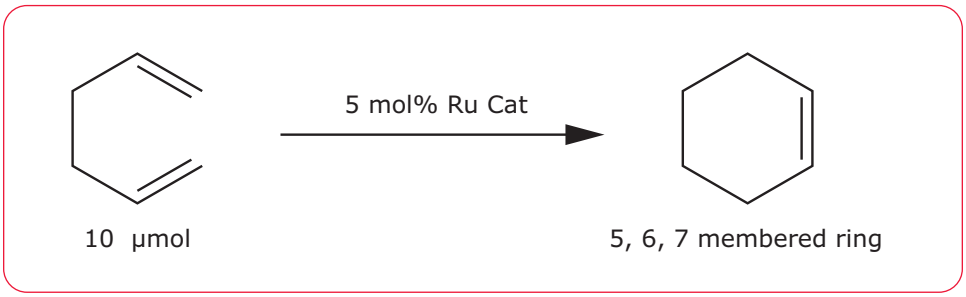
- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Step-by-Step Guide for KitAlysis™ High-Throughput Medium (5, 6, 7) Ring Closing Metathesis Reaction Screening Kit

6 pre-weighed catalysts (x4)

4 degassed solvents: Chlorobenzene (ClPh), Toluene (PhMe), Isopropyl Acetate (*i*-PrOAc), and Methyl-THF (Me-THF)

1 (optional) acid additive (degassed neat TFA)



Materials Required for Set-Up

- 1 mylar (foil) bag from the KitAlysis™ Grubbs Metathesis Reaction Screening Kit and you will use the following components:
  - 6 x 4 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - Four empty 4 mL substrate vials
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 1 (2 mL) ampule each of PhCl, PhMe, i-PrOAc, Me-THF, and TFA (optional) from supplied ampule boxes
- Four NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately). Provided with the KitAlysis™ 24-Well Reaction Block

Additional (not included) items needed:

- Pipette (0–100 μL) & tips
- 4 (1 mL) syringes with long needles
- Your olefin metathesis substrate
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

Note on Acid (TFA) additive:

Follow the instructions as outlined below, adding the acid additive (TFA) as the last step of the procedure directly to each individual reaction vial. **DO NOT ADD TFA DIRECTLY TO THE SUBSTATE STOCK SOLUTIONS** as this may cause your substrates to crash out of solution. It is advised to run a second screen in parallel without acid in order to compare the results.

When to use TFA: See the "TFA Additive Guidelines" below

- Preheat a hot plate to 60 °C (temperatures range but do not exceed 80 °C). It is recommended to use an oil bath or second reaction block to hold temperature and avoid spiking. Lower temperatures may be better but 60 °C is a great place to start.
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid and verify all holes, including the temperature probe hole, line up with the corresponding holes on the film.
- Check all screws to ensure they are not stripped. Replace any stripped screws with supplied replacements.
- Place the KitAlysis™ Benchtop Inertion Box, with tubing connected to inert gas onto the **second, non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details)
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for rest of set up.
- Prepare empty 4 mL substrate vials to make substrate mixtures. Label as:
  - "ClPh Substrate Mixture A,"
  - "Toluene Substrate Mixture B,"
  - "*i*-PrOAc Substrate Mixture C"
  - "Me-THF Substrate Mixture D."
- Add one stir bar to each of the 4 labeled substrate vials.
- Weigh your olefin metathesis substrate into each of the 4 substrate vials according to the substrate mixture recipe provided in the downloadable excel file. **If your cross-metathesis partners are volatile, add it last (after solvent mixtures have been added) directly to requisite reaction vials via pipette from a separate vial that has been quickly purged and then capped before placing into Inertion Box.**
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the "ClPh Substrate Mixture A," and the "Toluene Substrate Mixture B," in two of the holes located on the left-hand side of the Inertion Box diffuser tray. Place the "*i*-PrOAc Substrate Mixture C" and "Me-THF Substrate Mixture D." in two holes on the right-hand side of the plate. Ensure that one vial is placed in the center hole on both the left- and right-hand side of the diffuser tray. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block (A1 in upper left). **Leave the vial mat on.**
- Place 1 ampule each of ClPh, PhMe, *i*-PrOAc, and Me-THF into ampule holes located along the bottom of Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for rest of set up.
- Using the ampule cracker, open all ampules of solvent in the inertion box.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to corresponding substrate mixture vials.
- Stir mixtures until in solution (1–2 min). For slurries, see "additional tips" below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose stock solutions, **using a new pipette tip for each substrate mixture**. You may have a very small amount of excess solution remaining. Save it as a reaction standard for HPLC/TLC later.
  - Dose 100 µL of "ClPh Substrate Mixture A" to vials A1–A6.
  - Dose 100 µL "Toluene Substrate Mixture B" to vials B1–B6.
  - Dose 100 µL "*i*-PrOAc Mixture C" to vials C1–C6.
  - Dose 100 µL "Me-THF Substrate Mixture D" to vials D1–D6.
- **If using TFA**, remove one empty solvent ampule from the inertion box. Replace it with an ampule of TFA.
- Using an ampule cracker, open the TFA.
- Using a pipette, dose 5 µL TFA to each reaction vial. (It helps to say the name of the vial (A1, A2, etc.) as you go along to ensure you stay on track as you dose.)

- After all substrate mixtures (and acid additive if using) have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 60 °C (and to no more than 80 °C) overnight stirring at or near 300 rpm.
- Turn off nitrogen flow to box and dispose of any unused chemicals.
- At reaction completion, follow the Work-Up Procedure provided below that will quench reactions, make them more suitable for analysis, and add the internal standard.

Quench Solution/Internal Standard Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit) There is excess in the bottle so be sure to weigh it out.

  Note: This recipe makes 50 mL which is enough stock solution for four screens. The amount of internal standard is 10 mol% per reaction (1 µmol). So, a big product peak to small internal standard indicates a good reaction. Integrate to compare reactions against one another (product/internal standard).

Work-Up Procedure and Analysis

- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each reaction vial, using a pipette for accuracy.
- Replace reaction block lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of HPLC Grade Acetonitrile to each 24 individually labeled (A1, A2, etc.) HPLC vial or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (use the upper left quadrant as it corresponds to the reaction block vial array). See "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.
- See "troubleshooting" below for additional help

Note on catalyst quenching: The internal standard quench should be sufficient in most cases, however an additional catalyst quench may be desired prior to the HPLC analysis step. Choice of quenching agent may depend on substrate compatibility. For options see:

1. *Blacquiere, J. M.; Jurca, T.; Weiss, J.; Fogg, D. E. Adv. Synth. Catal.* ***2008****, 350, 2849 – 2855.*
2. *Yee, N. K.; Farina, V.; Houpis, I. N.; Haddad, N.; Frutos, R. P.; Gallou, F.; Wang, X.-j.; Wei, W.; Simpson, R. D.; Feng, X.; Fuchs, V.; Xu, J.; Tan, J.; Zhang, L.; Xu, J.; Smith-Kennan, L. L.; Vitous, J.; Ridges, M. D.; Spinelli, E. M. Johnson, M. J. Org. Chem. 2006, 71, 7133-7145.*

TFA Additive Guidelines

Two essential scenarios where an acid additive should be considered:

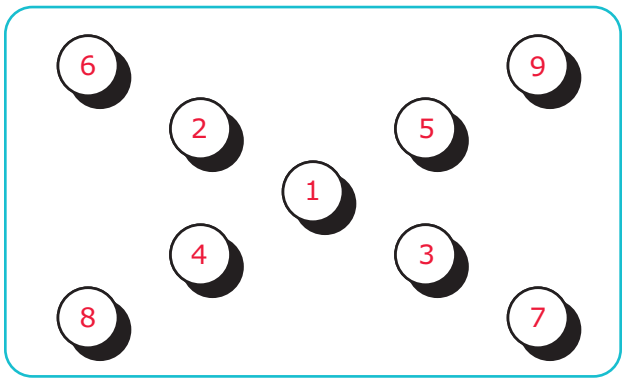
1. Strongly coordinating group anywhere in the molecule (primary/secondary amine, thiol, urea, isonitrile etc.). Additive should be matched to the pKa or Lewis basicity of the coordinating group (amines – HCl/TFA, ureas – boron or aluminum Lewis acids)
2. Weakly coordinating groups that are near the catalyst initiation site (amides, esters, ketones, alcohols that are 5 or 6 bonds away from the olefin).

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light, even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern supplied below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



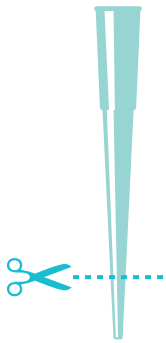
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring well while you draw aliquots for dosing into the corresponding reaction vial.



Reaction Troubleshooting

If no conversion is observed (starting material is not consumed), try one or more of the following:

1. Increase reaction temperature
2. Assess starting material for potential coordinating groups and screen acid additives (see above)
3. Assess starting material purity—could it contain a peroxide or impurity that is highly coordinating?

If the starting material is consumed but the reaction mixture is messy, double bond isomerization or other unwanted side reactions may have occurred. Try one or more of the following:

1. Reduce reaction temperature
2. Reduce reaction time
3. Reduce catalyst loading
4. Add isomerization inhibitors (see **Metathesis Guide** for more detail)

Scale-Up Guide: Medium (5, 6, 7) Ring Closing Metathesis

Important Note

All the catalysts used in the kit are sensitive to air and moisture in their commercially available form. Please see the catalyst SDS or product detail page for details on handling and storage. To best enable scale-up success, the use of glove box is highly recommended. However, modified bench top techniques are supplied below.

General Guidelines for Catalytic Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental-Glovebox Set-Up, Hood Reaction

The olefin substrate was weighed on a bench top balance and added to a cooled, oven dried flask equipped with an oven dried stir bar. The reaction was capped and then transferred to a standard chemical glove box. Once in the glove box, anhydrous Sure/Seal™ solvent was added via syringe. If use of acid additive was desired, this was added to the olefin solution. The catalyst (removed from the glove box fridge or brought in the box from a bench fridge) was then weighed and added to the reaction mixture, the mixture capped, and then removed from the glove box. Upon arrival at the bench, a reflux condenser, fitted with a septum and connected to a nitrogen-fed manifold needle (to allow the safe escape of ethylene without the loss of solvent while keeping the reaction under inertion) was connected to the reaction flask. The reaction was then added to a pre-heated oil bath and allowed to stir at the required temperature for the duration of the reaction.

Example Experimental-Bench Top Set-Up, Hood Reaction

The olefin substrate was weighed on a bench top balance and added to a cooled, oven dried two-neck flask equipped with an oven dried stir bar. A reflux condenser, fitted with a septum and connected to a nitrogen-fed manifold needle (to allow the safe escape of ethylene without the loss of solvent was attached to the vertical neck of the flask and the other neck was fitted with a septum. The reaction set-up was then evacuated and backfilled with nitrogen (3x, making sure to allow time as the set-up is larger than a single reaction flask). Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. If use of acid additive was desired, this was added to the olefin solution via syringe through the septa. The catalyst was removed from the fridge, quickly weighed and then added to the reaction by opening the septa for a short interval. (A stock solution of the catalyst in a portion of the reaction solvent can also be used to add the catalyst via syringe or cannula). The reaction was then allowed to stir at the required temperature for the duration of the reaction. The catalyst bottle was flushed with nitrogen and placed back in the fridge for storage.

KitAlysis™ High-Throughput Miyaura Borylation Reaction Screening Kit

Kit Design

The Miyaura Borylation Kit was designed to provide the best possible chance of finding good reaction conditions for cross-coupling reactions:

- pre-weighed base or base solution (provided)
- 10–20 mmol coupling partner (user supplied)
- 10 mmol aryl halide substrate (user supplied)
- Catalysts (user supplied).
- 6 solvents (toluene, DMA. THF, *n*-butanol, MeOH, MeCN) (provided)

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

The step-by-step user guide is designed for the first-time user and should be read completely before getting started; and used in conjunction with the **video**. This guide includes troubleshooting tips, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Excel Sheet:

The downloadable excel files are specific to the kit being run and can be found within each **Step-by-Step User Guide**. They have the following features:

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included in Your KITALYSIS-BOR-2PAK High-Throughput Screening Kit

Contents in each of the 2 individually sealed Mylar (foil bags):

- 22 (11 x 2) pre-weighed bases in glass vials loaded with stir bars, topped with cap mat.
- 4 empty reaction vials with lids (to make stock solutions)

The screening sets come pre-loaded with 30 µmol of base in each vial according to the following design.

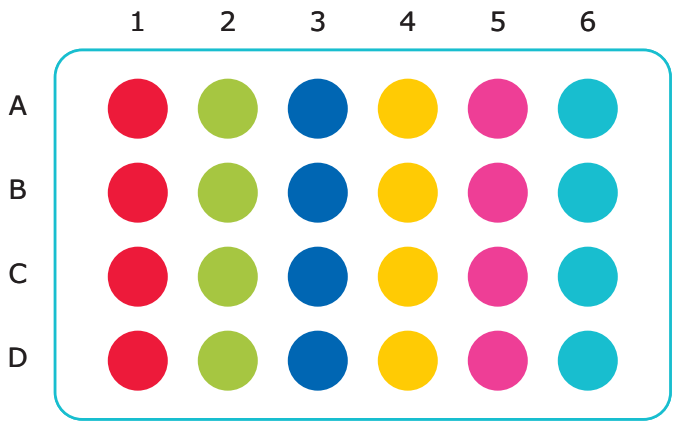


Figure 1. KitAlysis™ Ring Closing Metathesis Well Plate.

Description	Cat. No.	Vial
SPhos Pd G4	**804282**	A1, B1, C1, D1
XPhos Pd G4	**804274**	A2, B2, C2, D2
CataCXium Pd G4	**900349**	A3, B3, C3, D3
RuPhos Pd G4	**804290**	A4, B4, C4, D4
PCy3 Pd G4	**764175**	A5, B5, C5, D5
DTBPF Pd G	**804975**	A6, B6, C6, D6

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes:

- 4 empty 4 mL reaction vials
- 2 (2 mL) ampule each of the following: ethylene glycol, DIPEA
- 4 (2 mL) ampule each of the following: toluene, DMA, THF, *n*-butanol. MEOH, MECN

Internal Standard:

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the **step-by-step guide**
- KitAlysis™ 24-well Reaction Block Replacement Films-2EA
- Pack of 2 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

Stir Bars-8 Individually Packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures.

Additional Recommended Materials (Sold Separately)

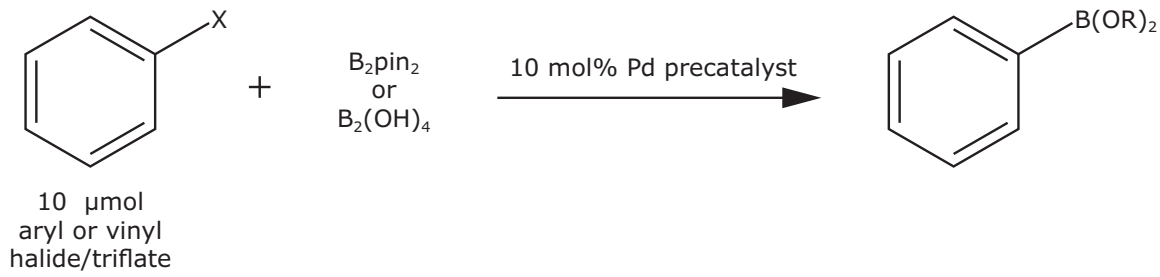
- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Step-by-Step Guide for KitAlysis™ High-Throughput Miyaura Borylation Reaction Screening Kit

2 bases (KOAc, DIEA)

6 degassed solvents (toluene, DMA, THF, *n*-butanol, MeOH, MeCN)

6 pre-weighed catalysts (x4)



Materials Required

- 1 mylar bag from the KitAlysis™ High-Throughput Miyaura Borylation Reaction Screening Kit and you will use the following components
  - 6 x 4 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 4 substrate stock solution vials (4 mL) pre-loaded with KOAc
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- $B2(OH)_4$ acid or B_2pin_2
- 1 (2 mL) ampule each of 4 of the following: MeOH, toluene, DMA, THF, *n*-butanol, MeCN (in ampule boxes)
- 1 (2 mL) ampule of DIEA (if desired as substitute for KOAc)
- 1 (2 mL) ampule of ethylene glycol (if desired as additive)
- 4 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 4 (1 mL) syringes with long needles
- Your (het)aryl or vinyl halide/triflate substrate
- empty 4 mL substrate stock solution vials (if using DIEA as base)
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

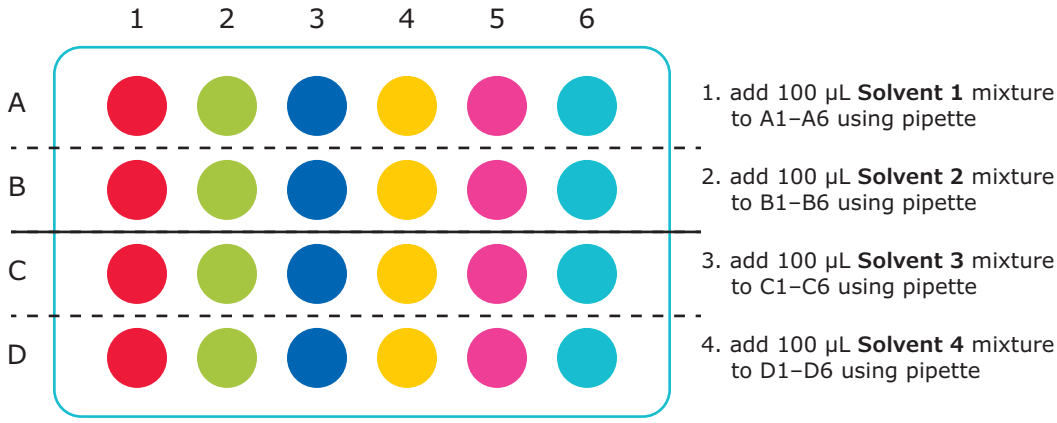
Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 60 °C (use oil bath or second reaction block to hold temperature and avoid spiking). Reaction temperatures for the Miyaura Borylation reaction can range from room temperature to 120 °C but 60 °C is a general and very good starting point.
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid (make sure all holes, including the temperature probe hole, line up with the corresponding holes on the film).
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block **with lid** into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. **Leave nitrogen flowing for remainder of set up.**
- Weigh substrate and boron source directly into all 4 substrate stock solution vials (if using KOAc as base, use the provided preloaded vials; if using DIEA as base, use user supplied vials) according to recipe (provided in the downloadable excel file) **omitting solvent**. **Add one stir bar to each vial mixture.** Label as “Solvent 1 Substrate Mixture A”, “Solvent 2 Substrate Mixture B”, “Solvent 3 Substrate Mixture C”, and “Solvent 4 Substrate Mixture D”).
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place “Solvent 1 Substrate Mixture A” and “Solvent 2 Substrate Mixture B” in two of the holes located on the left hand side of the Inertion Box diffuser tray. Place “Solvent 3 Substrate Mixture C” and “Solvent 4 Substrate Mixture D” in two holes on the right hand side of the plate. Ensure that one vial is placed in the center hole on either side. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block. Leave the mat on.
- Using an ampule cracker, open 1 ampule each of the 4 selected solvents quickly place into the holes located along the bottom of the Inertion Box, below the Reaction Block. Then open 1 ampule of DIEA (if desired as substitute for KOAc) and 1 ampule of ethylene glycol (if desired as additive) and place them in the remaining holes on either side of the plate.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials. **DO NOT add DIEA or ethylene glycol to any substrate mixture—they are added directly to each of the 24 reaction vials as the last step, if desired.**
- Stir mixtures until in solution (1–2 min). For slurries, see “additional tips” below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded bases in the reaction block.
- Dose stock solutions
  - Dose 100 µL of “Solvent 1 Substrate Mixture A” to vials A1–A6.
  - Dose 100 µL of “Solvent 2 Substrate Mixture B” into vials B1–B6.
  - Dose 100 µL of “Solvent 3 Substrate Mixture C” to vials C1–C6.
  - Dose 100 µL of “Solvent 4 Substrate Mixture D” to vials D1–D6.

    You may have a very small amount of excess solution remaining for each mixture. Save it as a reaction standard for HPLC/TLC later.
  - Dose 5 µL of DIEA to each vial (if necessary).
  - Dose 2 µL of ethylene glycol to each vial (for use with bis-boronic acid, if desired)



5. add 5 µL **DIEA** to each vial (if using in place of KOAc) and 2 µL ethylene glycol to each vial (if desired as additive with bis-boronic)

- After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check as you go along to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 60 °C for desired reaction time stirring at or near 300 rpm. Higher or lower temperatures may be optimal, but 60 °C is a good starting point.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.
- At reaction completion, follow the below Work-Up Procedure to quench reactions, make them more suitable for analysis, and add internal standard.
- Pinacol is provided if derivatization of the boronic acid (when using bis-boronic acid) to the boronic ester is desired.

Quench Solution/Internal Standard Recipe

- 24.5 mL CH_3CN
- 0.5 mL AcOH
- 7.7 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit) There is excess in the bottle so be sure to weigh it out.

  Note: This recipe makes 25 mL which is enough stock solution for both screening sets in the KitAlysis™ Miyaura Borylation Reaction Screening Kit. The amount of internal standard is 10 mol% per reaction, so a big product peak to small internal standard indicates a good reaction. Integrate to compare reactions against one another (product/internal standard).

Work-Up Procedure and Analysis

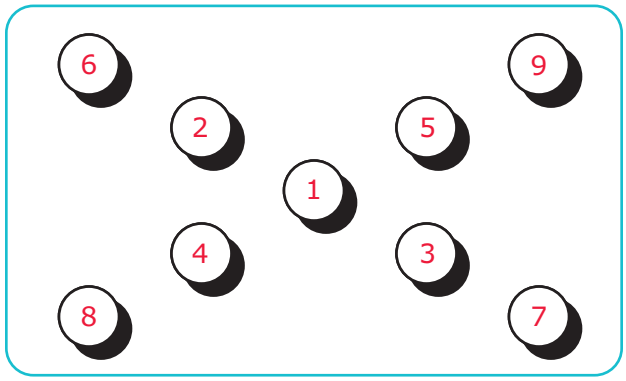
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of acetonitrile to each 24 individually labeled (A1, A2 etc,) HPLC vials or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (see "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.

Additional Tips:

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light, even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



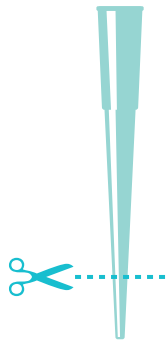
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring well while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: Miyaura Borylation Reaction

Important Note

To best enable scale-up success, the use of glove box is highly recommended. However, modified bench top techniques are provided below.

General Guidelines for Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental

All solids (base, substrates, catalyst) were weighed on a bench top balance and added to a cooled, oven dried flask[1,2] equipped with an oven dried stir bar. The reaction was capped and then purged and backfilled with nitrogen (3x) with a nitrogen-fed manifold needle. Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. If a liquid bases was required, it was added as the final component via syringe. When all constituents were added, the nitrogen line was removed[3] and the reaction allowed to stir at the required temperature for the duration of the reaction.

1. *See the* **Cross-Coupling Reaction Guide** *for more detailed additional guidance and troubleshooting tips.*
2. *Reaction vials capable of being sealed with a microwave cap are very useful as well. By design, the caps do not need to be replaced once punctured.*
3. *For higher temperatures, a reflux condenser operated under nitrogen can be used.*

KitAlysis™ High-Throughput 24 Palladium Precatalyst Reaction Screening Kit

Kit Design

The 24 palladium precatalyst cross-coupling kit was designed to provide the best possible chance of finding good reaction conditions for a wide range of cross-coupling reactions and is meant to widen the chemical space offered in more tailored kits (e.g., Suzuki, Buchwald-Hartwig) or to allow you to customize a reaction of your choice.

A step-by-step user guide and an excel sheet for stock solution recipes can be downloaded within the **Step-by-Step User Guide**, below.

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up Guide:

As this kit is designed to allow the user to customize based upon their needs, there is no specific user guide provided but some best practices, order of operations, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Additionally, consulting the existing detailed user guides may be helpful if you are looking to extend an existing kit (for example **Suzuki-Miyaura** & **Buchwald-Hartwig**) or to get inspiration. **Consulting the Cross-Coupling Guide** will also provide you with additional places to start. There is also a useful **video** to aid in set-up. All these resources, combined or alone, can provide general troubleshooting. these resources either combined or in isolation can provide general troubleshooting.

Excel Sheet:

The downloadable excel file is general, allowing the user to design their experiment to their specifications. For the most general reaction types, guidelines are provided in **the detailed reaction guides for the kits already available in the KitAlysis™ portfolio and in the Cross-Coupling guide. The excel sheet provides:**

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included In Your KITALYSIS-24PD-2PK High-Throughput Screening Kit

Contents in each of the 2 individually sealed Mylar (foil bags):

- 24 pre-weighed catalysts in glass vials loaded with stir bars, topped with cap mat.
- 4 empty reaction vials with lids (to make stock solutions)

The screening sets come pre-loaded with 1 µmol of catalyst in each vial according to the following design.

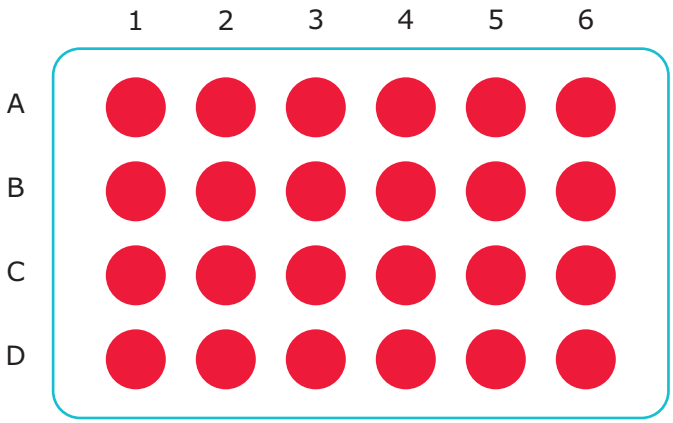


Figure 1. KitAlysis™ Well Plate Map

Description	Cat. No.	Vial
AdBrettPhos Pd G3	**776106**	A1
APhos Pd G3	**764183**	A2
rac-BINAP-Pd-G3	**804967**	A3
BrettPhos Pd G4	**804355**	A4
cataCXium® A Pd G3	**761435**	A5
CPhos Pd G3	**763004**	A6
CyJohnPhos Pd G3	**900621**	B1
DavePhos-Pd-G3	**804959**	B2
DPPF Pd G3	**804983**	B3
JackiePhos Pd G3	**762830**	B4
Josiphos SL-J009-1 Pd G3	**747130**	B5
meCgPPh Pd G3	**762822**	B6
4MetBuXPhos Pd G3	**900620**	C1
MorDalphos Pd G3	**792357**	C2
$P(Cy_3)$ Pd G3	**764175**	C3
$P(t\text{-}Bu)_3$ Pd G2	**756482**	C4
Pd-PEPPSI™-IPent catalyst	**732117**	C5
RuPhos Pd G4	**804290**	C6
SPhos Pd G4	**804282**	D1
tBuBrettPhos Pd G3	**745979**	D2
tBuXPhos Pd G3	**762229**	D3
XantPhos Pd G3	**763039**	D4
XPhos Pd G4	**804274**	D5
$PdCl_2(PPh_3)_2$	**412740**	D6

Internal Standard:

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the hyperlinked screen types in the "**Step-by-Step Guide**" section below.

KitAlysis™ 24-well Reaction Block Replacement Films-2EA

- Pack of 2 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

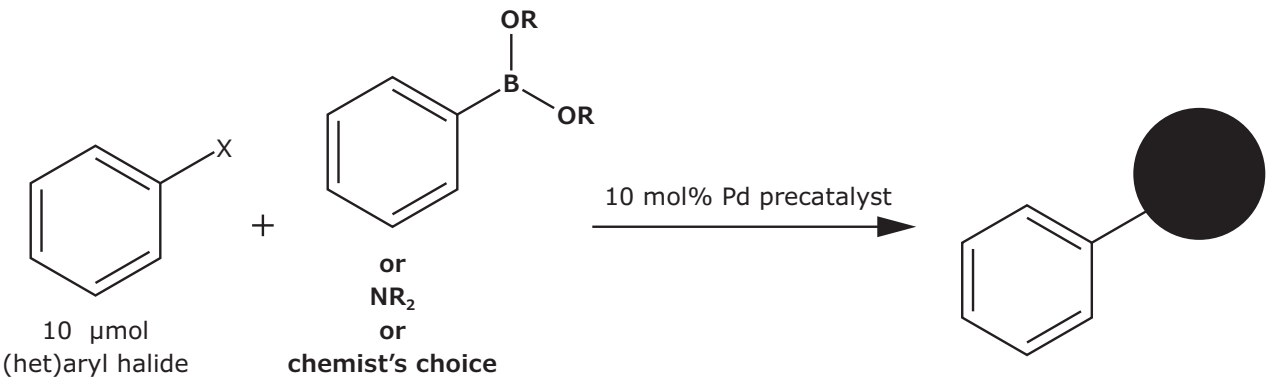
Stir Bars-3 individually packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures prior to dosing.

Additional Recommended Materials (Sold Separately)

- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Step-by-Step Guide for KitAlysis™ High-Throughput 24 Palladium Precatalyst Cross-Coupling Reaction Screening Kit



Useful for: Suzuki-Miyaura; Buchwald-Hartwig; Negishi; Heck; Sonagashira; Borylation; Ni/Pd co catalyst screen

Materials Required for Set-Up:

Depending upon your design, you need at a minimum:

- 1 mylar bag from the KitAlysis™ 24 Pd Precatalyst Reaction Screening Kit and you will use the following components:
  - 24 x 1 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat (included)
  - 2 empty 4 mL reaction vials (included)
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film (included)
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately)

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 1 (1 mL) syringes with long needles
- Your substrates
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates.

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure:

- Preheat your hot plate to the desired temperature. It is highly recommended to use an oil bath or second reaction block to hold temperature and avoid spiking.
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid and verify all holes, including the temperature probe hole, line up with the corresponding holes on the film.
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details)
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Weigh all substrates and solid bases into the empty 4 mL reaction vials according to recipe (provided in the downloadable excel file) **omitting solvent and liquid bases**. **Add one stir bar to each vial mixture.** Label vials to keep track.
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Ensure that one vial is placed in the center hole on both sides of the inertion box. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the capped, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram on the packaging and the Reaction Block (A1 in top left corner). Leave the mat on.
- Load solvent ampules into ampule holes located along the bottom of the Inertion Box, below the Reaction Block. You can also use anhydrous Sure/Seal™ 100 mL bottles of solvent with a nitrogen fed manifold needle attached. Rigorously degassed, ampulized solvents are available for many solvents, specially made for KitAlysis™ and sold separately to allow customization.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials from either ampules or Sure/Seal™ bottles. **DO NOT** add liquid base solutions to any substrate mixture-it is added directly to each reaction vial separately as the last step.
- Stir mixtures until in solution (1–2 min). For slurries, see “additional tips” below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose stock solutions according to your reaction design (100 µL of 0.1M solutions to each vial is standard). Dose 100 µL of “DMAc Substrate Mixture A” to vials A1–A6 according to scheme below.
  - You may have a very small amount of excess solution remaining for each mixture. Save it as a reaction standard for HPLC/TLC later.
- After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the “additional tips” section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check as you go along to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Make sure the probe is all the way into the bottom of the block to avoid overheating. Heat at desired temperature overnight stirring at or near 300 rpm.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.
- At reaction completion, follow the below Work-Up Procedure

Quench Solution Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit)

Note: This recipe makes 50 mL which is enough stock solution for all one screening sets in the KitAlysis™ 24 Pd Precatalyst Reaction Screening Kit. The amount of internal standard is 10 mol%. So, a big product peak to small internal standard indicates a good reaction.

Work-Up Procedure and Analysis

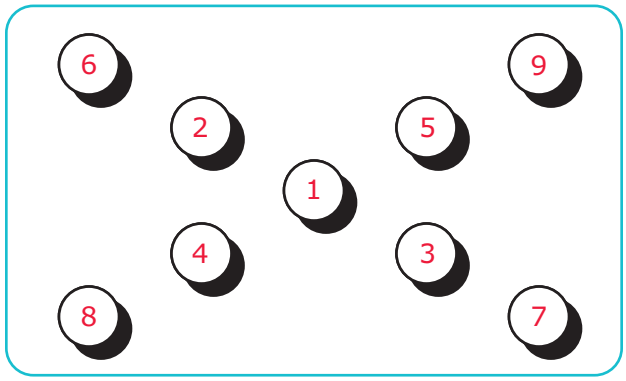
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw, and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of acetonitrile to each 24 individually labeled (A1, A2 etc.,) HPLC vials or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (see "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.

Additional Tips:

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light, even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



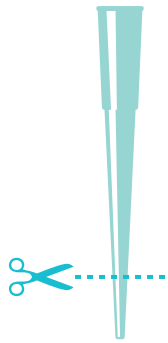
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring well while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: Palladium Precatalyst Cross-Coupling Reaction Screening Kit

Important Note

All the catalysts used in the kit are sensitive to air and moisture in their commercially available form. Please see the catalyst MSDS or product detail page for details on handling and storage. To best enable scale-up success, the use of glove box is highly recommended. However, modified bench top techniques are provided below.

General Guidelines for Catalytic Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental

All solids (base, substrates, catalyst) were weighed on a bench top balance and added to a cooled, oven dried flask[1,2] equipped with an oven dried stir bar. The reaction was capped and then purged and backfilled with nitrogen (3x) with a nitrogen-fed manifold needle. Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. If a liquid bases was required, it was added as the final component via syringe. When all constituents were added, the nitrogen line was removed[3] and the reaction allowed to stir at the required temperature for the duration of the reaction.

1. *See the* **Cross-Coupling Reaction Guide** *for more detailed additional guidance and troubleshooting tips.*
2. *Reaction vials capable of being sealed with a microwave cap are very useful as well. By design, the caps do not need to be replaced once punctured.*
3. *For higher temps, a reflux condenser operated under nitrogen can be used.*

KitAlysis™ High-Throughput Copper C-N Cross-Coupling Reaction Screening Kit

Kit Design

Cu C-N (Buchwald-Hartwig) cross-coupling kit was designed to provide the best possible chance of success and is run with:

- 10 µmol aryl halide
- 15 µmol amine (primary, secondary), use 20 µmol if the material is not too precious
- 4 µmol ligand per vial (1:4, Cu:Ligand); 6 in this system (40 mol%)
- 30 µmol base (K_3PO_4)
- 0.1 M solvent concentration (Dioxane, DMAc, Toluene, & DMSO)
- 1 µmol CuI (soluble source, 10 mol%)

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all of the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

Designed for the first-time user and should be read completely before getting started. Best if used in conjunction with the **video** as not all steps are outlined in the video in great detail. This guide includes trouble-shooting tips, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Excel Sheet:

Each sheet is designed to be used with the specific experimental design chosen by you depending upon the attributes of your substrates. The downloadable excel files are specific to the kit being run and can be found within each **Step-by-Step User Guide**. They have the following features:

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included In Your KITALYSIS-CN-2PK High-Throughput Screening Kit

Contents in each of the 2 individually sealed Mylar (foil bags):

- 24 (6 x 4) pre-weighed ligands in glass vials loaded with stir bars, topped with cap mat.

The screening sets come pre-loaded with 4 µmol of ligand in each vial according to the following design.

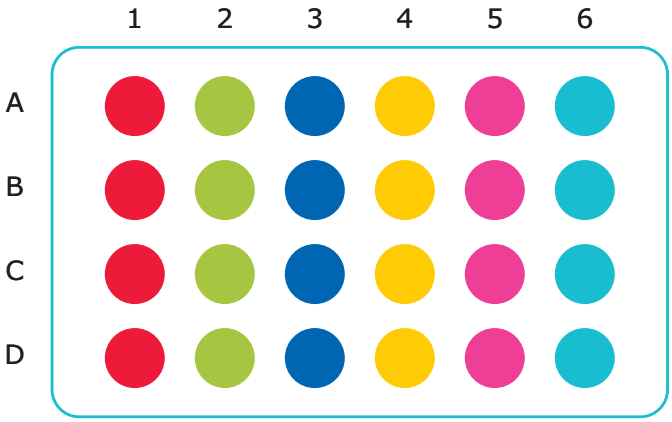


Figure 1. KitAlysis™ C-N Well Plate

Description	Cat. No.	Vial
2,6-Dimethylanilino(oxo)acetic acid	**806412**	A1, B1, C1, D1
2,6-Dimethoxyanilino(oxo)acetic acid	**806455**	A2, B2, C2, D2
2,6-Difluoroanilino(oxo)acetic acid	**806439**	A3, B3, C3, D3
2,3,4,5,6-Pentafluoroanilino(oxo) acetic acid	**806447**	A4, B4, C4, D4
3,5-Bis(trifluoromethyl) anilino(oxo)acetic acid	**806420**	A5, B5, C5, D5
2-Fluoro-6-(piperidine-1-sulfonyl)anilino(oxo)acetic acid	**805491**	A6, B6,C6, D6

- Potassium phosphate: 4 pre-weighed vials with product labels and lids
- CuI (soluble source) in pre-weighed vial

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes

- Dioxane: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- DMAc: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- Toluene: 4 x 2 mL degassed anhydrous Sure/Seal™
- DMSO: 4 x 2 mL degassed anhydrous Sure/Seal™

Internal Standard

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the hyperlinked screen types in the "Step-by-Step Guide" section below.
- KitAlysis™ 24-well Reaction Block Replacement Films-2EA
- Pack of 2 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

Stir Bars-8 Individually Packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures.

Additional Recommended Materials (Sold Separately)

- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Step-By-Step Guide for Kitalysis™ High-Throughput Copper C-N Cross-Coupling Reaction Screening Kit

1 base (K_3PO_4, pre-weighed solid)

4 solvents (DMAc, Toluene, DMSO, and Dioxane)

6 pre-weighed ligands

1 Cu catalyst (Soluble CuI source)

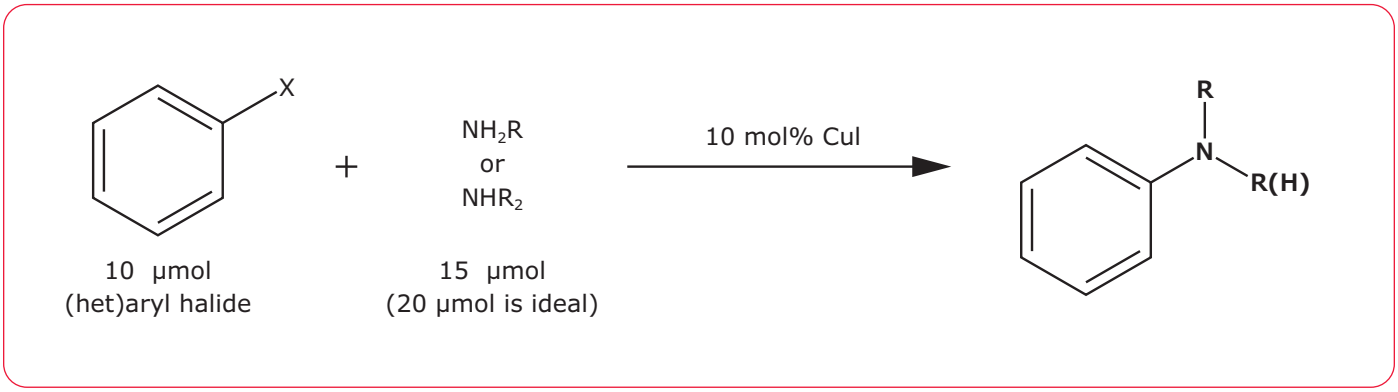


Figure 2.

Materials Required for Set-Up

- 1 mylar bag from the KitAlysis™ Cu C-N Cross-Coupling Reaction Screening Kit and you will use the following components
  - 6 x 4 pre-weighed ligands in glass vials loaded with stir bars and topped with cap mat
  - 4, 4 mL reaction vials with pre-weighed K_3PO_4 base
  - 1 pre-weighed vial of soluble CuI
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 1 (2 mL) ampule each of Toluene, Dioxane, DMSO, and DMAc (in ampule boxes)
- 4 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately). Provided with the KitAlysis™ 24-Well Reaction Block

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 4 (1 mL) syringes with long needles
- Your (het)aryl halide and amine
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates

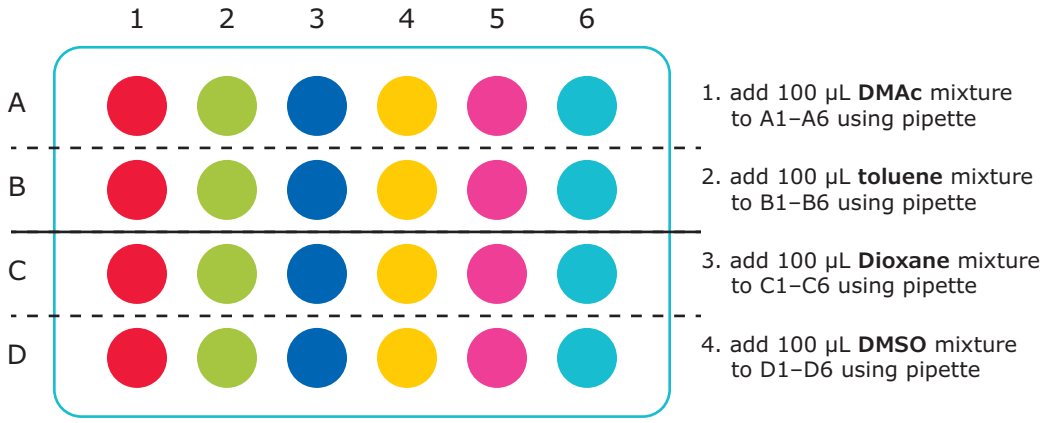
Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 100 °C. It is highly recommended to use an oil bath or second reaction block to hold temperature and avoid spiking. Reaction temperatures for the Cu CN reaction can range from room temperature to 120 °C but 100 °C is a general and very good starting point.
- Place a NEW KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid and verify all holes, including the temperature probe hole, line up with the corresponding holes on the film.
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details)
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Weigh both substrates directly into all of the 4 mL reaction vials (that contain the pre-weighed K_3PO_4) according to recipe provided in the downloadable excel file **omitting solvent**. **Add one stir bar to each vial mixture.** Label as “DMAc Substrate Mixture A”, “Toluene Substrate Mixture B”, “Dioxane Substrate Mixture C”, and “DMSO Mixture D”).
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the “DMAc Substrate Mixture A” and the “Toluene Substrate Mixture B” in two of the holes located on the left hand side of the Inertion Box diffuser tray. Place the “Dioxane Substrate Mixture C”, and “DMSO Substrate Mixture D” in two holes on the right hand side of the plate. Take the vial of CuI from the mylar bag and place into the bottom right vial hole in the Inertion box— NOTE there will be no stir bar added to the CuI vial. (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed). **Ensure that one vial is placed in the center hole on either side. This vial placement allows for the best flow of inert gas.**
- Transfer the **capped**, 24-vial, preloaded catalysts into the reaction block making sure to **load it according to the matching diagram on the packaging and the Reaction Block**. Leave the mat on.
- Place 1 ampule each of DMAc, Toluene, Dioxane, and DMSO into ampule holes located along the bottom of the Inertion Box, below the Reaction Block.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Using ampule cracker, open all ampules while still resting in the holes in the Inertion box (keeping them in the box under the flow of nitrogen).
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe
- Add required solvent amounts to open substrate mixtures and CuI vials following recipe according to the excel file.
- Stir mixtures until in solution (1–2 min). For slurries, see “additional tips” below (there is no stir bar in the CuI but will go into solution with a little shake of the vial).
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose stock solutions
    1. Dose 100 µL of “DMAc Substrate Mixture A” to vials A1–A6 according to scheme below.
    2. Dose 100 µL “Toluene Substrate Mixture B” into vials B1–B6 according to scheme below.
    3. Dose 100 µL “Dioxane Substrate Mixture C”, to vials C1–C6 according to scheme below.
    4. Dose 100 µL and “DMSO Substrate Mixture D” into vials D1–D6 according to scheme below.

       You may have a very small amount of excess solution remaining for each mixture. Save it as a reaction standard for HPLC/TLC later.
    5. Dose 20 µL of the CuI solution to *each* of the 24 reaction vials A1–D6. It is helpful to say the vial location to yourself as you go along to keep track (A1, A2, etc).



5. add 20 µL **CuI/DMSO** mixture to each vial A1–D6

- After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check as you go along to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 100 °C overnight stirring at or near 300 rpm. Higher or lower temperatures may be optimal, but 100 °C is a good starting point.
- Make the quench solution in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.
- At reaction completion, follow the below Work-Up Procedure

Quench Solution Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit)

  Note: This recipe makes 50 mL which is enough stock solution for all four screening sets in the KitAlysis™ Suzuki-Miyaura Cross-Coupling Reaction Screening Kit. The amount of internal standard is 10 mol%. So a big product peak to small internal standard indicates a good reaction.

Work-Up Procedure and Analysis

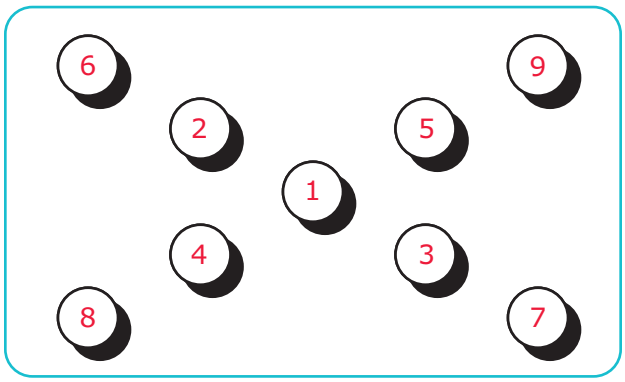
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of acetonitrile to each 24 individually labeled (A1, A2 etc,) HPLC vials or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (see "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light, even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the cross pattern provided below. Check to see that the lid is evenly sealed onto the base on all sides. Do this visual check to avoid having to unscrew the lid and screw again.
3. **Tighten:** Repeat the same pattern until the KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



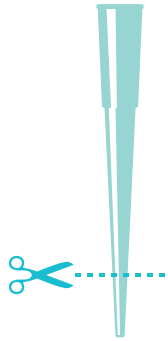
Screw Pattern for 24-Well Reaction Plates

Probe for hot plate does not fit into reaction block hole

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring well while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: Copper C-N Cross-Coupling Reaction

Important Note

All the catalysts used in the kit are sensitive to air and moisture in their commercially available form. Please see the catalyst MSDS or product detail page for details on handling and storage. To best enable scale-up success, the use of glove box is highly recommended. However, modified bench top techniques are provided below.

General Guidelines for Catalytic Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental-Glovebox Set-Up, Hood Reaction

The olefin substrate was weighed on a bench top balance and added to a cooled, oven dried flask equipped with an oven dried stir bar. The reaction was capped and then transferred standard chemical glove box. Once in the glove box, anhydrous Sure/Seal™ solvent was added via syringe. If use of acid additive was desired, this was added to the olefin solution. The catalyst (removed from the glove box fridge or brought in the box from a bench fridge) was then weighed and added to the reaction mixture, the mixture capped, and then removed from the glove box. Upon arrival at the bench, a reflux condenser, fitted with a septum and connected to a nitrogen-fed manifold needle (to allow the safe escape of ethylene without the loss of solvent while keeping the reaction under inertion) was connected to the reaction flask. The reaction was then added to a pre-heated oil bath and allowed to stir at the required temperature for the duration of the reaction.

Example Experimental-Bench Top Set-Up, Hood Reaction

The olefin substrate was weighed on a bench top balance and added to a cooled, oven dried two-neck flask equipped with an oven dried stir bar. A reflux condenser, fitted with a septum and connected to a nitrogen-fed manifold needle (to allow the safe escape of ethylene without the loss of solvent was attached to the vertical neck of the flask and the other neck was fitted with a septum. The reaction set-up was then evacuated and backfilled with nitrogen (3x, making sure to allow time as the set-up is larger than a single reaction flask). Anhydrous solvent (Sure/Seal™ed and punctured with a nitrogen-fed manifold needle) was then added via syringe. If use of acid additive was desired, this was added to the olefin solution via syringe through the septa. The catalyst was removed from the fridge, quickly weighed, and then added to the reaction by opening the septa for a short interval. (A stock solution of the catalyst in a portion of the reaction solvent can also be used to add the catalyst via syringe or cannula). The reaction was then allowed to stir at the required temperature for the duration of the reaction. The catalyst bottle was flushed with nitrogen and placed back in the fridge for storage.

KitAlysis™ Suzuki-Miyaura Cross-Coupling Reaction Screening Kit

Kit Design

The Suzuki-Miyaura cross-coupling kit was designed to provide the best possible chance of finding good reaction conditions and is run with:

- 10 µmol (het)aryl halide
- 15–20 µmol (het)aryl boronate
- 1 µmol catalyst per vial (1:1 Pd:Ligand); 6 in this system
- 30 µmol base (1.5M K_3PO_4)
- 0.1 M solvent concentration (DMAc, Toluene, *n*-butanol, and THF)
- 60 °C

Use of the Provided Tools

Multiple tools have been created to ensure your success with kit set up. Start with the more detailed guide to ensure you are comfortable with all of the steps before using the quick guides on the excel worksheet. Remember that while the technique is new, it is still organic chemistry and so the steps will seem easy once you try just one kit. It is just a new way of approaching something you are already very good at.

Detailed Set-Up User Guide:

The step-by-step user guide is designed for the first-time user and should be read completely before getting started; and used in conjunction with the **video**. This guide includes trouble-shooting tips, how-to's for the Labware, and work-up recipes with procedures. Everything you need to set up a kit with confidence every time.

Excel Sheet:

The downloadable excel files are specific to the kit being run and can be found within each **Step-by-Step User Guide**. They have the following features:

- Calculations for substrate recipes depending upon the molecular weight of your substrates
- Quick directions for the more avid user
- Print button to allow you to take recipe to the lab
- Contains all info such that they can be saved as a pdf and appended to ELN for experimental information

Materials Included in Your KITALYSIS-SM-4PAK High-Throughput Screening Kit

Contents in each of the 4 individually sealed Mylar (foil bags):

- 4 empty reaction vials with lids (to make stock solutions)
- 24 (6 x 4) pre-weighed catalysts in glass vials loaded with stir bars, topped with cap mat.

The screening sets come pre-loaded with 1 µmol of catalyst in each vial according to the following design.

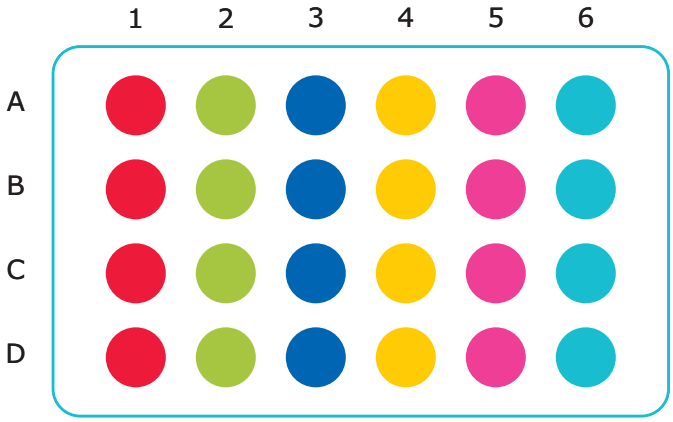


Figure 1. KitAlysis™ Well Plate Map

Description	Cat. No.	Vial
XPhos-Pd-G3	**763381**	A1, B1, C1, D1
SPhos-Pd-G2	**753009**	A2, B2, C2, D2
cataCXium® A Pd G3	**761435**	A3, B3, C3, D3
APhos-Pd-G3	**764183**	A4, B4, C4, D4
$P(Cy_3)$-Pd-G3	**764175**	A5, B5, C5, D5
Pd-PEPPSI™ -IPent Catalyst	**732117**	A6, B6, C6, D6

All contents in the foil bag are weighed, plated, packed, and sealed in a glove box under nitrogen.

Ampule Boxes:

- Toluene: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- DMAc: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- THF: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- *n*-BuOH: 4 x 2 mL of degassed, anhydrous Sure/Seal™
- 1.5M aqueous K_3PO_4: 4 x 2 mL of degassed solution

Internal Standard:

- Biphenyl (30 mg). To be added to the reaction during the work-up. Recipe for the work-up with the internal standard can be found within the hyperlinked screen types in the "**Step by Step Guide**" section below.
- KitAlysis™ 24-well Reaction Block Replacement Films-4EA
- Pack of 4 enables a new film to be used with each kit ensuring a tight, cross-contaminate-free seal every time.

Stir Bars-16 individually packed

- Fit perfectly into supplied vials to ensure proper stirring of substrate mixtures prior to dosing.

Additional Recommended Materials (Sold Separately)

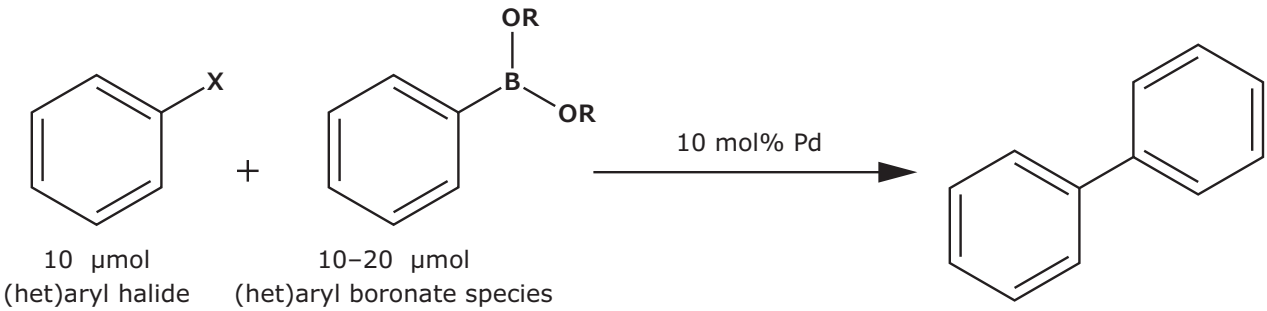
- 96-well plate for automated HPLC analysis
- 96-well plate cap mat for automated HPLC analysis
- 10–100 µL pipette
- 2–200 µL pipette tip refill
- 100–1000 µL pipette
- 50–1000 µL pipette tip refill
- 2-inch needles for ease in ampule solvent extraction
- 1 mL needle for accurate solvent volume extraction from ampule

Step-by-Step Guide for KitAlysis™ Suzuki-Miyaura Cross-Coupling Reaction Screening Kit

1 base (K_3PO_4, 1.5 M solution in degassed water)

4 degassed solvents (DMAc, Toluene, *n*-Butanol, and THF)

6 pre-weighed catalysts (x4)



10 µmol (het)aryl halide

10–20 µmol (het)aryl boronate species

Materials Required for Set-Up:

- 1 mylar bag from the KitAlysis™ Suzuki-Miyaura Reaction Screening Kit and you will use the following components
  - 6 x 4 pre-weighed catalysts in glass vials loaded with stir bars and topped with cap mat
  - 4 empty 4 mL substrate stock solution vials
- 1 NEW KitAlysis™ 24-Well Reaction Block Replacement Film
- 1 (2 mL) ampule **each** of Toluene, THF, *n*-Butanol, DMAc, and aq. 1.5 M K_3PO_4 (in ampule boxes)
- 4 NEW stir bars
- KitAlysis™ 24-Well Reaction Block (sold separately)
- KitAlysis™ Benchtop Inertion Box (sold separately) or glove box/glove bag
- KitAlysis™ Torque Screwdriver Set (sold separately).

Additional (not included) items needed:

- Pipette (0–100 µL) & tips
- 4 (1 mL) syringes with long needles
- Your (het)aryl halide and (het)aryl boronate
- Nitrogen (or Argon): from hood line or tank
- 1 hot plate with stir capabilities
- 1 stir plate (or hot plate to be used without heat)
- HPLC vials, 96-well HPLC auto sampler block, or TLC plates

Solutions & slurries to make:

Go to the online user set-up page to enter molecular weights into the downloadable **excel file**.

Set-Up Procedure

- Preheat a hot plate to 60 °C (use oil bath or second reaction block to hold temperature and avoid spiking). Reaction temperatures for the Suzuki-Miyaura reaction can range from room temperature to 120 °C but 60 °C is a general and very good starting point.
- Place a new KitAlysis™ 24-Well Reaction Block Replacement Film on reaction block lid (make sure all holes, including the temperature probe hole, line up with the corresponding holes on the film).
- Check all screws to ensure they are not stripped. Replace any stripped screws with provided replacements.
- Place the KitAlysis™ Benchtop Inertion Box with tubing connected to inert gas onto the second, **non-heated stir plate** (see **KitAlysis™ Benchtop Inertion Box Set-up** for details).
- Place KitAlysis™ 24-Well Reaction Block with lid into KitAlysis™ Benchtop Inertion Box. Start nitrogen flow and **purge 5 minutes**. **Leave nitrogen flowing for remainder of set up.**
- Weigh both substrates directly into all of the empty 4 mL reaction vials according to recipe provided in the downloadable excel file **omitting solvent and base solution**. **Add one stir bar to each vial mixture.** Label as “DMAc Substrate Mixture A”, “Toluene Substrate Mixture B”, “*n*-butanol Substrate Mixture C”, and “THF Substrate Mixture D”).
- Partially open the lid on the KitAlysis™ Benchtop Inertion Box. Place the “DMAc Substrate Mixture A” and the “Toluene Substrate Mixture B” in two of the holes located on the left hand side of the Inertion Box diffuser tray. Place the “*n*-butanol Substrate Mixture C”, and “THF Substrate Mixture D” in two holes on the right hand side of the plate. Ensure that one vial is placed in the center hole on either side. This vial placement allows for the best flow of inert gas (remove lids from the solid mixtures before placing them into the recommended holes, keeping the lids in the Inertion Box for later use if needed).
- Transfer the **capped**, 24-vial, preloaded catalysts into the reaction block making sure to load it according to the matching diagram (A1, top Left corner) on the packaging and the Reaction Block. Leave the cap mat on.
- Using an ampule cracker, open 1 ampule each of DMAc, Toluene, *n*-butanol, and THF and *quickly* place into ampule holes located along the bottom of the Inertion Box, below the Reaction Block. Then open 1 ampule of the base solution and place it in the remaining hole on either side of the plate.
- Once all components are in the KitAlysis™ Benchtop Inertion Box, close the lid and **purge for an additional 5 minutes**. Leave nitrogen flowing for remainder of set up.
- Purge needle and syringe in a nitrogen diffuser hole 2x by pulling and then pushing plunger. Using purged needle and syringe, add required solvent amounts to open substrate mixture vials. DO NOT add base solution to any substrate mixture-it is added directly to each of the 24 reaction vials as the last step.
- Stir mixtures until in solution (1–2 min). For slurries, see “additional tips” below.
- While mixtures are stirring, carefully remove the cap mat from the 24-vial, preloaded catalysts in the reaction block.
- Dose stock solutions (using a new pipette tip each time)
  - Dose 100 µL of “DMAc Substrate Mixture A” to vials A1–A6 according to scheme below.
  - Dose 100 µL “Toluene Substrate Mixture B” into vials B1–B6 according to scheme below.
  - Dose 100 µL “*n*-butanol Substrate Mixture C”, to vials C1–C6 according to scheme below.
  - Dose 100 µL and “THF Substrate Mixture D” into vials D1–D6 according to scheme below.

  You may have a very small amount of excess solution remaining for each mixture. Save it as a reaction standard for HPLC/TLC later.
  - Dose 20 µL of the aq. 1.5 M K_3PO_4 solution mixtures to all vials A1–D6. It is helpful to say the vial location to yourself as you go along to keep track (A1, A2, etc).

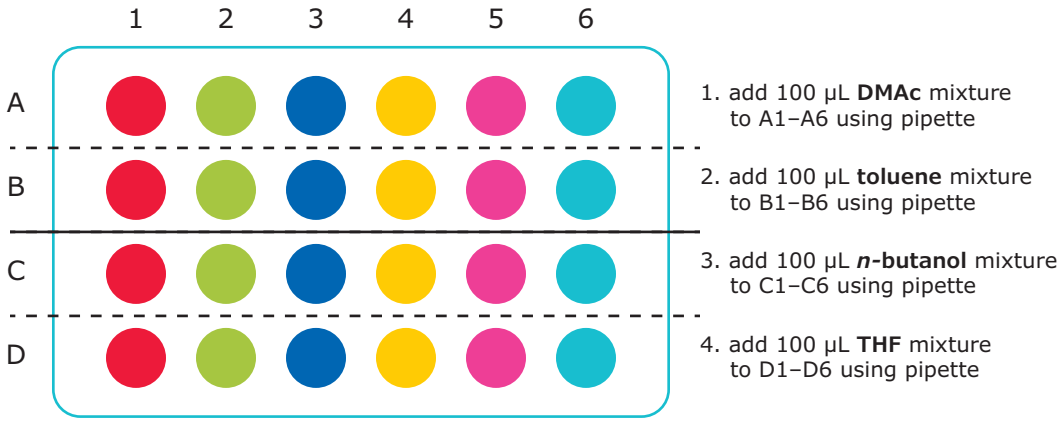


Figure 1. Dose instructions for DMAc, Toluene, *n*-butanol, THF, and K_3PO_4

- After all substrate mixtures and base have been dosed according to recipe, take the KitAlysis™ 24-Well Reaction Block lid and line up the screws with the holes in the plate. **Ensure the temperature probe holes line up on both the lid and the block.**
- Screw on lid according to directions and pattern shown in the "additional tips" section below. Before removing KitAlysis™ 24-Well Reaction Block from the Inertion Box, ensure that the lid is evenly sealed onto the base. Do this visual check as you go along to avoid having to unscrew the lid and screw again.
- Once completely sealed, remove the KitAlysis™ 24-Well Reaction Block from the Inertion Box. Place on preheated hot plate with probe through the lid and inserted into the block. Heat at 60 °C overnight stirring at or near 300 rpm. Higher or lower temperatures may be optimal, but 60 °C is a good starting point.
- Make the quench solution/internal standard in a bottle with a replaceable lid following the recipe below for HPLC analysis. TLC is also possible.
- At reaction completion, follow the below Work-Up Procedure to quench reactions, make them more suitable for analysis, and add internal standard.

Quench Solution/Internal Standard Recipe

- 49 mL CH_3CN
- 1 mL AcOH
- 15.4 mg Biphenyl (KitAlysis™ Internal Standard provided in the kit) There is excess in the bottle so be sure to weigh it out.

  Note: This recipe makes 50 mL which is enough stock solution for all four screening sets in the KitAlysis™ Suzuki-Miyaura Cross-Coupling Reaction Screening Kit. The amount of internal standard is 10 mol% per reaction. So a big product peak to small internal standard indicates a good reaction. Integrate to compare reactions against one another (product/internal standard).

Work-Up Procedure and Analysis

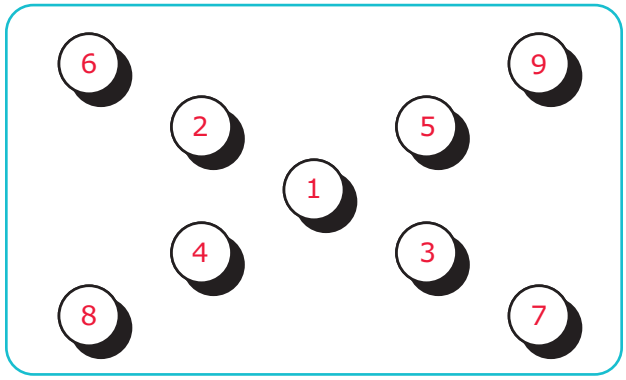
- Cool Reaction Block. Remove lid using small, non-torque KitAlysis™ screwdriver.
- Check each vial for solvent loss, and record.
- Aliquot 500 µL of prepared quench solution to each vial using a pipette for accuracy.
- Replace lid, tighten middle screw and stir on stir plate (**NO HEAT**) for 2–3 minutes. ***DO NOT INVERT BLOCK.***
- After 2–3 minutes of stirring, let plate rest (without stirring) for 5 minutes to allow insoluble material to settle out of solution to the bottom of the vials.
- While plate is resting, add 700 µL of acetonitrile to each 24 individually labeled (A1, A2 etc,) HPLC vials or to each of 24 wells of a 96-well HPLC/UPLC auto sampler block (see "additional recommended materials" below for suggestions on the auto sampler block and cap mat.
- Remove lid on KitAlysis™ 24-Well Reaction Block carefully.
- Using a clean pipette each time, remove a 25 µL aliquot from each vial into corresponding HPLC vials or HPLC block. **Be careful to pull material from the top of the vials to avoid any precipitate.**
- Run on HPLC auto sampler. You may need to adjust the amount of acetonitrile from the suggested 700 µL to accommodate your unique HPLC system.

Additional Tips

Sealing the Plate-screwing down the cover

Sealing the plate properly is critical to success. The key is light, even pressure to the lid of the block to keep the cover flat while sealing.

1. Line-up screws making sure that the base and lid temperature probe holes line-up in the KitAlysis™ 24-Well Reaction Block.
2. **Initially flush:** Using your thumb and forefinger, press the lid of the box until it becomes flush with the vials. Then, using the KitAlysis™ Torque Screwdriver, insert screws until flush, but not tight, with the top of the box, following the c KitAlysis™ Torque Screwdriver "clicks" indicating complete tightness. Go around the block once more for a final check to ensure all screws are tight.



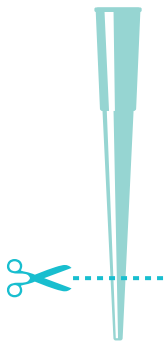
Screw Pattern for 24-Well Reaction Plates

Probe for Hot Plate Does Not Fit into Reaction Block Hole

Use a small oil bath or other metal block (for best results) in the back of the hot plate and place the probe in there. Place reaction block as close to the center of the hot plate as possible for more even stirring.

Slurry Additions

Due to narrow opening of pipette tips, slurry additions will result in blockages. To aid in uniform dosing, simply snip off the end of the tip at the first marker (~10 mm). To ensure an evenly dispersed aliquot, it is critical that the mixture is stirring well while you draw aliquots for dosing into the corresponding reaction vial.



Scale-Up Guide: Suzuki-Miyaura Cross-Coupling Reaction

Important Note

All of the preformed catalysts used in the kit are air and moisture stable complexes in their commercially available form. Once activated by base under the reaction conditions they become sensitive to air. To best enable scale-up success, the use of standard Schlenk technique is recommended.

General Guidelines for Catalytic Reaction Scale-Up

- Oven dry all glassware and stir bars
- Preheat oil bath to avoid temperature spiking
- Use dry, high purity reagents (and substrates)[1]
- Use unopened Sure/Seal™ anhydrous solvents (or newly degassed)[1]
- Keep reactions under an atmosphere of nitrogen
- Purge needle/syringe with nitrogen prior to use

Example Experimental

All solids (substrates/catalyst) were weighed on a bench top balance and added to a cooled, oven dried flask[1,2] equipped with an oven dried stir bar. The reaction was capped and then purged and backfilled with nitrogen (3x) with a nitrogen-fed manifold needle. Anhydrous solvent (unopened Sure/Seal™ bottle punctured with a nitrogen-fed manifold needle) was then added via syringe. The aq. base was first degassed and then it was added as the final component via syringe. When all constituents were added, the nitrogen line was removed[3] and the reaction allowed to stir at the required temperature for the duration of the reaction.

1. *See the* **Cross-Coupling Reaction Guide** *for more detailed additional guidance and troubleshooting tips.*
2. *Reaction vials capable of being sealed with a microwave cap are very useful as well. By design, the caps do not need to be replaced once punctured.*
3. *For higher temps, a reflux condenser operated under nitrogen can be used.*

Catalyst Screening followed by TLC-MS for Reaction Progress Analysis using KitAlysis™

Introduction

Catalyst screening is an important prerequisite in organic synthesis. KitAlysis™ High-Throughput Screening Kits showcase the most widely used catalytic methods of synthetic chemists, translated into an off-the-shelf screening system to quickly and efficiently identify or optimize suitable catalytic reaction conditions.

To evaluate the screening an analysis tool is needed. Thin layer chromatography (TLC) allows for a fast and cost-effective determination of the reaction progress by parallel analysis of the samples on one plate. This setup perfectly fits to the parallel synthesis utilizing KitAlysis™.

This application note displays the use of the KitAlysis™ High-Throughput Buchwald-Hartwig Amination Reaction Screening Kit for the optimization of the coupling of aryl bromide and diphenylamine combined with an evaluation of the result with TLC-MS analysis.

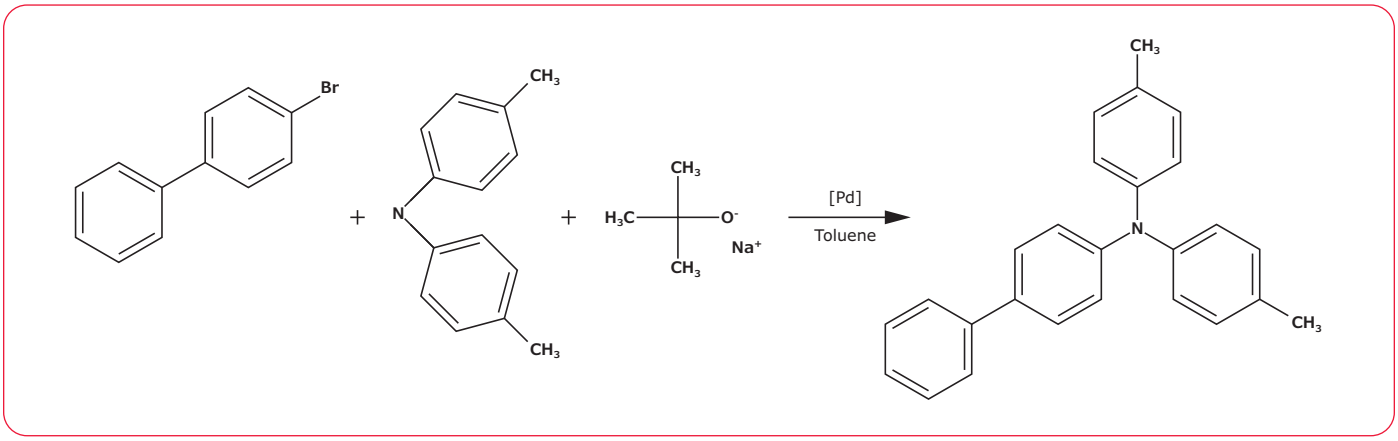


Figure 1. Schematic reaction showing the coupling of aryl bromide (1) and diphenylamine (2) to form biphenyl-4-yl-di-p-tolyl-amine (3), where the product has a molecular weight of 349.468 g/Mol.

Results and Discussion

The two reactants, aryl bromide and diphenylamine, were applied in addition to the samples. The samples were clearly visible under UV-light, therefore no derivatization was necessary. Figures 2a, 2b and 2c show the developed plate under white light, at 254 nm and at 366 nm.

Good reaction progress was observed in tracks 1–5 and 8–12. Byproduct formation somewhat different in the lower Rf value region as well as amine educt amount (Rf 0.19). Significant concentrations of educts were observed in tracks 6 and 7 (MorDalPhos Pd G3, BINAP Pd G3).

Track	Samples / Catalysts KITALYSIS-CN	Concentration	Application volume	Rf of product and educts
1	BrettPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
2	CPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
3	DavePhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
4	$P(tBu)_3$ Pd G2	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
5	Josiphos SL-J009-1 Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
6	MorDalPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
7	BINAP Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
8	RuPhos Pd G2	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
9	SPhos Pd G2	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
10	tBuXPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
11	XantPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
12	XPhos Pd G3	KitAlysis™ sample filled up with 1 mL THF	2.0 µL	0.5
13	amine educt	1 mg/mL	1.0 µL	0.19
14	Ar-Br educt	1 mg/mL	1.0 µL	0.77

Figure 2a. Developed plate under white light

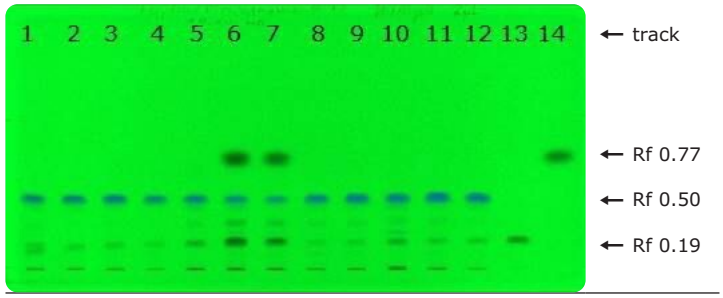


Figure 2b. Developed plate at 254 nm

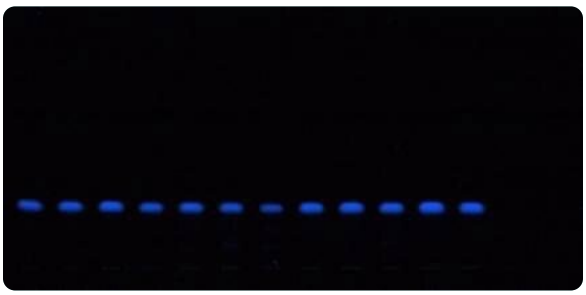


Figure 2c. Developed plate at 366 nm

Confirmation of reaction product by mass spectrometry: Formation of ACN adduct via radical ionization

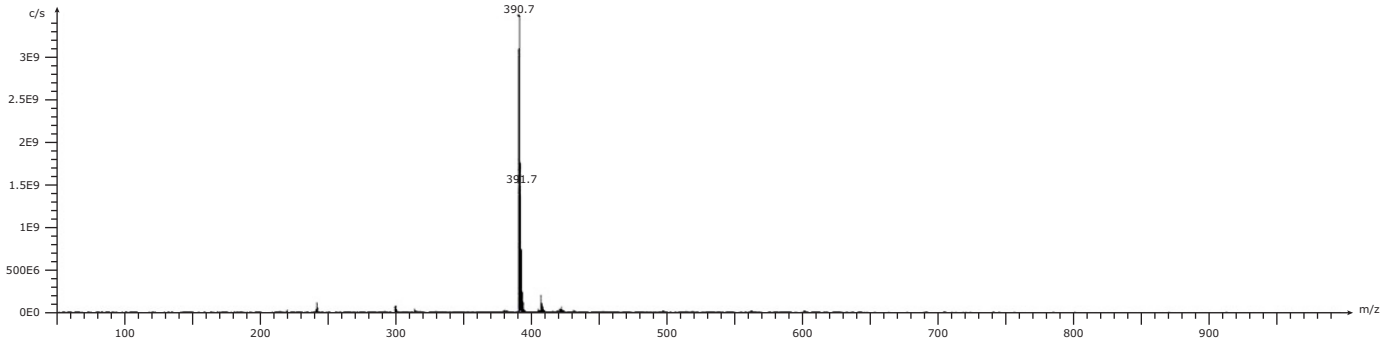


Figure 3. Mass spectrum (ESI+) of product [M+ACN]

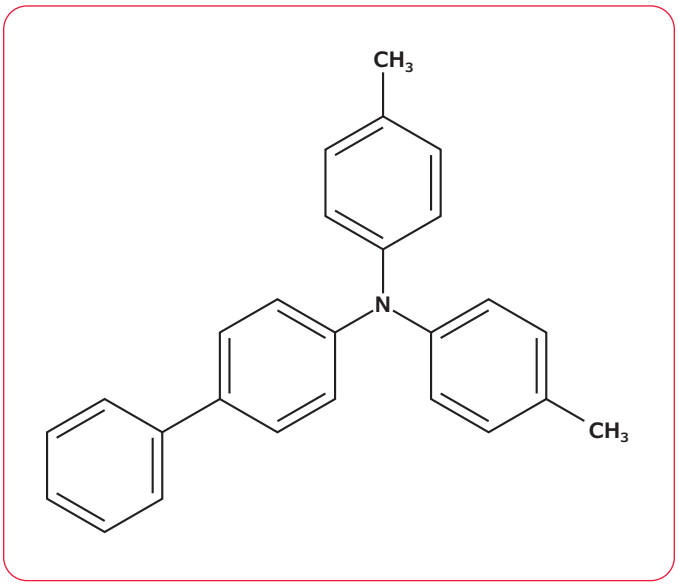


Figure 4. Biphenyl-4-yl-di-p-tolyl-amine (M = 349.568 g/Mol)

Chromatography	Plate	HPTLC Silica gel 60 F_{254} MS-grade
	Sample preparation	Fill up the KiTAlysis™ samples with 1mL tetrahydrofuran (THF) Educts: Dissolve 1 mg each in 1 mL THF
	Sample application	ATS 4 sample applicator (Camag), 6 mm bandwise
	Application volume	1.0 µL track 13–14 / 2.0 µL track 1–12
	Mobile phase	*n*-Heptane/dichloromethane 6/1 (v/v) + 0.1% trifluoroacetic acid
	Migration distance	5 cm
	Migration time	10–11 min
Extraction	Extraction equipment	Plate Express (Advion)
	Extraction solvent	Acetonitrile/water 95/5 (v/v) + 0.1 % formic acid
	Extraction flow	0.2 mL/min
Detection	Documentation equipment	TLC Visualizer (Camag)
	Wavelength	scan at white light, 254 nm, 366 nm
	Staining	–
	MS equipment	Single quadrupole mass spectrometer Expression CMS (Advion)
	MS detection	ESI(+) mode (m/z 50–1200)

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