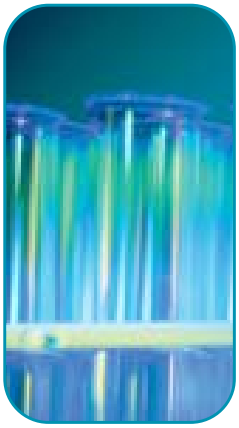
PRODUCT GUIDE



Instruction Manual

Catch and Release® v2.0

Reversible Immunoprecipitation System

Cat. #17-500 & #17-500A

For laboratory research use only.

Not intended for human or animal diagnostic, therapeutic or other clinical uses.

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contents

I. Introduction and Principle

Immunoprecipitation (IP) is a frequently used method to purify specific proteins from complex samples such as cell lysates or extracts. Traditional IP protocols use Protein A or Protein G coupled to an insoluble resin, such as agarose beads, to capture an antigen:antibody complex in solution. The complex is then "precipitated" by centrifugation. Limitations of traditional IP include sample handling and processing difficulties, the inability to release native antigen from the beads for functional assays and poor reproducibility and recovery due to multiple wash steps.

Catch and Release® overcomes many of the limitations associated with traditional IP. Its unique Spin-Column format was designed to make IP faster, simpler and more reproducible. Catch and Release® enables the elution of the antigen:antibody complex without denaturation, while ensuring minimal contamination by non-specific proteins in the eluate. In addition, the convenient Spin-Column format of Catch and Release® improves performance and makes higher throughput processing of samples possible.

Catch and Release® Columns contain a proprietary resin in a microfuge-compatible Spin Column secured by a screw cap top and a breakaway closure on the bottom. An Antibody Capture Affinity Ligand, for binding the

antigen:antibody complex, is also provided and serves as a tether between the complex and the resin. Most lysate proteins and antibodies will exhibit little or no binding to the resin without the Antibody Capture Affinity Ligand. It is this feature that enables the fast and simple elution of the antigen:antibody complex from the resin in either a denatured or native form.

Catch and Release® has been successfully tested on a number of proteins with both rabbit and mouse antibodies (see table 1, page 6). As little as 30 minutes of incubation with most antibodies can provide the desired results, demonstrating a significant time advantage to researchers as compared with traditional IP methods.

Table 1: Select Proteins Tested with Catch and Release®

Protein	Mol. Weight (kDa)	Upstate Ab Cat. #	Host
Akt-1	65	**	
AUF1	45	**	
Caspase 3	32	06-735	Rb
Caspase 6	34	06-691	Rb
cdk2	33	06-505	Rb
CREB	43	06-863	Rb
Cyclin B1	58	05-373	M
D4-GDI	28	06-731	Rb
FAK	125	05-537	M
FRA-1	39	**	
GSK3β	42	**	
HDAC1	65	06-720	Rb
Jak2	130	06-255	Rb
MEK1	45	06-269	Rb
Mib1	110	**	
Myosin Heavy Chain	220	05-716	M
NKG2D	50	**	
Nrf	120	**	

Protein	Mol. Weight (kDa)	Cat. #	Host
Paxillin	68	**	
phospho-Tyrosine	–	05-777	M
phospho-Tyrosine	–	06-427	Rb
PLC-γ-1	135	05-163	M
PP2A	36	05-421	M

M-mouse, Rb-rabbit

*** tested using investigator-supplied antibody*

Proteins have also been tested with antibodies to the following epitope tags:

- Flag
- GFP
- HA
- Myc

Note: *Catch and Release® is not suitable to immunoprecipitate proteins expressing 6X Histidine epitope tagged fusion proteins (i.e., His-tagged fusion proteins) due to non-specific interactions with the resin.*

II. System Components

A. Reagents Supplied*

- **Antibody Capture Affinity Ligand:**
  One vial containing 60 µg Antibody Capture Affinity Ligand in 500 µl PBS containing 10% glycerol and 2 mM PMSF. Store at 4°C. Stable for 6 months.
- **Catch and Release® Wash Buffer, 10X:**
  One vial containing 15 ml of 10X buffer, pH 7.4 containing the following detergents: 10% NP-40 and 2.5% deoxycholic acid. Liquid at 4°C. Stable for two years. ***Note:*** *If crystallization occurs when buffer is stored at 4°C, warm to room temperature and vortex briefly before use.*
- **Non-denaturing Elution Buffer, 4X:**
  One vial containing 10 ml of 4X PBS based IP Elution Buffer. Store at 4°C. Stable for two years.
- **Denaturing Elution Buffer, 1X:**
  One vial containing 4 ml of 1X PBS based IP Elution Buffer. Add β-Mercaptoethanol (βME; not provided) to a final concentration of 5% (v/v) immediately before use. Store at 4°C. Stable for one year.

- **Catch and Release® Spin Columns:**
  50 columns containing 0.5 ml (20% w/v) of IP capture resin in suspension.
- **Catch and Release® Capture Tubes:**
  100 reservoir tubes.

* *The Catch and Release® Sample Pack (cat. # 17-500A) contains 1/10 the amount of reagents supplied in the regular pack size (cat. #17-500)*

B. Required Materials Not Provided

- Cell Lysates
- Specific primary antibodies
- Milli-Q® water
- Pipettes and tips
- Microcentrifuge
- Rotator or rocker
- Forceps
- Microcentrifuge tubes

If performing Western blots on immunoprecipitated material:

- SDS-PAGE reagents and apparatus
- Western blotting reagents and apparatus

- PVDF or other membrane
- Saran Wrap®
- Kimwipes®
- Specific primary antibodies
- Wash buffer
- Blocking buffer
- X-ray film and dark room or digital imaging system
- Ponceau Stain (optional)
- Stripping buffers (optional)
- Membrane incubation containers
- Timer

Other recommended kits:

1. For Western blot detection:
   - Visualizer™ Western Blot Detection Kit, mouse (cat. #64-201)
   - Visualizer™ Western Blot Detection Kit, rabbit (cat. # 64-202)
   - ChemiBlot™ Molecular Weight Markers, (cat. #2230)
   - BLOT - FastStain™ (cat. #2076)
   - ChemiBLOCKER™ (cat. #2170)

- ReBlot™ Western Blot Recycling Kit (cat. # 2060)
- ReBlot™ Plus Western Blot Recycling Kit (cat. #2500)

2. For negative immunoprecipitation (IP) species controls:
   - Normal Mouse IgG (cat. #12-371)
   - Normal Rabbit IgG (cat. #12-370)
   - Mouse Serum (cat. #S25-10ML)
   - Rabbit Serum (cat. #S20-100ML)

C. Preparation of Reagents

- Unless otherwise noted, all dilutions of stock reagents provided in the kit are to be done with high-quality water, such as Milli-Q® water.
- If gel electrophoresis is to be performed, it should be done according to the specifications set by the manufacturers of the gel and the apparatus, taking into consideration the specific protein(s) that need to be resolved.
- When transferring the resolved proteins to a membrane, follow the recommendations set by the manufacturer of the transfer apparatus.
- IP with Catch and Release® and Western blot detection with Visualizer™ is compatible with either nitrocellulose or PVDF membranes.

III. Catch and Release® Procedure

A. Optimizing Incubation Time and Temperature

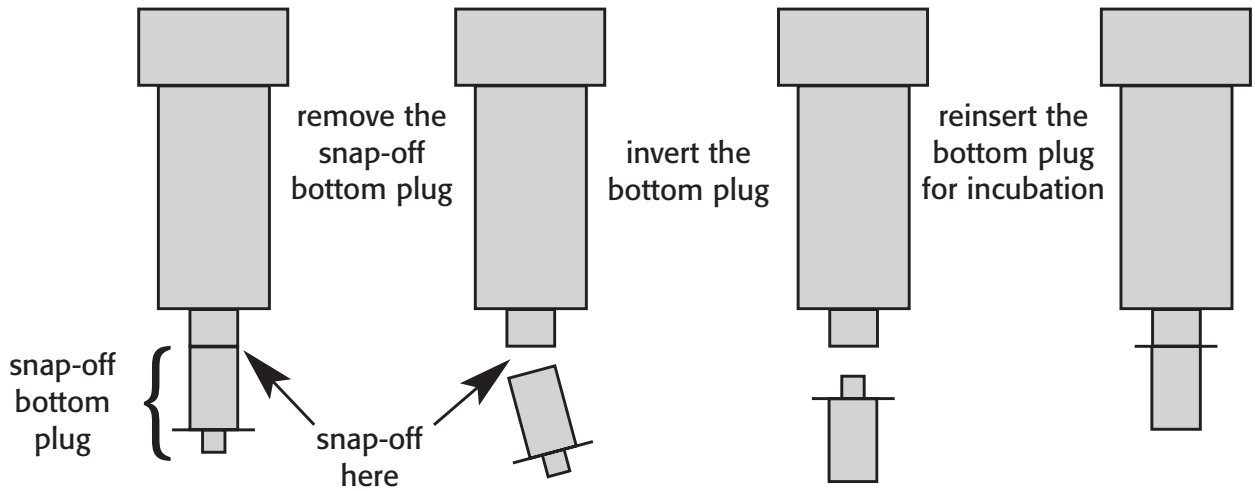
The Catch and Release® procedure as outlined below uses incubation times and temperatures that have been demonstrated to work well with antibodies for many proteins* (see table 1, page 6). If these conditions differ from the conditions used in your traditional IP procedure, you should **follow your traditional method** in your first use of the kit. For example, if you normally incubate a sample with your primary antibody for 1 hour at 4ºC, then you should do the same in Step 5 (see page 15). Once you have verified that Catch and Release® works with your antibody and protein, you may choose to optimize the procedure by adjusting incubation times. Some antibodies will exhibit optimal antigen binding in as little as 10-15 minutes, others may require an overnight incubation; some incubations will work at room temperature, while others are best performed at 4ºC. These two key parameters should be empirically determined by the researcher for every antibody, lysate and protein of interest.

** Catch and Release® should not be used to IP His-tagged proteins*

B. Catch and Release® Protocol

1. Dilute enough 10X Catch and Release® Wash Buffer to the 1X working concentration with Milli-Q® water for incubation and all washes. You will need approximately 2.5 ml for washes and some additional volume possibly for the antibody incubation (Step 4).
2. Label the Spin Columns, Capture Tubes and microcentrifuge tubes to be used. Remove the snap-off bottom plug (save for later use; see figure 1), and insert the Spin Column into a Capture Tube. Remove the screw-on cap and centrifuge at 5000 rpm (2000 xg) for 15-30 seconds to remove the resin slurry buffer. Wash the resin twice with 400 µl 1X Wash Buffer. Empty the Capture Tube, and plug the bottom end of the Column with the snap-off bottom plug.

Figure 1



3. Determine the volume of combined reagents:

   a. 500 µg of cell lysate. ***Notes: 1.*** *This is a recommended starting amount, but the optimal amount may need to be empirically determined for each individual antibody and antigen.* ***2.*** *High concentrations (>1mM) of reducing agents, such as dithiothreitol (DTT) or β-mercapto-ethanol (βME), may denature antigens or antibodies and prevent capture.*

   b. 1-4 µg of a specific primary antibody and negative control antibody. 5-10 µl of whole antiserum or ascites fluid. ***Note:*** *These are recommended starting amounts but the optimal amounts may need to be empirically determined for each individual antibody and sample containing antigen.* ***Upstate highly recommends performing corresponding negative IP controls (for any immunoprecipitation procedure) as side-by-side comparison.*** *Please see page 11 for a listing of recommended negative IP control antibodies.*

   c. 10 µl of Antibody Capture Affinity Ligand

   d. Sufficient 1X Wash Buffer to provide a final total volume of 500 µl

Component	Amount
Cell Lysate	_____ µl
Antibody	_____ µl
Affinity Ligand	10 µl
1X Wash Buffer	_____ µl
Total	**500 µl**

4. With the bottom end of the Spin Column plugged, add the reagents in the following order to the column:
   a. 1X Wash Buffer
   b. Cell lysate
   c. Specific primary antibody or negative control antibody
   d. Antibody Capture Affinity Ligand
5. Cap the top of the Column (using the screw-on cap), and incubate on a rotator or mixer at room temperature for 30 minutes (see page 12 for more information on incubation times and temperatures), ensuring that the slurry remains suspended during incubation.
6. Remove the snap-off bottom plug and discard. Place the Column in the Capture tube. Remove the screw-on cap and centrifuge at 5000 rpm (2000 xg) for 15-30 seconds to collect flow-through. Transfer the flow-through to a microcentrifuge tube and save for Western blot analysis, if desired (it may be useful for trouble-shooting, if necessary).

7. Wash the Column 3X with 400 µl of 1X Wash Buffer, spinning at 5000 rpm (2000 xg) 15-30 seconds for each wash. Washes may be saved (if desired) for Western blot analysis and trouble-shooting.

8. Place the Column into a fresh Capture Tube.

9. Proteins may be eluted from the Column in either a denatured form (i.e. for SDS-PAGE and Western blotting), or a native form, following **9A** or **9B**.

   9A. For elution of protein in its denatured, reduced form: Add 70 µl of 1X Denaturing Elution Buffer containing βME to the Spin Column. Centrifuge and save eluate for Western analysis. ***Note:*** *This can be done as an additional step after Step* ***9B****.*

   9B. For elution of protein in its native form: Dilute 4X Non-Denaturing Elution Buffer to 1X, and add 70 µl to the Spin Column. Centrifuge the Spin Column at 5000 rpm (2000 xg), and save the eluate for Western analysis or other assays. ***Notes: 1.*** *Additional elutions with 2X or 4X Non-Denaturing Elution Buffer can be done for maximum recovery of bound material.*
   2. *Successive elutions using either or both protocols (Steps* ***9A*** *and/or* ***9B****) may allow a more complete recovery of the antigen; see figures 1-3 (lanes 5-7) on pages 27-29.*

9C. Catch and Release® Immunoprecipitation Kinase Assay (IPK) Protocol

To perform a kinase assay using the eluted protein in its native form (i.e., non-denaturing elution listed in step 9B.), the Catch and Release® kit was used with Upstate's polyclonal antibody, Anti-Erk1/2 (cat. #06-182) which immunoprecipates active Erk1/2. Active Erk1/2 was eluted and used in conjunction with Upstate's non-radioactive MAP Kinase/Erk Assay Kit (cat. #17-191). The non-radioactive MAP Kinase/Erk assay kit is designed to measure the phosphotransferase activity of MAP (mitogen-activated protein) Kinase (also known as Erk1/2) in immunoprecipitates. The assay kit is based on phosphorylation of a specific substrate (myelin basic protein, MBP). The phosphorylated substrate is then analyzed by immunoblot analysis, probing with a monoclonal Phospho-specific MBP antibody.

Cell Lysate Preparation

Rat Pheochromocytoma (PC-12) cells grown to sub-confluence and serum starved overnight. To activate Map Kinase, the cells were stimulated with NGF (50 ng/ml for 10 mins at 37°C) and were lysed in ice cold buffer A (50 mM Tris, pH 7.5, 1 mM

EDTA, 1 mM EGTA, 0.5mM Na3VO4, 0.1% 2-mercaptoethanol, 1% Triton X-100, 50 mM sodium fluoride, 5mM sodium pyrophosphate, 10 mM sodium β-glycerol phosphate, 0.1mM PMSF, 1 mg/ml of aprotinin, pepstatin, leupeptin and 1mM Microcystin). Microcystin was added to ensure complete inactivation of cellular PP1 and PP2A phosphatases, which may dephosphorylate the active MAP Kinase.

Any insoluble material after cell lysis was pelleted by centrifugation at 16,000 xg for 10 minutes at 4°C. A Bradford assay, using BSA as a standard, was used to assess total protein concentration of the cell lysate. The cell lysate was diluted to 1 mg/ml with ice cold buffer A prior to performing the immunoprecipitation to maintain kinase activity.

Catch and Release® IPK (Protocol to IP Active Kinase)

4 µg of negative normal rabbit IgG and/or 4 µg of anti-Erk1/2 was used as described in the instruction manual; except that at step 7, page 16, the final column wash was performed with Kinase Assay Dilution Buffer (ADB) included in non-radioactive MAP Kinase/Erk assay kit (cat. #17-191), and the eluate was diluted 1X (i.e., the 70 µl eluate in step 9B was diluted equally with ADB).

All kinase reactions were performed using either normal IgG (negative control) or anti-Erk1/2, and were performed in a total volume of 50 µl. Reactions were at 30°C for 30 minutes with mixing:

Component	Amount
MAP Kinase Substrate Cocktail (cat. #20-166)	10 µl
Magnesium/ATP Cocktail (cat. #20-113)	10 µl
Assay Dilution Buffer (cat. #20-108)	10 µl
Eluate of IP material	20 µl
Total	**50 µl**

After the incubation time, 50 µl kinase reactions were diluted in 2X Laemmli sample buffer/reducing sample buffer for a final volume of 100 µl. The samples were boiled for 5 minutes, and 20 µl out of the 100 µl boiled sample was loaded on an Invitrogen 4-12% Bis-Tris gel using MES running buffer (concentration of MBP substrate loaded is 2 µg per lane), and protein was transferred to nitrocellulose and/or PVDF and probed with anti-phospho-MBP following the instructions contained

within the non-radioactive MAP Kinase/Erk assay kit's datasheet. ***Note:*** *An equal amount was used in all Catch and Release® and traditional immunoprecipitation conditions (500 µg total cellular protein was used). Please see IPK data on Figure 4; page 30 for both Catch and Release® and traditional IP. Experiments were normalized so that equivalent amounts of Catch and Release® eluate or traditional IP using Protein A agarose beads were used with the non-radioactive MAP Kinase Assay Kit.*

Traditional IPK (Protocol to IP Active Kinase)

4 µg of negative normal rabbit IgG and/or 4 µg of anti-Erk1/2 were bound to 30 µl of protein A beads for 30 minutes. Then 500 µg of PC12 lysate in Buffer A was added, and the antibody-agarose complex and incubated at 4°C for 1 hour on a rotator. The agarose complex was pelleted and washed 2 times with buffer A and once with 1X kinase assay dilution buffer.

The total volume of the Protein A beads was adjusted to match that of the eluate from the Catch and Release® kit. An equal volume of each immunoprecipitation added to the kinase reaction using non-radioactive MAP Kinase/Erk assay kit.

IV. Technical Support

A. Catch and Release: Frequently Asked Questions

1. Q: What is the blue eluate sometimes seen after initial washes?

A: This has been seen when spinning the columns at high speeds during the initial Spin Column resin washes. However, this should not affect column binding efficiency. Reducing centrifuge speed to 5000 rpm (2000 xg) should eliminate the blue color in the eluate.

2. Q: What should the user do if a viscous precipitate forms in the column during the initial incubation step?

A: Genomic DNA is present in the lysate. Try clarifying the lysate by spinning out genomic DNA at 15,000 rpm for 5 minutes at 4ºC. Some of the protein of interest might also be removed in the process. Re-check protein concentration of the clarified sample, and increase volume of lysate to use if necessary.

3. Q: Why did everything (i.e., my primary antibody and target protein) come through in the flow-thru and column wash fractions?

A: 1. Antibody Capture Affinity Ligand not added. Repeat incubation with Ligand.

2. Expired Antibody Capture Affinity Ligand. Check shelf life of Ligand. Obtain fresh lot of Antibody Capture Affinity Ligand if needed and repeat incubation.
3. No antibody added. Repeat incubation adding antibody.
4. Antibody chosen does not perform well for immunoprecipitation methods. Choose antibody that works for IP applications and repeat.

4. Q: Why are bands faint or not present in the elution fractions and flow-thru?

A: 1. Insufficient exposure time during Western blot detection procedure. Increase exposure time on x-ray film or digital imaging system.
2. Antibody concentration not sufficient or not optimized for either IP or Western detection. Repeat procedures with increased primary antibody concentration.
3. Secondary antibody concentration is not optimal. Optimize for Western detection, and re-probe blot after stripping.
4. Western detection reagents old or expired. Retry using fresh reagents.
5. Cell lysate contained low levels of antigen.
6. Antibody not optimal for IP. Re-evaluate it in a traditional IP.
7. Increase incubation time for IP.

B. Western Detection Problems: Troubleshooting

Smeared Pattern or Distorted Bands

- **Uneven contact between gel and membrane:** Cassettes used should allow a tight fit, leading to even pressure over the entire surface of the gel and membrane.
- **For Tris-Glycine gels, gel not equilibrated in buffer prior to transfer:** The gel should be soaked in Towbin transfer buffer containing methanol for 15-30 minutes before assembling the transfer sandwich. For precast gels, make sure to follow the manufacturer's instructions for gel preparation for transfer.

"Bald Spots"

- **Bubbles between gel and membrane:** Bubbles create areas of low transfer efficiency. Bubbles should be completely removed when putting together the transfer sandwich.

Incomplete Transfer

- **Incomplete protein transfer:** This often occurs with high molecular weight proteins, especially when using a methanol-based transfer buffer. One way to prevent this is by using a nylon membrane, which does not

require methanol in the transfer buffer. Adding SDS to the transfer buffer and using higher field strengths also improve protein transfer.

- **Proteins transferred through membrane:** This may occur when working with proteins of very low molecular weight. Optimizing/shortening transfer times and using a double layer of membrane usually enhances retention of small proteins. PVDF membranes are superior to nitrocellulose for preventing proteins from transferring through the membrane. In some cases, use of nitrocellulose or PVDF membranes with a 0.2 µm pore size works better for small proteins than membranes with the more common 0.45 µm pore size.
- **Inappropriate transfer buffer used:** The most stable and commonly used buffers are Tris-Glycine based (Towbin Transfer Buffer).
- **Impurities in the transfer buffer:** This will lead to a pattern on the membrane that mirrors the holes in the transfer cassette. Fresh buffer should be prepared for each transfer.
- **The wrong transfer buffer was used based on the isoelectric point (pI) of the protein being detected:** The pH of the transfer buffer used and the pI of the protein being detected will determine the direction of

the protein transfer. If the pH of the buffer and the pI of the protein are near equal, the protein will remain in the gel. If the pH of the buffer is lower than the pI of the protein, the protein will have a net negative charge and migrate towards the positive electrode. If the pH of the buffer is higher than the pI of the protein, the protein will have a net positive charge and migrate towards the negative electrode. Adding SDS to the buffer is one way to make sure all proteins in the gel have a net negative charge and, therefore, migrate towards the positive electrode. For pre-cast, neutral pH gels, consult the manufacturers instructions.

High Background

- **Cross-reactivity between blocking agent and primary antibody:** This will result in overall membrane background. Usually, the addition of detergent (Tween®-20) to the Washing Buffer will eliminate the problem. If background persists, changing the blocking agent is recommended. A blot performed without a primary but with the secondary may be useful in identification of background caused by the primary or secondary.
- **Concentration of either primary and/or secondary antibody too high or incubation time too long:**

The higher the antibody concentration and the longer the incubation time, the greater the non-specific binding. Raising the incubation temperature (e.g. to 37°C) is recommended over lengthening the incubation time. Also, many short washing steps are better than a few long ones.

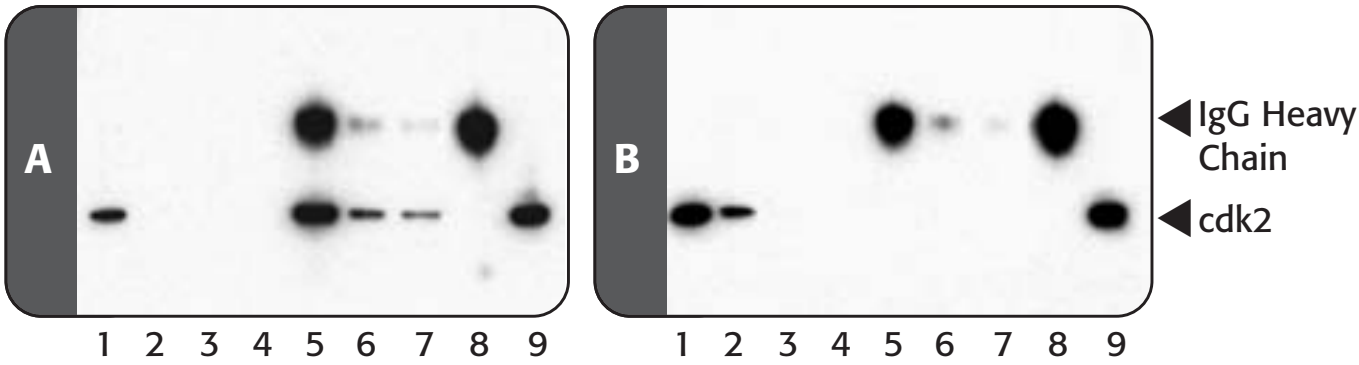
- **Membrane drying during incubation process:** Care should be taken to keep membrane from drying out during incubation.

Little or No Signal

- **Antigen is not recognized by primary antibody:** This can occur especially with monoclonal antibodies that were raised against a native protein. In some cases, a non-reducing gel system may need to be used.
- **Inhibition of secondary antibody conjugate:** HRP-labeled antibodies should not be used in conjunction with sodium azide or hemoglobin.
- **Detergent is too harsh:** SDS, Nonidet P-40 and Triton X-100 disrupt binding between proteins. Tween®-20 is the most commonly used and recommended detergent for washing and incubation solutions.

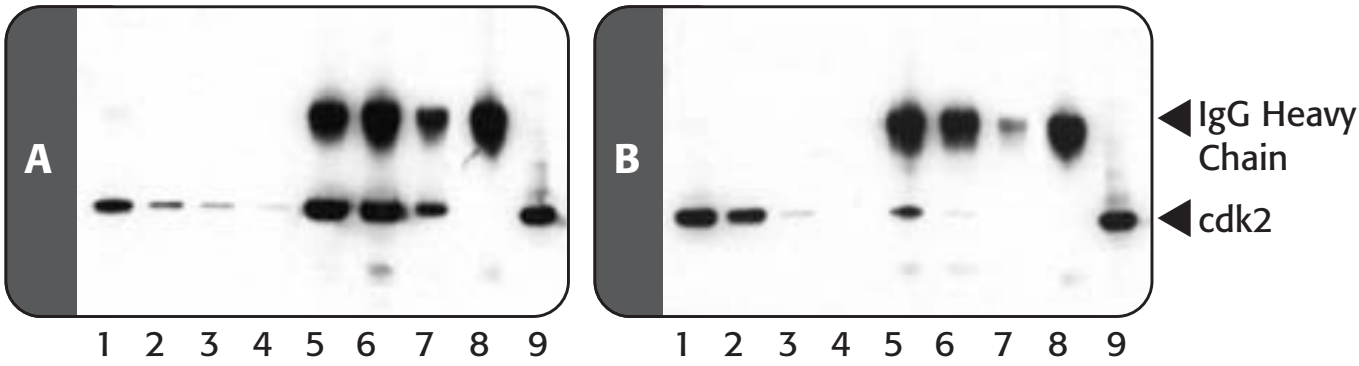
V. Catch and Release® vs. Traditional Immunoprecipitation Data

Figure 1. Catch and Release® with non-denaturing elution



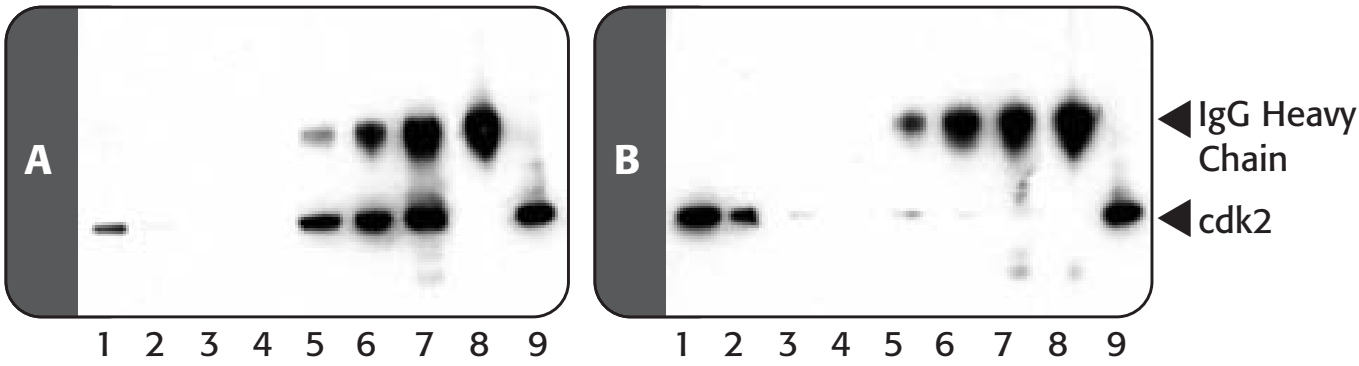
Legend. *Catch and Release® Spin columns and protocol were used with the Non-denaturing Elution Buffer to immunoprecipitate cdk2. HeLa nuclear extract was mixed with* ***A****. anti-cdk2 (cat. #06-505) or* ***B.*** *normal, rabbit IgG as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted. The upper band is the heavy chain of IgG and the lower band is cdk2.* ***Lane 1:*** *flow through,* ***Lane 2:*** *wash 1,* ***Lane 3:*** *wash 2,* ***Lane 4:*** *wash 3,* ***Lane 5:*** *elution 1,* ***Lane 6:*** *elution 2,* ***Lane 7:*** *elution 3,* ***Lane 8:*** *anti-cdk2,* ***Lane 9:*** *HeLa nuclear extract.*

Figure 2. Catch and Release® with denaturing elution



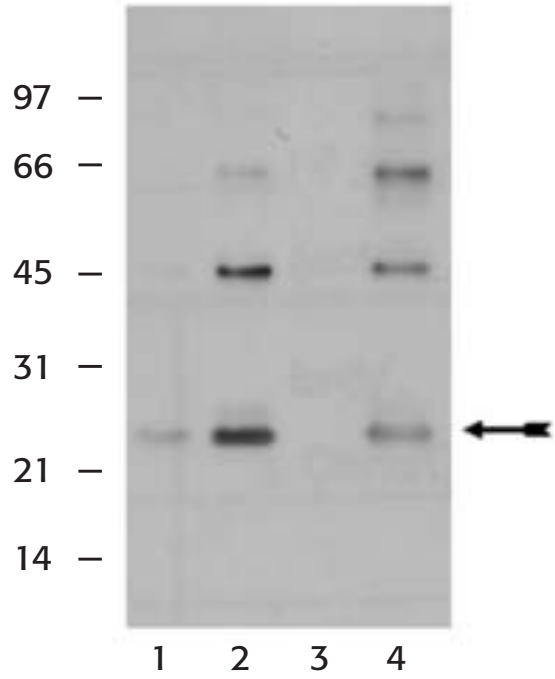
Legend. *Catch and Release® columns and protocol were used with the denaturing elution buffer to immunoprecipitate cdk2. HeLa nuclear extract was mixed with* ***A.*** *anti-CDK2 (cat. #06-505) or* ***B.*** *normal, rabbit IgG as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted. The upper band is the heavy chain of IgG and the lower band is cdk2.* ***Lane 1:*** *flow through,* ***Lane 2:*** *wash 1,* ***Lane 3:*** *wash 2,* ***Lane 4:*** *wash 3,* ***Lane 5:*** *elution 1,* ***Lane 6:*** *elution 2,* ***Lane 7:*** *elution 3,* ***Lane 8:*** *anti-CDK2,* ***Lane 9:*** *HeLa nuclear extract.*

Figure 3. Traditional IP with denaturing elution



Legend. *Traditional immunoprecipitation was performed for cdk2 using Protein A agarose and the denaturing elution buffer. HeLa nuclear extract was mixed with* ***A****. anti-cdk2 (cat. #06-505) or* ***B.*** *normal, rabbit IgG as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted. The upper band is the heavy chain of IgG and the lower band is cdk2.* ***Lane 1:*** *unbound,* ***Lane 2:*** *wash 1,* ***Lane 3:*** *wash 2,* ***Lane 4:*** *wash 3,* ***Lane 5:*** *elution 1,* ***Lane 6:*** *elution 2,* ***Lane 7:*** *elution 3,* ***Lane 8:*** *anti-CDK2,* ***Lane 9:*** *HeLa nuclear extract.*

Figure 4. Catch and Release® with non-denaturing elution to immunoprecipitate active kinase.



Legend. *Immunoprecipitation Kinase Assay was performed using NGF-treated PC-12 cells lysed with Buffer A (see Certificate of Analysis for cat. #06-182). Lysate (500 µg) was used for IP with 4 ug of either normal Rabbit IgG (lanes 1 and 3) or Anti-MAP Kinase (lanes 2 and 4). IP was performed with Catch and Release® (lanes 1 and 2) or by traditional IP (lanes 3 and 4). Catch and Release® was used as described in the instruction manual, except that the final wash used Assay Dilution Buffer (included in cat. #17-191), and the eluate was diluted 1X with Assay Dilution Buffer. Following IP, equivalent amounts of Catch and Release®*

eluate or agarose beads from the traditional IP were used for MAP Kinase assay using a non-radioactive MAP Kinase Assay Kit, which utilizes MBP as a substrate for MAP Kinase. ***Note:*** *IgG heavy chain is not detected because IP antibody was rabbit IgG and western detection used mouse monoclonal antibody.*

After the assay, reactions were stopped with SDS-PAGE sample buffer were used for SDS-PAGE and Western blot for phospho-MBP. The migration position of phosphorylated MBP is indicated by an arrow (the larger molecular weight proteins detected may be dimerized and/or unreduced phosphorylated MBP). It can be seen that immunoprecipitation using the Catch and Release® system, effectively immuno-precipitated active MAP Kinase. Interestingly, kinase obtained using Catch and Release® appeared to be significantly more active than kinase used from traditional IP, which was still in complex with agarose beads.

VI. References

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