User Guide

Fluorescent In Situ Hybridization (FISH) Probe

for Detection of Microbes (Bacteria or Fungi)

MBD0028 MBD0029 MBD0032 MBD0033 MBD0034 MBD0035 MBD0039 MBD0040
MBD0041 MBD0042 MBD0047 MBD0048 MBD0049 MBD0050 MBD0051 MBD0059 MBD0062

Intended Use

These Fluorescent In Situ Hybridization (FISH) probes are intended for the detection of the following microbes:

MBD0028 Porphyromonas gingivalis,
MBD0029 Porphyromonas gingivalis,
MBD0032 Eubacteria,
MBD0033 Eubacteria,
MBD0034 Bacterial negative control,
MBD0035 Bacterial negative control,
MBD0039 Proteus sp.,
MBD0040 Proteus sp.,
MBD0041 Akkermansia muciniphila,
MBD0042 Escherichia coli,
MBD0047 Enterococcus faecium,
MBD0048 Enterococcus faecium,
MBD0049 Escherichia coli,
MBD0050 Akkermansia muciniphila,
MBD0051 Yeast species
MBD0059 Alphaproteobacteria
MBD0062 Planctomycetota and Verrucomicrobiota

Optimization of the protocol may be necessary for certain strains/microorganisms.

Introduction

FISH is a technique used to identify and localize the presence or absence of specific DNA or RNA sequences in cells and tissues using fluorescently labeled probes. FISH is a powerful tool used in karyotyping, cytogenotyping, cancer diagnosis, bacteria species specification, and gene-expression analysis.[1]

Precaution and Disclaimer

The Fluorescent In Situ Hybridization (FISH) probes for the detection of bacteria and fungi are for research use only, not for drug, household, or other uses. Please consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices. Formamide and Paraformaldehyde are irritants. May cause damage to organs (blood) through prolonged or repeated exposure if swallowed (oral). Use only in a fume hood. Avoid ingestion, contact with skin, eyes and clothing. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagents.

Equipment and Reagents Required

(Not provided)

Equipment

- 15 mL Corning® tubes
- 50 mL Corning® tubes
- Sterile Eppendorf® tubes
- Sterile tips
- Filters 0.2 µm
- PTFE diagnostic microscope slides 10 well, 6.7 mm or equivalent
- Coverslips
- 48 °C water bath
- Hybridization oven system
- Centrifuge
- Vortex
- Magnetic stirrer and stirring bars
- Fluorescence microscope equipped with appropriate filter for fluorescence detection
- Kimwipes® wipers
- Tweezers
- Histology slide container and holder
- Aluminum foil
- Timer
- Gloves
- Eye goggles

Reagents

- 0.01 M, Phosphate buffered saline (PBS) pH 7.2-7.4.
- Paraformaldehyde (PFA) 16%
- Ethanol
- Tris-EDTA buffer solution
- DAPI ready-made solution with Antifade
- Formamide
- 10% SDS
- 1 M Tris-HCl, pH 8.0
- 0.5 M EDTA
- Milli-Q® Reagent Grade Water
- NaCl

Preparation

1. Prepare (for fixation step only) PFA 4% in PBS - Dilute PFA 16% in PBS.
2. Dilute ethanol to 50% in PBS. Store at -20 °C.
3. Dilute 50%, 80%, and 98% ethanol in distilled and filtered 0.2 µm water (250 mL each) in glass bottles.
4. Dilute the fluorescently labeled probe to suitable concentration (10 µM as described below).
5. Prepare 5 M NaCl, 146.1 g into 500 mL water and filter 0.2 µm.
6. Prepare hybridization buffer with appropriate % formamide according to **Table 1** and **2**. This can be prepared in advance and later stored at -20 °C.

Example

For 20% formamide hybridization buffer:

- 5 M NaCl, 360 µL
- 1 M Tris-HCl pH 8.0, 40 µL
- dH_2O, 1198 µL
- Formamide, 400 µL
- 10% SDS, 2 µL

Table 1. Recommended hybridization conditions for each probe

Description	Catalogue Number	Recommended % formamide for hybridization	Recommended probe concentration (micromolar) for hybridization	Recommended hybridization time (hours)
Porphyromonas gingivalis FISH probe, ATTO488	MBD0028	20	1	2
Porphyromonas gingivalis FISH probe, Cy3	MBD0029	20	1	2
Eubacteria FISH probe, ATTO488	MBD0032	0-40	1	2
Eubacteria FISH probe, Cy3	MBD0033	0-40	1	2
Bacterial negative control FISH probe, ATTO488	MBD0034	0-40	1	2
Bacterial negative control FISH probe, Cy3	MBD0035	0-40	1	2
Proteus FISH probe, ATTO488	MBD0039	20	1	2
Proteus FISH probe, Cy3	MBD0040	20	1	2
Akkermansia muciniphila FISH probe, Cy3	MBD0041	30	1	2

Description	Catalogue Number	Recommended % formamide for hybridization	Recommended probe concentration (micromolar) for hybridization	Recommended hybridization time (hours)
Escherichia coli FISH probe, Cy3	MBD0042	30	1	2
Enterococcus faecium FISH probe, ATTO488	MBD0047	10	2	3
Enterococcus faecium FISH probe, Cy3	MBD0048	10	2	3
Escherichia coli FISH probe, ATTO 488	MBD0049	30	1	2
Akkermansia muciniphila FISH probe, ATTO	MBD0050	30	1	2
Yeast FISH probe, Cy3	MBD0051	30	1	2
Alphaproteobacteria FISH probe-Cy3	MBD0059	20	1	2
Eubacteria FISH probe extension set	MBD0062	20	1	2

Table 2. Hybridization Buffer
Formulation based upon 2 mL solution of hybridization buffer. Add in the following order:

Name	Formulation Amount	Actual Amount
5 M NaCl	360 µL	
1 M Tris-HCl, pH 8.0	40 µL	
dH_2O 0.2 µm filtered according to formamide volume*		
Formamide volume is determined for each probe*		
10% SDS	2 µL	

* Formamide and water volumes for hybridization buffer according to % (See Table 3).

Table 3. Formamide volumes for hybridization buffer[2]

% Formamide	Formamide volume x (µL)	Milli-Q® water volume y (µL)
0	0	1598
5	100	1498
10	200	1398
15	300	1298
20	400	1198
25	500	1098
30	600	998
35	700	898
40	800	798
45	900	698
50	1000	598

- Allow the formamide to reach room temperature from 4 °C storage temperature by placing it in the fume hood. Prepare the required amount of hybridization buffer (buffer can be prepared on the bench but formamide must be added in a fume hood and SDS 10% added last). Add SDS last to avoid precipitation with the concentrated NaCl.
- Divide into 400-500 µL aliquots (seal with parafilm) and store in -20 °C in the dark up to 6 months for future use.
- For each FISH experiment allow the required amount of hybridization buffer aliquot to reach room temperature in the fume hood before use.

7. Prepare fresh wash buffer (NaCl, 20 mM, pH 7.2 Tris-HCl, EDTA, 0.01% SDS) according to **Tables 4** and **5**. Add SDS last to avoid precipitation with the concentrated NaCl. Total volume of 250 mL is recommended. Store at room temperature (enough for 4 slides).

Example

For 20% formamide add:

- Filtered dH_2O 0.2 µm, 1300 µL
- 5 M NaCl, 2150 µL
- 1 M Tris-HCl pH 8.0, 1 mL
- EDTA 0.5 M, 500 µL
- 10% SDS, 50 µL

Table 4. Formulation based upon 50 mL solution of washing buffer. Add in the following order:

Name	Formulation Amount	Actual Amount
Filtered dH_2O 0.2 µm in order to complete to 50 mL		
5 M NaCl*		
1 M Tris-HCl, pH 8.0	1 mL	
0.5 M EDTA*		
10% SDS	50 µL	

* Optimal volume depends on the % formamide in the hybridization buffer (See Table 5).

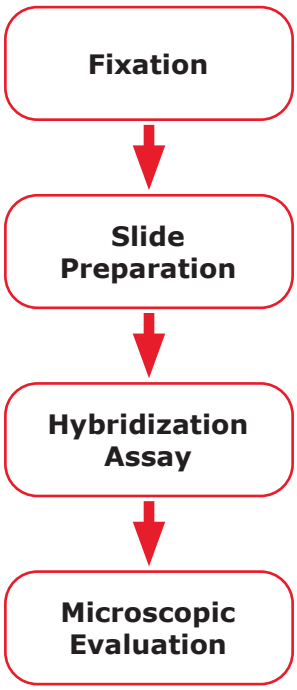
Table 5. NaCl and EDTA concentrations in the washing buffer.[3]

% Formamide (hybridiz. buffer)	5 M NaCl (=y) µL	0.5 M EDTA (=z) µL
0	9000	-
5	6300	-
10	4500	-
15	3180	-
20	2150	500
25	1490	500
30	1020	500
35	700	500
40	460	500
45	300	500
50	180	500
55	100	500
60	40	500
65	-*	500
70	-	350
75	-	250
80	-	175
85	-	125
90	-	88
95	-	62

* Enough NaCl in EDTA

Procedures

During the whole process refer to the procedure flow chart below and to product specifications.



Plan your test and clearly label slides noting species, probe name, dilution or concentration and date.

Fixation

1a. **Grow bacteria or yeast**
Grow bacteria or yeast to logarithmic phase accordingly to appropriate growth curve and OD measurement to obtain approximately 10^7-10^9 CFU/mL.

2a. **Harvest**
At least 10 mL culture in 15 mL Corning® tubes, centrifuge 4500 rpm for 10 minutes.

3a. **Wash Pellet**
Discard the supernatant and wash the pellet with 10 mL PBS. Make sure that the supernatant is clear. Check all the pellet is suspended, especially on the sides of the tube.

4a. **Centrifuge**
Centrifuge at 4500 rpm for 10 minutes.

5a. **Wash Pellet**
Discard the supernatant and wash the pellet with PBS. Repeat stage 4a-5a, two more times (3 washes total).

6a. **Add PBS**

- Add 1 mL of PBS to pellet and suspend.
- Add 3 mL of freshly prepared PFA 4% in PBS solution and resuspend thoroughly.

7a. **Incubate**
Incubate sample on ice for 1.5-2 hours.

8a. **Centrifuge**
Centrifuge at 4500 rpm for 10 minutes and discard the PFA supernatant into chemical waste.

9a. **Resuspend**
Resuspend in PBS thoroughly (if difficult due to harsh pellet continue with resuspension) and centrifuge 4500 rpm for 10 minutes, discard the supernatant into chemical waste.

10a. **Repeat stage 9a.**
Two washes total.

11a. **Suspend pellet**
Suspend the pellet in 10 mL ethanol at 50% in PBS. The suspension should be homogenous, if not, allow to recover overnight in water 2-8 °C before dividing into 1 mL aliquots in sterile Eppendorf Tubes®.

12a. **Storage**
Storage in -20 °C recommended for several months.

13a. **Colony Count**
You may want to perform colony counting.

14a. **Viability test**
Viability test is suggested.

Slide Preparation

DAPI test for fixed cells before FISH assay to verify fixation and dilution

1b. **Vortex**
Briefly vortex-mix fixed samples to resuspend settled material and prepare the following dilutions:

- 1:1 (directly from stock)
- 1:5 and 1:10 in 50% ethanol in filtered PBS

Apply 20 µL of sample per well on a Teflon™-coated multi-well slide. It is possible to immobilize different types on the same slide in different wells, make at least one duplicate.

2b. **Air-dry**
Air-dry thoroughly (to prevent cells detaching in subsequent steps) in the chemical hood (approximately 1-1.5 hours).

3b. **Dehydrate**
Place slides in histology slide container and dehydrate slides in an ethanol series (3 min each in 50%, 80%, and 98% ethanol in filtered water - 0.2 µm [250 mL]). Work in chemical hood. Ethanol solutions can be reused up to 5 times.

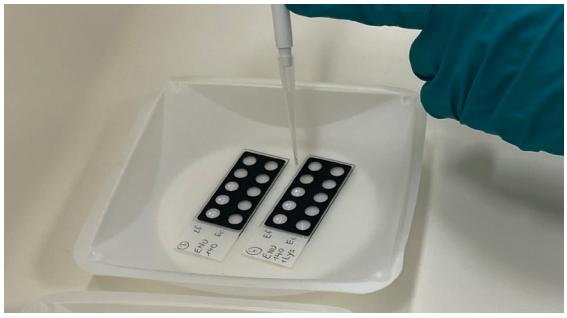
4b. **Air-dry**
Air-dry slides on a weighing boat in chemical hood.

5b. **Apply DAPI Mounting media**
Gently angle down coverslip and place it in position while avoiding air bubbles and allow the slides to recover in the dark for 2 hours. Store slides in 4 °C.

6b. **Microscope Observation**
Observe under microscope. If the cells on slide appear too dense/far apart, repeat the steps with higher/lower dilution of stock.

Preparation of slides for FISH assay

1c. **Vortex**
Briefly vortex-mix fixed samples to resuspend settled material and prepare the suitable dilution if needed in 50% ethanol in filtered PBS. Apply 20 µL of sample per well on a Teflon™-coated multi-well slide. It is possible to immobilize different types of microorganisms on the same slide in different wells, make at least one duplicate.



2c. **Perform steps 2b-4b**
Perform steps 2-4 from previous Slide Preparation section.

3c. **Storage and Hybridization**
Slides can be stored at -20 °C, but preferably should be hybridized on the same day after cell immobilization.

Probe Dilution

Prepare working probe solution of 10 µM with freshly prepared Tris-EDTA X 1 or pure water and store in aliquots of 10 µL in -20 °C in the dark.

Hybridization Assay

1e. **Prepare before**
Before FISH experiment make the following preparations:

- Set the water bath to 48 °C.
- Bring a required frozen aliquot amount of hybridization buffer to room temperature in the fume hood.

2e. **Hybridization Program**
Set the hybridization program to 46 °C for 2 hours.

3e. **Prepare FISH Probe**
Prepare the FISH probe and hybridization buffer mixture:

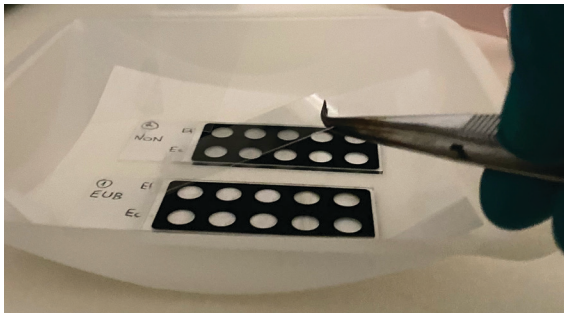
- 1 µL probe (from 10 µM working solution) to 9 µL hybridization buffer per well on the slide.
- Add the required probe amount to the tube in advance and then add the hybridization buffer in the fume hood.
- Pipette the hybridization buffer carefully before adding it to the probe (do not vortex).

4e. **Place Slide**
Place the slide with immobilized cells on a Corning® dish (with Kimwipes® inside to soak potential hybridization buffer spill) in the fume hood.

5e. **Add Probe-Hybridization Buffer**
Carefully add 10 µL of probe-hybridization buffer mixture per well.

NOTE: Use only one type of probe per slide to avoid cross contamination between wells.

6e. **Place Coverslip**
Using tweezers carefully place a coverslip on top of the slide without disturbing the probe-hybridization buffer mixture.



7e. **Place in Hybridization Oven**
Using tweezers carefully place the slide(s) in the hybridization oven and turn on the hybridization program.

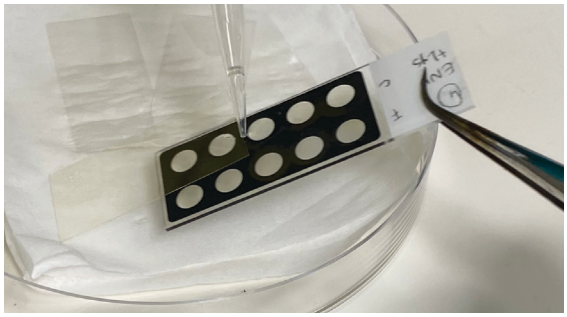
8e. **Prepare Beaker**
During hybridization time place a beaker of distilled (approximately 750 mL) water on ice.

9e. **Divide Wash Buffer**
Divide wash buffer to 50 mL tubes: 45 mL each per slide and additional 45 mL for washes, and place in water bath at 48 °C.

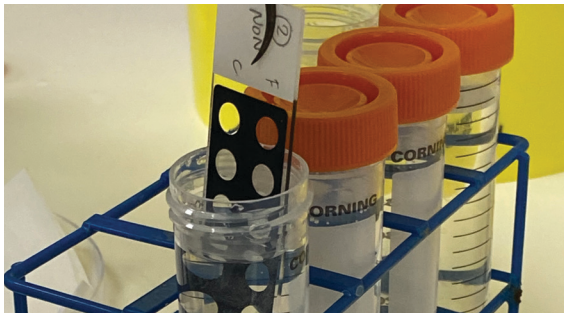
10e. **Upon Completion of Hybridization Program**
Open the hybridization oven, if your system allows it do not terminate the program since termination may reduce the temperature and this can interfere with probe binding.

11e. **Place Tubes**
Place the 50 mL tubes with the wash buffer inside the fume hood. Prepare a tube for waste.

12e. **Place Slides**
Using tweezers, carefully place the slide on the Corning® dish with Kimwipes® and using pipette and tip remove the coverslip into the dish. Allow the used coverslips and dish to dry before placing in Formamide waste container.



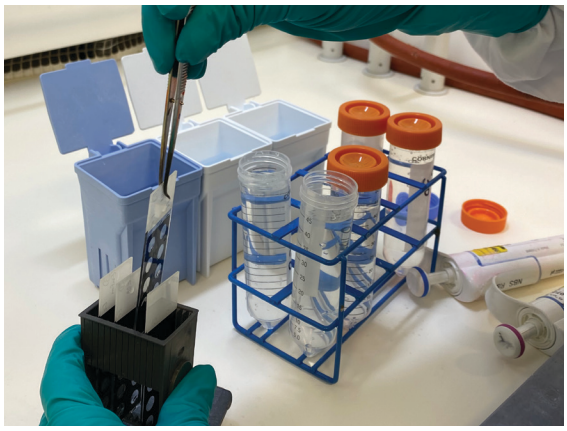
13e. **Rinse Slides**
Using tweezers carefully place the slide over a 50 mL tube for waste and rinse the slide three times with 1 mL pre-warmed wash buffer, then carefully place the slide in a new tube containing 45 mL prewarmed wash buffer. Pay attention that all the slide is covered in the buffer.



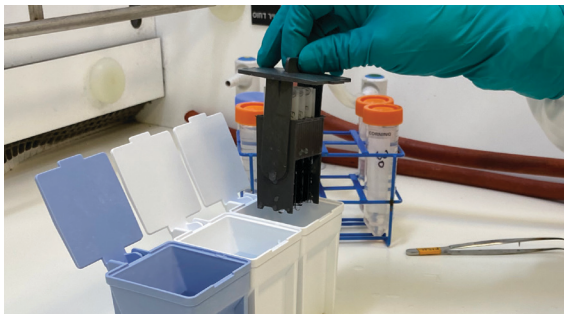
14e. **Place Tubes with Slides**
Place in the water bath at 48 °C for 15 min.

15e. **Divide Water**
During washing incubation divide the water (2-8 °C) prepared in step 8e to three histology slide containers (approximately 250 mL each).

16e. **Remove the Slides**
Using tweezers remove the slide from the tube and place in slide holder before soaking in water.

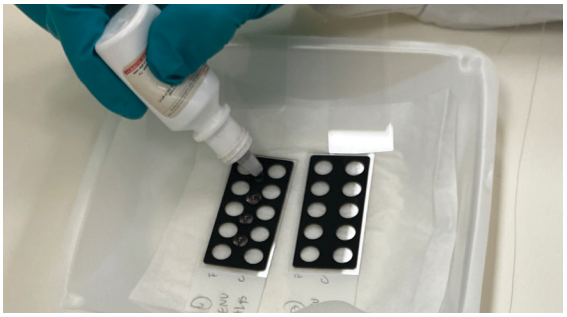


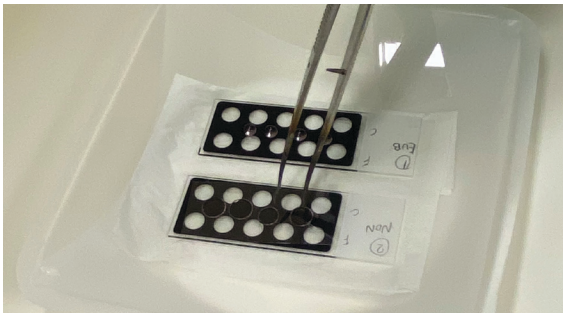
Quickly rinse (10 seconds each) the slide in water (2-8 °C) by soaking the slides subsequently in the three containers. Pay attention that all the slides are covered in water.



17e. **Place Slides on Weighing Boat**
Place the slides on a weighing boat and dry immediately using Kimwipes®.

18e. **Apply DAPI Mounting Medium**
Apply DAPI mounting medium (four drops per slide) and gently angle down coverslip and place it in position while avoiding air bubbles.





19e. **Dry Slides**
Keep the slides in the dark at room temperature for at least 2 hours to allow the mounting medium to dry.

20e. **Store Slides**
Store in the dark at 4 °C and observe at least on the next day using a fluorescence microscope, equipped with appropriate filter for fluorescence detection.

21e. **Dispose of Washing Buffer**
Dispose all formamide solid and liquid waste in the appropriate containers and allow the tubes to dry in fume hood before placing in plastic Formamide waste container.

Microscope Viewing

1f. **Evaluate Slides**

Examine the slides using a fluorescence microscope, equipped with appropriate filter for fluorescence detection.

Make sure that the microscope is optimally aligned, lenses are clean and correct filters are set.

Avoid prolonged or intense slide illumination.

2f. **Slide Storage**
Store representative stained slides for future reference at 2-8 °C in the dark (weeks to several months).

Product Ordering

Description	Catalogue Number
Porphyromonas gingivalis FISH probe, ATTO488	MBD0028
Porphyromonas gingivalis FISH probe, Cy3	MBD0029
Eubacteria FISH probe, ATTO488	MBD0032
Eubacteria FISH probe, Cy3	MBD0033
Bacterial negative control FISH probe, ATTO488	MBD0034
Bacterial negative control FISH probe, Cy3	MBD0035
Proteus FISH probe, ATTO488	MBD0039
Proteus FISH probe, Cy3	MBD0040
Akkermansia muciniphila FISH probe, Cy3	MBD0041
Escherichia coli FISH probe, Cy3	MBD0042
Enterococcus faecium FISH probe, ATTO488	MBD0047
Enterococcus faecium FISH probe, Cy3	MBD0048
Escherichia coli FISH probe, ATTO488	MBD0049
Akkermansia muciniphila FISH probe, ATTO488	MBD0050
Yeast FISH probe, Cy3	MBD0051
Alphaproteobacteria FISH probe, Cy3	MBD0059
Eubacteria FISH probe extension set, Cy3	MBD0062

Related Products

Description	Catalogue Number
Pure water	95284
Tris-EDTA buffer solution	93283
PBS	D8537
DAPI ready-made solution with Antifade	MBD0020
10% SDS	71736
1 M Tris-HCl, pH 8.0	T2694
0.5 M EDTA	E7889
NaCl	S9888
Kimwipes®, 4.5 in. x 8.5 in.	Z188956

References

1. Wang, D. O. et al. A quick and simple FISH protocol with hybridization-sensitive fluorescent linear oligodeoxynucleotide probes. Rna 18, 166–175 (2012).
2. Hugenholtz, P., Tyson, G. W. & Blackall, L. L. Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence in situ hybridization. Methods Mol. Biol. 179, 29–42 (2002).
3. Nistico, L. et al. Fluorescence 'in situ' hybridization for the detection of biofilm in the middle ear and upper respiratory tract mucosa. Methods Mol. Biol. 493, 191–213 (2009).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/Terms.

Technical Assistance

Visit the tech service page on our web site at SigmaAldrich.com/techservice.