For life science research only.
Not for use in diagnostic procedures.

Anti-HA Affinity Matrix
from rat IgG_1 (clone BMG 3F10)

Version: 10
Content Version: June 2021

Rat monoclonal antibody (Clone 3F10), immobilized

Cat. No. 11 815 016 001 1 ml
settled resin volume

Store the matrix at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-HA Affinity Matrix	Immobilized Anti-HA High Affinity rat monoclonal antibody. ▪ Antibody is covalently coupled to the matrix (agarose beads) and is supplied as a 2 ml slurry containing 1 ml of beads in 1 ml of buffer (phosphate-buffered saline and 0.09% sodium azide as preservative). ▪ 3.5 mg of antibody is reacted per 1 ml of beads in the coupling reaction. ▪ Includes plastic column with top and bottom caps.	1 ml settled resin volume

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the matrix is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Anti-HA Affinity Matrix	Store at +2 to +8°C. ⚠ ***Do not freeze.***
2	Empty column	Store at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For immunoprecipitation

See section, **Working Solution** *for additional information on preparing solutions.*

- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Pipettes
- Positive-displacement pipettes
- Pipette tips, including wide-bore and fine-bore types
- End-over-end rocker

For affinity purification

See section, **Working Solution** *for additional information on preparing solutions.*

- Rack or stand suitable for gravity collection of samples
- 23G needle for adjustment of flow rate
  - *Recommended needle size for 0.5 to 1.0 ml column bed-volumes, adjust size for larger or smaller columns.*

Western blot

See section, **Working Solution** *for additional information on preparing solutions.*
- PVDF Western Blotting Membranes*
- Anti-HA-Peroxidase, High Affinity (3F10)*
- Western Blocking Reagent*
- Lumi-Light Western Blotting Substrate*
- Lumi-Film Chemiluminescent Detection Film*
- Tween 20*
- Methanol
- Plastic wrap
- Tris buffered saline (TBS), pH 7.5

1.4. Application

Anti-HA Affinity Matrix is suitable for:
- Immunoprecipitation of HA-tagged proteins from mammalian, yeast, and bacterial cell extracts.
- Affinity purification of HA-tagged proteins from crude protein extracts.

Following immunoprecipitation or purification, the tagged protein of interest may be analyzed by:
- Western blotting using the Anti-HA antibody.
- Silver staining or similar protein stain.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Sample preparation

Prepare protein extracts containing the HA-tagged protein of interest using a standard method.
See section, **Working Solution** *for additional information on preparing solutions.*

General recommendations

To obtain optimal performance of the affinity column:
- Centrifuge extracts at a minimum of 15,000 × *g* for 30 minutes to remove insoluble particulates prior to column loading.
- Use protease inhibitors to reduce proteolytic activity. Use cOmplete Protease Inhibitor Cocktail Tablets* for most applications.
- Do not overload column, this may decrease the purity of the final product.

Working Solution

Sample extracts

Solution	Preparation/Composition	For use in...
Lysis buffer for mammalian extracts	50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40*, cOmplete Protease Inhibitor Cocktail Tablets*. *Other buffers containing 1% Nonidet P-40* and 0.5% sodium deoxycholate, such as RIPA buffer may also be used.*	Preparation of the samples
Lysis buffer for bacterial extracts	20 mM Tris, pH 8.0, 100 mM NaCl, cOmplete Protease Inhibitor Cocktail Tablets* (followed by freeze-thaw).	

Other cell lysis buffers may be more appropriate for individual applications.

Immunoprecipitation

Solution	Preparation/Composition	For use in...
Electrophoresis sample buffer	20 mM Tris*, pH 7.5, 2 mM EDTA, 5% SDS*, 0.02% bromophenol blue, 20% glycerol, 200 mM DTT*.	Gel electrophoresis

Affinity purification

Prepare the following reagents before beginning the affinity purification. One hundred milliliters of each buffer should be adequate for several purifications

Solution	Preparation/Composition	Storage and Stability	For use in...
Equilibration buffer	20 mM Tris*, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA.	Store for 1 month at +2 to +8°C.	Equilibration of column
Wash buffer	20 mM Tris*, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA, 0.05% Tween 20*.		Washing of column
Elution buffer	HA Peptide* reconstituted to 1 mg/ml in Equilibration buffer.	Store in aliquots for 1 month at −15 to −25°C. ***Thaw before use.***	Elution of proteins
Column storage buffer	20 mM Tris*, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA, 0.09% sodium azide.	Store for 1 month at +2 to +8°C.	Storage column
Regeneration buffer	0.1 M glycine, pH 2.0.		Regeneration column for reuse

Equilibrate all the buffers to +15 to +25°C before beginning column purification.

Western blot

Solution	Preparation/Composition	For use in...
Transfer buffer	20% methanol, 25 mM Tris-base*, 194 mM glycine.	Equilibration of membrane and protein transfer
Blocking buffer	10 mM Tris*, pH 7.5, 150 mM NaCl, containing 1x Western Blocking Reagent*	Blocking membrane
TBS-Tween (TBST)	10 mM Tris*, pH 7.5, 150 mM NaCl, containing 0.05% Tween 20*.	Washing steps

2.2. Protocols

Immunoprecipitation

See section, **Working Solution** *for additional information on preparing solutions.*

1. Aliquot 0.5 to 1 ml of cold lysate in Lysis buffer to a microcentrifuge tube.
   Typical amount of total protein is 1 to 100 µg. Lysate protein concentration may be adjusted with Lysis buffer, depending on the relative abundance of the tagged protein.

2. Add 50 to 100 µl of resuspended Anti-HA Affinity Matrix into each microcentrifuge tube.
   Use a positive-displacement pipette and wide-bore pipette tips for dispension. Carefully resuspend matrix prior to dispensing by inverting the vial several times. Do not resuspend matrix using a vortex.

3. Incubate samples at +2 to +8°C on a rocker for 30 minutes.
   – Longer incubations of two hours to overnight may be beneficial for the co-precipitation of protein complexes.

4. Pellet matrix at full speed in a microcentrifuge for 5 to 10 seconds.
   – Carefully remove supernatant.
   Do not disturb the matrix when removing the supernatant. Use fine-bore pipette tips to remove the last approximately 100 µl, leaving approximately 20 µl on the pelleted matrix.

5. Wash matrix three times with 1 ml of cold Lysis buffer, carefully pelleting and removing the supernatant at each wash step.
   – Resuspend matrix in next wash solution.

6. Add 1 to 2 matrix volumes of Electrophoresis sample buffer to final washed matrix pellet.
   – Boil for 5 minutes.
   – Pellet matrix again before gel electrophoresis.
   – Load supernatant fraction on gel.

Affinity purification

Column preparation

See section, **Working Solution** *for additional information on preparing solutions.*

1. Attach lower cap to bottom of column.
   – Place column on rack or stand above the desired collection tube.

2. Gently invert Anti-HA Affinity Matrix several times to thoroughly resuspend beads.

3. Pipette the desired volume of slurry into column; suggested final settled bead volume is 0.5 to 1.0 ml.

4. Replace lower cap with the 23G needle.

5. Drain column storage buffer into the collection tube.
   Do not allow column to dry out. Adjust column flow rate to approximately 0.3 to 0.5 ml/minute. If flow rate is faster, adjust with a smaller gauge needle.

6. Immediately add a 10-fold bead volume of Equilibration buffer, for example 1 ml for a 1 ml column.
   – Allow buffer to drip through column; prepare the protein extract to be loaded during this step.

Loading preparation

See section, **Working Solution** *for additional information on preparing solutions.*

1. Determine amount of crude protein extract to be purified.
   – Optimal starting volume is 1 to 4 ml for a 1 ml column.
   – Crude extract may be diluted in Equilibration buffer with protease inhibitors.
   – Amount of total protein is typically 1 to 5 mg. Optimal total protein concentration depends on expression levels of tagged protein.
   Column may become ineffective when excessive total protein is loaded, or insoluble materials have not been adequately removed from the extract. Total protein loaded may be increased gradually with successive column runs if desired. Use a column flow rate of 0.1 ml/min or greater.

2. Load the protein extract on the column after equilibration.

3. Collect the protein extract flow through in a clean collection tube.
   – Save this fraction at +2 to +8°C.

Column elution

See section, **Working Solution** *for additional information on preparing solutions.*

1. Wash column with a minimum of 20 bead volumes of Wash buffer at +15 to +25°C to remove nonspecifically bound protein.
   Perform an OD_{280} reading at the end of this step to verify that the final wash fractions contain no protein and are close to baseline levels (Wash buffer alone). Save wash fractions if desired.

2. Immediately replace needle with lower cap and add 1 bead volume of Elution buffer to the column.
   – Incubate for 15 minutes at +37°C.
   Elution may be performed at lower temperatures, leading to lower yields of purified protein result.

3. Replace needle and collect the elution fraction in a clean microfuge or equivalent collection tube.

4. Repeat Steps 2 and 3, two more times.

5. Read OD_{280} for each fraction and pool as desired.

6. Store samples at +2 to +8°C until analysis.

Column reuse, regeneration, and storage

See section, **Working Solution** *for additional information on preparing solutions.*
When peptide elution is performed, strip the column between each use to remove HA peptide bound to the column.

1. Strip column by running 20 bead volumes of Regeneration buffer through the column.

2. Immediately equilibrate with 20 bead volumes of Equilibration buffer.

3. Store tightly capped at +2 to +8°C in 2 bead volumes of Column storage buffer.

Yield

Yield of purified protein is dependent upon expression levels within crude extract.

- Typical results from a 1 ml column range from 2 to 8 nmol of purified protein.
- Using a whole-cell bacterial extract, which expressed HA-tagged bacterial alkaline phosphatase, the column capacity was determined to be approximately 9 nmol protein/ml resin. Quantity of tagged-protein increased when additional total protein was loaded, however, a decrease in the purity of the elution was also noted, as evidenced by additional protein bands on a silver-stained gel.

Reuse

Poured columns containing the affinity matrix may be used at least 10 times.

- Column purification using the suggested procedures with peptide elution followed by glycine stripping, was performed ten times with a bacterial extract containing HA-tagged bacterial alkaline phosphatase. No loss in column activity was observed.
- Activity of the column for more than ten uses should be determined by the user and may be dependent upon the tagged protein or cell extract.

Western blotting

See section, **Working Solution** *for additional information on preparing solutions.*

1. Perform gel electrophoresis according to standard protocols.
   – Wet PVDF membrane in 100% methanol and equilibrate the membrane in Transfer buffer.
   – Perform western transfer onto the PVDF membrane.

2. After transfer, block the membrane for 1 hour at +15 to +25°C in Blocking buffer with gentle shaking.
   10 ml of Blocking buffer covers a 10 cm × 10 cm PVDF membrane.

3. Combine 10 ml Blocking buffer with 10 ml TBS solution.

4. Prepare working solution of Anti-HA-Peroxidase (final antibody concentration, 10 to 50 mU/ml) by diluting Anti-HA-Peroxidase stock solution with the solution prepared in Step 3.

5. Incubate the blocked membrane with Anti-HA-Peroxidase working solution for 30 minutes at +15 to +25°C with gentle shaking.

6. Wash the membrane five times, 5 minutes per wash with TBST solution.

7. Prepare Lumi-Light Western Blotting Substrate (POD)* according to the Instructions for Use.
   – Incubate membrane in this solution for 5 minutes.

8. Drain excess detection solution from membrane and wrap in plastic wrap.
   – Expose the membrane to X-ray film or Lumi-Film* for 60 seconds according to the method provided with the substrate.
   Substrate development and X-ray film exposure conditions may vary for each experiment.

2.3. Parameters

Affinity/Binding Capacity

Anti-HA High Affinity clone 3F10 and Anti-HA clone 12CA5 are recognizing the same epitope. Higher specific affinity of clone 3F10 compared to other anti-HA antibodies is due to reduced cross-reactivity.

Specificity

Anti-HA 3F10 recognizes the 9-amino acid sequence YPYDVPDYA, derived from the human influenza hemagglutinin (HA) protein. The antibody recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins by epitope tagging.

3. Results

Western blots after immunoprecipitation

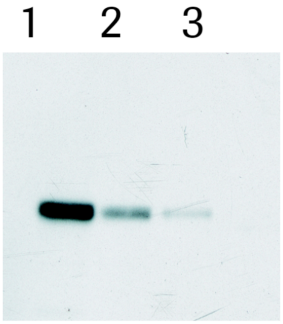

Fig. 1: Western blot following immunoprecipitation of a rat cell lysate (approximately 1 mg/ml total protein) expressing low levels of stable transfected HA:protein phosphatase 2A (38 kD).
Lane 1: Lysate diluted 1:2 before IP
Lane 2: Lysate diluted 1:8 before IP
Lane 3: Lysate diluted 1:32 before IP

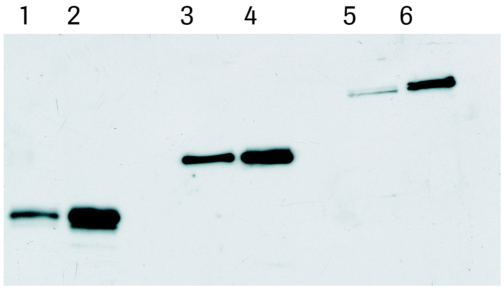

Fig. 2: Western blot demonstrating immunoprecipitation of HA-tagged protein from bacterial, mammalian, and yeast cell lysates. Blots were probed with Anti-HA-Peroxidase, High Affinity.
Lane 1: Crude bacterial lysate containing expressed HA:GFP (30 kD)
Lane 2: Immunoprecipitated HA:GFP
Lane 3: Crude mammalian lysate containing expressed HA:BAP (50 kD)
Lane 4: Immunoprecipitated HA:BAP
Lane 5: Crude yeast lysate containing expressed HA:cdc53p (100 kD)
Lane 6: Immunoprecipitated HA:cdc53p

Western blots of HA-tagged proteins after purification

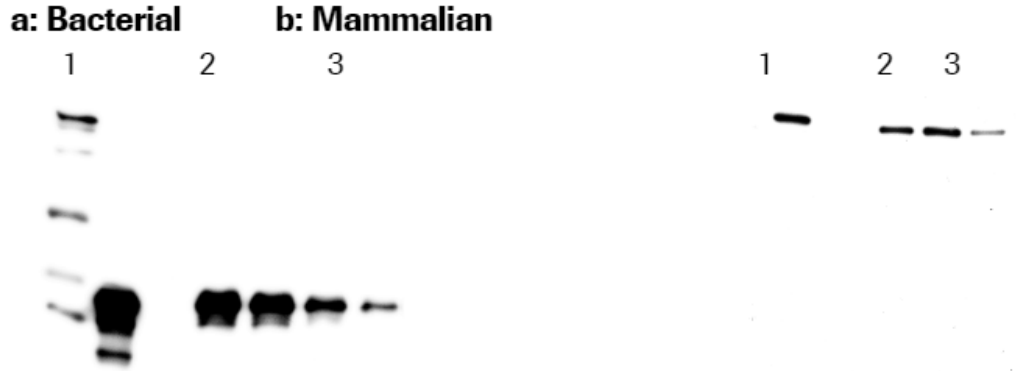

Fig. 3: Western blots of HA-tagged proteins purified on 0.5 ml Anti-HA Affinity Matrix columns, showing purified product from three successive peptide elutions (blots were probed with Anti-HA-Peroxidase, High Affinity).
3a: HA:GFP purified from 1.1 mg of total bacterial extract.
3b: HA:BAP bacterial alkaline phosphatase purified from 1 mg of mammalian extract from transiently transfected COS-1 cells.
Lane 1: Multi-Taq-Marker
Lane 2: Crude extract
Lane 3: Column flow through

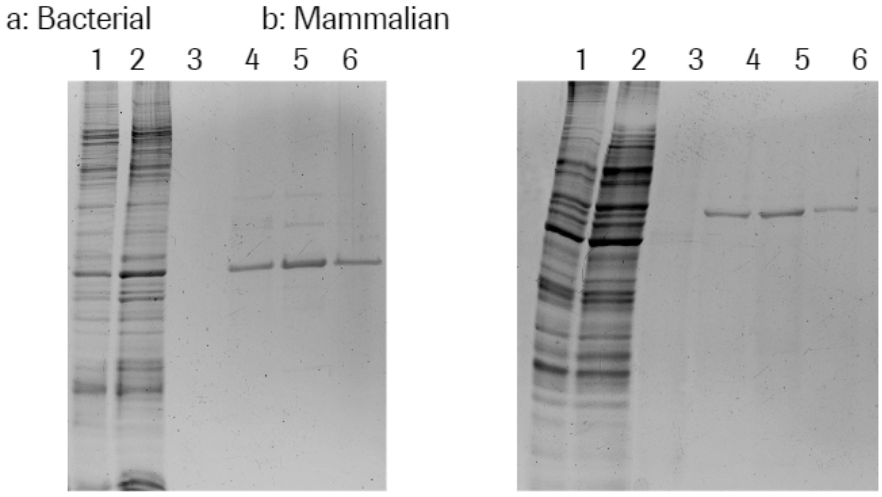

Fig. 4: Silver-stained gels showing HA-tagged proteins purified on 0.5 ml Anti-HA Affinity Matrix columns depicting purified product from three successive peptide elutions.
4a: HA:BAP purified from 0.75 mg of total protein.
4b: HA:BAP purified from 2.3 mg of mammalian extract from transiently transfected COS-1 cells.
Lane 1: Crude extract
Lane 2: Column flow through (unbound proteins)
Lane 3: Last wash fraction
Lanes 4 to 6: Purified protein (elutions 1 to 3)

4. Troubleshooting

Observation	Possible cause	Recommendation
Little or no HA-tagged protein is eluted.	Tagged protein is degraded.	Include protease inhibitors and perform purification at +2 to +8°C.
	Tagged protein not fully eluted.	If working at less than +37°C, increase temperature, time, and/or number of elutions.
		Try batch mixing of peptide solution with matrix.
	Tagged protein expression is absent.	Check for expression of protein in crude extract by western blot or biochemical assay.
	Tagged protein expression is very low.	Load larger volume of extract. Run column several times, pool, and concentrate final eluates.
Large quantities of tagged protein remain in the flow through sample.	Column is overloaded.	Decrease amount of loaded protein extract.
	Column not regenerated after use.	Regenerate column.
Column flow stops.	Column is overloaded.	Decrease amount of loaded protein extract.
	Starting extract contains insoluble materials.	Preclear starting extract by high-speed centrifugation or filtration.
	Air bubble in needle.	Replace needle or place gentle pressure on column by briefly covering top of column with gloved hand.
Tagged protein appears degraded; a smear or multiple lower molecular weight bands on western blotting.	Protease activity during procedure.	Increase protease inhibitors in protein extract sample.
		Perform all steps at lower temperatures.
Tagged protein appears with multiple bands on a silver-stained gel.	Column is overloaded.	Decrease amount of loaded protein extract.
	Wash step is insufficient.	Include detergent in Wash buffer.
Nonspecific reactivity on western blot, especially with high total protein loading.	Too much tagged protein loaded on gel.	Titer down total protein loaded in each lane. *The sensitive Anti-HA High Affinity antibody detects picogram quantities of tagged protein in western blots and less protein needs to be loaded than with many other antibodies.*
	Cross-reactivity from an anti-rat secondary antibody.	Use Anti-HA-Peroxidase, High Affinity* or Anti-HA-Biotin, High Affinity* for detection. If an anti-rat secondary antibody is chosen, check carefully for cross-reactivity from secondary antibody by running controls using secondary antibody alone.
Diminished reactivity on western blot.	Inappropriate detection system, such as use of an anti-mouse secondary antibody for detection.	Choose appropriate detection system.

5. Additional Information on this Product

5.1. Test Principle

Background information

Epitope tagging

Epitope tagging, the fusion of a short stretch of amino acids to a protein of interest by recombinant techniques, is a widely used method for the surveillance of the fusion protein with tag-specific monoclonal antibodies. The epitope tagging approach permits:

- the size, cellular localization, and abundance of proteins produced by newly discovered genes.
- post-translational modifications of proteins.
- the movement of proteins within cells.
- the identity of proteins within functional protein complexes.
- the function of proteins that are unstable, difficult to purify, or share epitopes with a number of other proteins, and
- eliminates the need to generate specific antibodies recognizing the protein of interest.

Anti-HA antibodies in epitope tagging

Among the different epitope tags described in the literature, the most commonly used tag is derived from the hemagglutinin of the influenza virus. Several antibodies have been described that react with this epitope tag, the most prominent of which is Anti-HA (Clone12CA5). However, these antibodies are restricted by requiring additional amino acids adjacent to the HA tag or by recognizing HA-tagged proteins with only moderate affinity, as demonstrated by cross-reacting bands that have been reported in certain western blot experiments.

Anti-HA, High Affinity

The Anti-HA, High Affinity antibody (clone 3F10) recognizes the same epitope as clone 12CA5. The high affinity and low working concentrations of this monoclonal result in less cross-reactivity than with other anti-HA antibodies. The 3F10 antibody has been demonstrated to work effectively in a variety of model systems and for a variety of applications, including western blotting, immunoprecipitation, and immunofluorescence. It has proven to be useful in combination with other tag and non-tag antibodies for co-localization of proteins and in studies of protein:protein interactions. The Anti-HA Affinity Matrix provides a simple and effective method for the immunoprecipitation of HA-tagged proteins from crude protein mixtures. It is also useful for the rapid immunoaffinity purification of tagged proteins under nondenaturing elution conditions with the HA peptide. The high affinity of the 3F10 antibody enables even rarely expressed tagged proteins to be identified, and the covalent linkage between the antibody and matrix beads results in a final immunoprecipitated or purified product which does not contain large quantities of antibody that may interfere with detection.

5.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Anti-HA-Peroxidase, High Affinity	25 U, 25 µg	12 013 819 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Lumi-Light Western Blotting Substrate	1 kit, 4,000 cm^2 membrane, 400 blots with 10 x 10 cm	12 015 200 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Tris	custom fill	10 153 265 103
HA Peptide	5 mg	11 666 975 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
1,4-Dithiothreitol (DTT)	custom fill	10 197 785 103
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
cOmplete	20 tablets in a glass vial, for 50 ml each	11 697 498 001
	3 x 20 tablets in glass vials, for 50 ml each	11 836 145 001
Anti-HA-Biotin, High Affinity	50 µg	12 158 167 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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