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

Genopure Plasmid Midi Kit

Version: 14
Content Version: March 2024

For the isolation of plasmid DNA from bacterial cultures

Cat. No. 03 143 414 001 1 kit
20 preparations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	colorless	Genopure Plasmid Midi Kit, Suspension Buffer	For suspension of bacterial cell pellets.	1 bottle, 100 ml
2	blue	Genopure Plasmid Midi Kit, RNase A	▪ Lyophilized ▪ To be dissolved in Suspension Buffer. ▪ For removal of RNA.	1 bottle, 12 mg
3	green	Genopure Plasmid Midi Kit, Lysis Buffer	▪ For bacterial cell lysis. ▪ Ready-to-use solution.	1 bottle, 100 ml
4	white	Genopure Plasmid Midi Kit, Neutralization Buffer	▪ To form a stable cellular debris precipitate. ▪ Ready-to-use solution.	1 bottle, 100 ml
5	red	Genopure Plasmid Midi Kit, Equilibration Buffer	▪ Equilibrates the columns prior to use. ▪ Ready-to-use solution.	1 bottle, 70 ml
6	blue	Genopure Plasmid Midi Kit, Wash Buffer	▪ Removes residual impurities. ▪ Ready-to-use solution.	2 bottles, 125 ml each
7	yellow	Genopure Plasmid Midi Kit, Elution Buffer	▪ For plasmid elution. ▪ Ready-to-use solution.	1 bottle, 125 ml
8	bag	NucleoBond AX 100 Columns	For isolation step.	1 bag, 20 columns
9	bag	Folded filters	▪ For removal of cellular debris. ▪ Eliminates a centrifugation step.	1 bag, 20 filters
10	bag	Sealing rings	Fixes the columns in test tubes.	1 bag, 10 rings

⚠ ***All solutions are clear.***

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped at ambient temperature.
When stored at +15 to +25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle / Bag	Cap	Label	Storage
1	colorless	Suspension Buffer	Store at +15 to +25°C. ⚠ ***Keep bottle tightly capped to ensure stability of the pH values.***
2	blue	RNase A	Store at +15 to +25°C.
3	green	Lysis Buffer	Store at +15 to +25°C. ⚠ ***Keep bottle tightly capped to ensure stability of the pH values.*** ⚠ ***SDS in Lysis Buffer may crystallize at temperatures <+20°C. If precipitates are present, warm in a +37°C water bath until dissolved. Mix well before use.***
4	white	Neutralization Buffer	Store at +15 to +25°C. ⚠ ***Keep bottle tightly capped to ensure stability of the pH values.*** ⚠ ***If precipitates are present, warm at ambient temperature (>+20°C) or in a +37°C water bath until dissolved. Mix well before use.***
5	red	Equilibration Buffer	Store at +15 to +25°C. ⚠ ***Keep bottles tightly capped to ensure stability of the pH values.***
6	blue	Wash Buffer	
7	yellow	Elution Buffer	
8	bag	NucleoBond AX 100 Columns	Store at +15 to +25°C. ⚠ ***Do not freeze columns or expose to extended heat for long periods of time.***
9	bag	Folded filters	Store at +15 to +25°C.
10	bag	Sealing rings	

1.3. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Centrifuge and tubes for harvesting bacterial cultures, capable of ≥15,000 × *g*
- Tubes for collecting and precipitating eluted plasmid DNA
- Funnel for clearing of lysates by folded filters

For Plasmid Isolations

- Isopropanol
- 70% ethanol
- TE buffer or other low-salt buffer
- Double-distilled water

1.4. Application

The Genopure Plasmid Midi Kit is used to prepare purified plasmid DNA in medium quantities (midi preps). Using a modified alkaline lysis method, highly purified plasmid DNA from *E. coli* (free of RNA contamination according to current quality control procedures) is generated. The kit is designed for the isolation of up to 100 µg plasmid DNA from bacterial culture.
Plasmid DNA isolated with this kit is suitable for all molecular biology applications, such as:

- Transfection
- PCR
- Restriction analysis
- Southern blotting
- Sequencing
- Cloning

1.5. Preparation Time

Assay Time

Hands-on time: approximately 10 minutes
Total time: 60 minutes, including a filtration step after alkaline lysis.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use 5 to 30 ml *E. coli* culture, transformed with a high-copy-number plasmid, or
- 10 to 100 ml *E. coli* culture, transformed with a low-copy-number plasmid.

⚠ ***Harvest cultures at a density between 2.0 and 6.0 A_{600} units per ml bacterial culture.***

General Considerations

Media

The isolation method is optimized for cultures grown in LB media. Other rich media may require increased volumes of Suspension Buffer, Lysis Buffer, and Neutralization Buffer, and an additional wash step.

Plasmid Size

The isolation procedure is suitable for all plasmid sizes. Lysates of larger constructs (up to 100 kb) should be cleared by filtration to avoid shearing.

Preparation of Overnight Culture

1. Transfer a single bacterial colony from a selective plate.

2. Inoculate a starter culture in 1 to 5 ml LB medium containing the proper antibiotic in an appropriately sized, loosely capped tube.

3. Incubate the culture at +37°C for 12 to 16 hours with vigorous shaking (220 to 250 rpm).

4. Inoculate 30 to 100 ml selective LB medium with 1 ml of the overnight starter culture.

5. Incubate at +37°C for 12 to 16 hours with vigorous shaking (220 to 250 rpm).
   – Typically, the cell density is approximately 2 to 6 A_{600} units.

⚠ ***Higher culture volumes can cause overloading of the column resulting in lower yields due to inefficient cell lysis and clogging of the column.***

Number of Tests

The kit is for 20 plasmid midi preparations. Up to eight samples can be processed at a time.

⚠ ***The stated number of isolations is valid for the isolation of high-copy-number plasmids.***

When isolating P1 constructs or other low-copy plasmids using alkaline lysis protocols, incomplete bacterial lysis is the number one problem resulting in low yield. To improve alkaline lysis when isolating low-copy-number plasmids, use the Genopure Buffer Set for Low Copy Number Plasmids* as a supplement to the Genopure Plasmid Kits.

Safety Information

Precautions

Lysis Buffer (Bottle 3) is irritating to eyes and skin. Use the following precautions when handling the buffer:
- Keep away from food, drink, and animal feed.
- If contact with eyes, rinse immediately with plenty of water and seek medical attention.
- Wear suitable protective clothing.
- If swallowed, immediately seek medical attention. Provide bottle with label to medical personnel.

Working Solution

Suspension Buffer/RNase A

Prepare the working solution according to the following steps.

1. Pipette 1 ml of Suspension Buffer (Bottle 1) into the RNase A (Bottle 2).

2. Insert the rubber stopper into Bottle 2 and invert until all lyophilizate including any that sticks to the rubber stopper is dissolved.

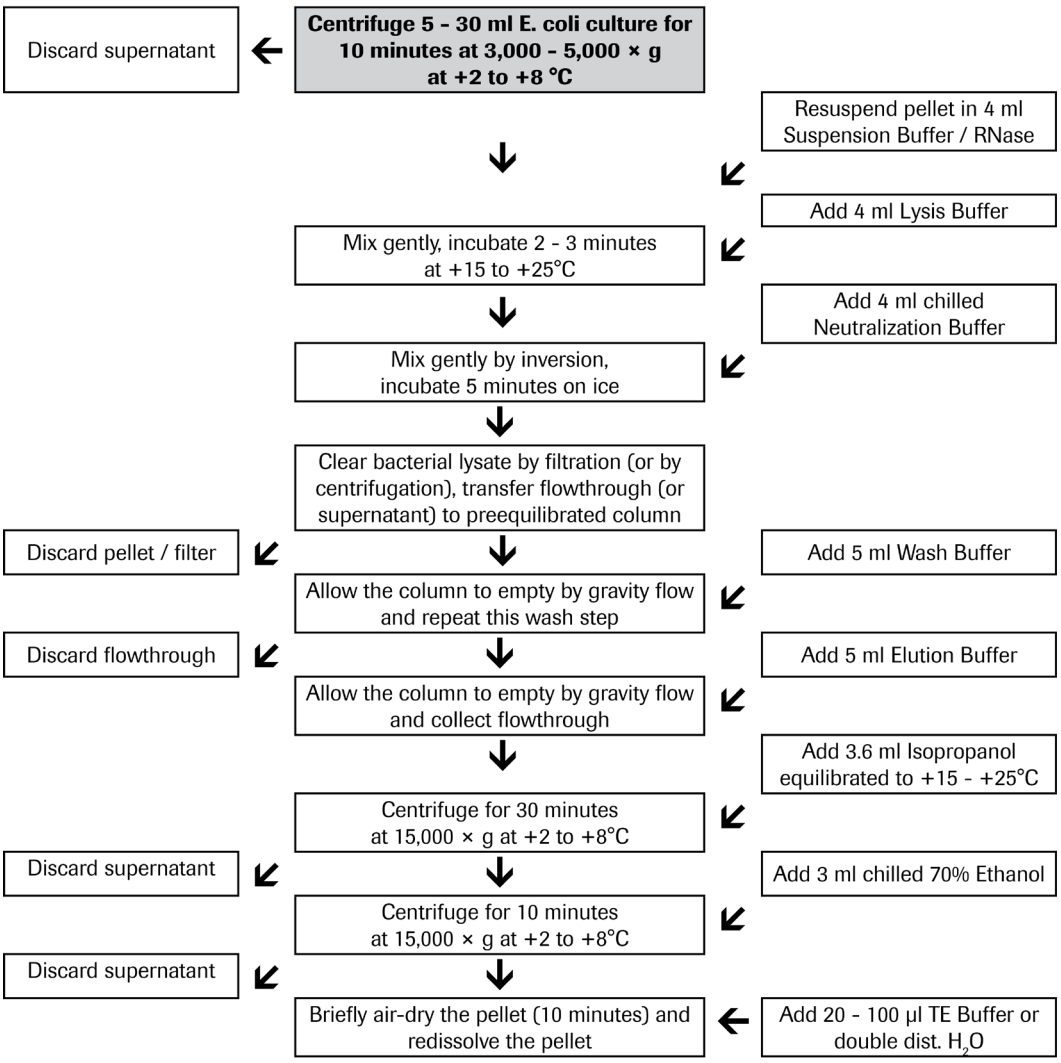
3. Transfer the dissolved enzyme back into the Suspension Buffer (Bottle 1).
   – This is sufficient working solution for 60 Midi preps (isolation of up to 100 µg plasmid DNA/preparation).

   When preparing aliquots of the working solution, the final concentration of RNase A in the working solution must be 100 µg/ml.

4. Store reconstituted buffer for 6 months at +2 to +8°C.

2.2. Protocols

Experimental Overview



High-Copy-Number Plasmids

1. Centrifuge bacterial cells from 5 to 30 ml *E. coli* culture grown in LB medium for 5 to 10 minutes at 3,000 to 5,000 × *g* at +2 to +8°C.
   – Discard the supernatant.
   – Carefully resuspend the pellet in 4 ml Suspension Buffer plus RNase and mix well.

2. Add 4 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times.
   – Incubate 2 to 3 minutes at +15 to +25°C.

   ⚠ ***To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 minutes.***

3. Add 4 ml chilled Neutralization Buffer to the suspension.
   – Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogeneous suspension is formed.
   – Incubate the tube 5 minutes on ice.

   i *The solution becomes cloudy and a flocculent precipitate forms.*

4. Clear the lysate by either centrifugation or by filtration according to the following table.

Centrifugation	Filtration
Centrifuge at high speed for >30 minutes at >12,000 × *g* at +2 to +8°C.	Place a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
Immediately and carefully, remove the supernatant from the white precipitate and proceed to Step 5.	Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
	Load the lysate onto the wet, folded filter and collect the flow through.

   ⚠ ***The SDS precipitates with cellular debris when Neutralization Buffer is added. This white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after Step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.***

5. Mount the sealing ring to the column (Fig. 1) to fix the column in the collection tube.
   – Insert one column into one collection tube.
   – Equilibrate the column with 2.5 ml Equilibration Buffer.
   – Allow the column to empty by gravity flow.
   – Discard the flow through.

6. Load the cleared lysate from Step 4 onto the equilibrated column.
   – Allow the column to empty by gravity flow.
   – Discard the flow through.

7. Wash the column with 5 ml Wash Buffer.
   – Allow the column to empty by gravity flow.
   – Discard the flow through.

8. Repeat Step 7.
   – Discard flow through and collection tube.

9. Insert the column into a new collection tube capable of withstanding high-speed centrifugation (≥15,000 × *g*).
   – Elute the plasmid with 5 ml prewarmed Elution Buffer (+50°C).
   – Allow the column to empty by gravity flow.
   – The collected flow through contains the plasmid.

10 Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C).
– Centrifuge immediately for 30 minutes at ≥15,000 × *g* at +2 to+ 8°C.
– Carefully discard the supernatant.

11 Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol.
– Centrifuge for 10 minutes at >15,000 × *g* at +2 to +8°C.
– Carefully remove ethanol from the tube using a pipette tip.
– Air dry the plasmid DNA pellet for 10 minutes.

12 Carefully dissolve the plasmid DNA pellet in 20 to 100 µl TE buffer or sterile double-distilled water.

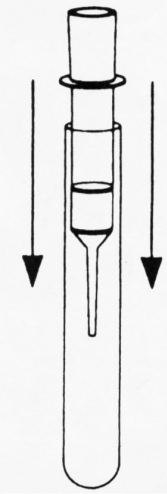


Fig. 1: Mounting the sealing ring to the column.

Sample Storage

If you plan to continue with your experiments, use the purified plasmid DNA immediately. If you want to analyze plasmid DNA at another time, store DNA at +2 to +8°C or −15 to −25°C.

Low-Copy-Number Plasmids

1 Centrifuge bacterial cells from 10 to 100 ml *E. coli* culture grown in LB medium for 5 to 10 minutes at 3,000 to 5,000 × *g* at +2 to +8°C.
– Discard the supernatant.
– Carefully resuspend the pellet in 8 ml Suspension Buffer plus RNase and mix well.

2 Add 8 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times.
– Incubate 2 to 3 minutes at +15 to +25°C.

⚠ ***To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 minutes.***

3 Add 8 ml chilled Neutralization Buffer to the suspension.
– Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogeneous suspension is formed.
– Incubate the tube 5 minutes on ice.

ⓘ *The solution becomes cloudy and a flocculent precipitate forms.*

4 Clear the lysate by either centrifugation or by filtration according to the following table.

Centrifugation	Filtration
Centrifuge at high speed for >30 minutes at >12,000 × *g* at +2 to +8°C.	Place a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
Immediately and carefully, remove the supernatant from the white precipitate and proceed to Step 5.	Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
	Load the lysate onto the wet, folded filter and collect the flow through.

⚠ ***The SDS precipitates with cellular debris when Neutralization Buffer is added. This white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after Step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.***

5 Mount the sealing ring to the column (Fig. 2) to fix the column in the collection tube.
- Insert one column into one collection tube.
- Equilibrate the column with 2.5 ml Equilibration Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow through.

6 Load the cleared lysate from Step 4 onto the equilibrated column.
- Allow the column to empty by gravity flow.
- Collect the flow through and load it a second time onto the column.
- Allow the column to empty by gravity flow.
- Discard the flow through.

7 Wash the column with 4 ml Wash Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow through.

8 Repeat Step 7.

9 Repeat Step 7.
- Discard flow through and collection tube.

10 Insert the column into a new collection tube capable of withstanding high-speed centrifugation (≥15,000 × *g*).
- Elute the plasmid with 2.5 ml prewarmed Elution Buffer (+50°C).
- Allow the column to empty by gravity flow.
- Collect the flow through.

11 Elute a second time with 2.5 ml prewarmed Elution Buffer (+50°C) and combine eluates.
- Allow the column to empty by gravity flow.
- The collected flow through contains the plasmid DNA.

12 Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C).
- Centrifuge immediately for 30 minutes at ≥15,000 × *g* at +2 to +8°C.
- Carefully discard the supernatant.

13 Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol.
- Centrifuge for 10 minutes at >15,000 × *g* at +2 to +8°C.
- Carefully remove ethanol from the tube using a pipette tip.
- Air dry the plasmid DNA pellet for 10 minutes.

14 Carefully dissolve the plasmid DNA pellet in 20 to 50 µl TE buffer or sterile double-distilled water.

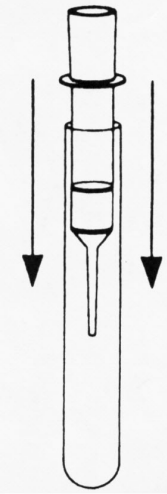


Fig. 2: Mounting the sealing ring to the column.

Sample Storage

If you plan to continue with your experiments, use the purified plasmid DNA immediately. If you want to analyze plasmid DNA at a later time, store DNA at +2 to +8°C or −15 to −25°C.

2.3. Parameters

Purity

Plasmid DNA is free of all other bacterial components, including RNA, according to the current quality control procedures (see section, **Quality Control**).

3. Results

Yield

The yield of plasmid DNA preparations is comparable to traditional purification methods. The yield also depends on several parameters: the *E. coli* strain, density of cell culture, quality of the bacterial culture growth, amount of culture suspension used for the preparation, type of plasmid used, etc.

- Typical yield of a high-copy-number plasmids is approximately 3 to 5 µg of DNA per ml of original bacterial culture (pUC, pTZ, pGEM in common host strains such as XL-1 blue, HB101, JM 109).
- Typical yield of low-copy-number plasmids is approximately 0.2 to 1 µg of DNA per ml of original bacterial culture.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity.	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		After adding RNase to Suspension Buffer, store aliquots of the working solution at +2 to +8°C.
		Close all reagent bottles tightly after each use to ensure stability, correct pH, and freedom from contamination.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
		Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.
Low recovery of nucleic acids after elution.	Wrong reagent used for elution. *Salt is required for optimal elution.*	Use the Elution Buffer from the kit.
Low plasmid yield.	Too few cells in starting material.	Grow *E. coli* to an absorbance (A_{600}) of 2 to 6 before harvest.
	Incomplete cell lysis.	Be sure the *E. coli* pellet is completely resuspended in Suspension Buffer.
		Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer).
		Make sure a cloudy white precipitate forms when Neutralization Buffer is added to the lysate.
	Lysate did not bind completely to column.	Pre-equilibrate the column by adding Equilibration Buffer before adding sample.
RNA present in final product.	RNase not completely dissolved.	To reconstitute the lyophilized RNase completely, see section, **Working Solution, Suspension Buffer/RNase A**.
	Too many cells in starting material.	Do not overload the column.
Genomic DNA present in final product.	Genomic DNA sheared during lysis step.	Do not vortex the preparation after adding Lysis Buffer.
Additional band running slightly faster than supercoiled plasmid is seen on gels.	Denatured plasmid in final product.	Reduce the incubation time during Step 2 (lysis step) of the protocol.

5. Additional Information on this Product

5.1. Test Principle

How this Product Works

The isolation procedure is based on a modified alkaline lysis protocol. Bacteria are partially lysed, allowing the plasmid DNA to escape the cell wall into the supernatant. The larger *E. coli* chromosomal DNA is trapped in the cell wall. The lysate is cleared of cellular debris and the plasmid DNA-containing fraction is added to the column. The bound plasmid DNA is washed to remove contaminating bacterial components. The plasmid DNA is eluted and precipitated to remove salt and to concentrate the eluate. This is a commonly used method that generates highly purified plasmid DNA free of RNA contamination according to current quality control procedures.

An overview of the steps is shown below:

1. Cell harvest and disruption.
2. Precipitation of chromosomal DNA.
3. Clarification of bacterial lysate.
4. Adsorption of the plasmid DNA to the matrix.
5. Wash to remove residual impurities.
6. Elute plasmid DNA with high-salt buffer.
7. Concentration and salt removal by alcohol precipitation.

Joint Cooperation

The column matrix developed by Macherey-Nagel GmbH and Co. KG, and the proven optimal buffer composition of Roche Diagnostics, have been combined to provide you with a state-of-the-art plasmid isolation product.

5.2. Quality Control

- Plasmid DNA purified using this kit has been tested for restriction digestion. pUC 19 was isolated from transformed HB101 as described in the protocol. 1 µg of plasmid was completely digested with 1 U *Msp* I for 2 hours at +37°C as shown by agarose gel analysis.
- Plasmid recovery was tested with 50 µg purified plasmid. The recovery was >90% with more than 80% in supercoiled form.
- The yield of plasmid DNA was determined by isolating pBS from DH5α cells. From 30 ml culture volume with a density of A_{600}, between 3 and 6 was obtained >85 µg plasmid DNA.
- The purity checked by the ratio of A_{260}/A_{280} is 1.8 ± 0.2.
- RNA contamination was analyzed using 3 µg pBS purified with the standard procedure, and checked by electrophoresis on an agarose gel. No RNA was detected.
- Kit components have been tested for the absence of nucleases, according to current Quality Control procedures.

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

Removed information related to the REACH Annex XIV.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
RNase, DNase-free	500 µg, 1 mL	11 119 915 001
Genopure Plasmid Maxi Kit	1 kit, 10 preparations	03 143 422 001
Genopure Buffer Set for Low-Copy Number Plasmids	1 set, buffer for 20 maxi preps or 60 midi preps	04 634 772 001
RNase A	25 mg	10 109 142 001
	100 mg	10 109 169 001

6.4. Trademarks

GENOPURE is a trademark of Roche.
All other 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:
Product Disclaimers.

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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