SUPELCO®
Solutions within.™

HybridSPE®-Phospholipid Technology

Effectively remove phospholipids and proteins for accurate and reproducible results

NEW – HybridSPE-PLus

LC-MS Workflow Solutions

Plates, Cartridges and Accessories

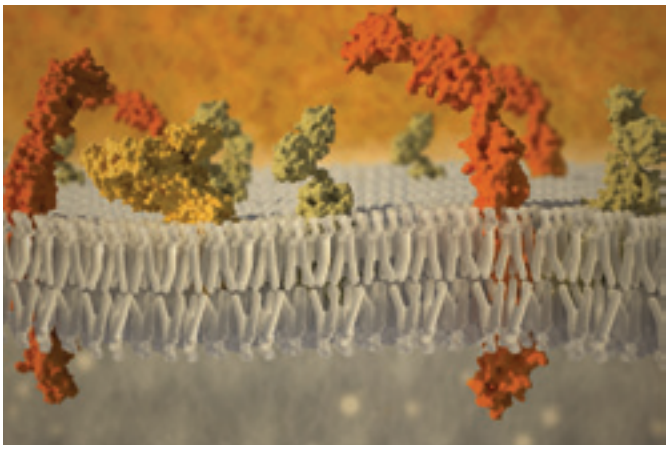
HybridSPE-Phospholipid Technology

Key Features and Benefits

- More accurate results
  - Unique chemistry that can effectively separate hydrophobic analytes (such as vitamins) from phospholipids, unlike competitive product chemistries
  - Elimination of proteins and phospholipid-induced ion suppression
- Simultaneous removal of proteins and phospholipids
  - Simple, standardized methodology, analogous to traditional protein precipitation
  - Alternative to complex traditional SPE method development
- Reproducible, consistent performance reduces need for reprocessing
- Less instrument downtime and longer column life
- Decreased run times by eliminating the need for gradients to clean columns between samples
- High throughput processing that is automatable and compatible with most common robotic systems
- Ready-to-use, no preconditioning required

Phospholipids: A Concern for LC-MS Analysis of Small Molecules in Biological Matrices

Phospholipids are present as a major component of all cell membranes. They are therefore present in all biological sample matrices including serum, plasma and whole blood and can be a problem in LC-MS analysis of small molecules because they often co-elute and ionize along with the analytes of interest. This co-elution results in ion suppression (an erroneous decrease) of the mass spec signal that can cause variability and impact LC-MS result accuracy (**Figure 1**).



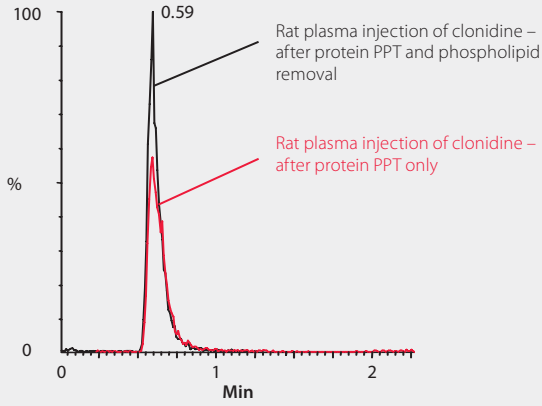
Importance of Accurate Results and Fast Answers in Bioanalysis

At Supelco, we understand that the results of your analyses can have a significant impact on the lives of many others. This puts pressure on you to ensure the data you produce is as accurate as possible. Not only do you need quick answers, you need answers you can trust. The complexity of the types of samples with which you work does not make your job any easier. Proteins and phospholipids inherently present, to varying degrees in your samples, can add variability to your analytical results when using sensitive techniques such as LC-MS or LC-MS/MS. At Supelco, we have over four decades of chromatography expertise to help you navigate the complexity of your sample prep options.

Limitations of Traditional Biological Sample Cleanup Methods

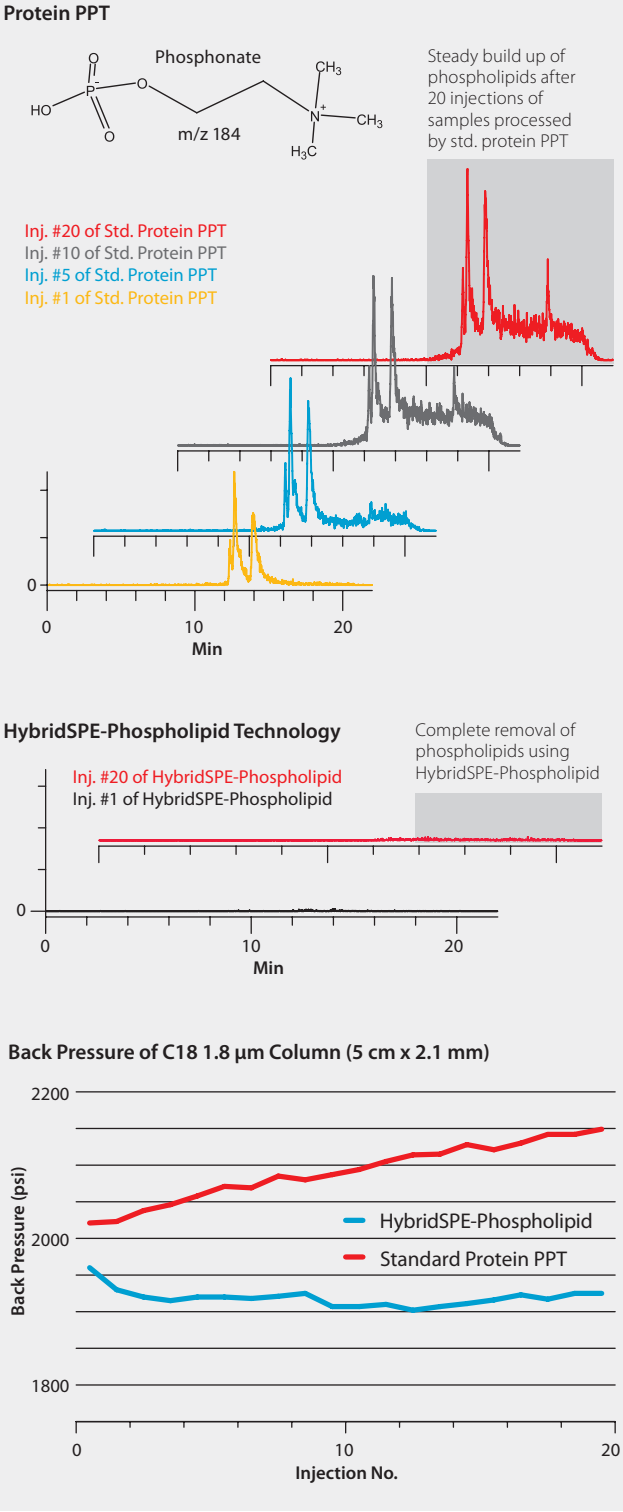
Most clinical and biological researchers use traditional methods such as protein precipitation and liquid-liquid extraction to cleanup their samples prior to analysis. While these techniques allow for inexpensive and quick removal of proteins, they completely fail to address the problem of phospholipid-induced ion suppression.

Figure 1. Phospholipid Effect on Ionization of Clonidine



Even if phospholipids do not co-elute with the analyte of interest, they can accumulate on your analytical column and elute from the column sporadically in downstream analyses. This can cause unpredictable ion suppression and poor reproducibility, thereby putting the accuracy of your results at risk (**Figure 2**).

Figure 2. Gradient RP LC-MS of Blank Plasma Samples Prepared by Standard Protein PPT vs. HybridSPE-Phospholipid



Phospholipid Removal Techniques

To overcome the problem of phospholipid-induced ion suppression, some analysts try traditional SPE. Traditional SPE often requires time-consuming and complex method development, but still only removes nominal amounts of phospholipids. Remaining phospholipids can impact the accuracy of your results, accumulate on your analytical column to impact future analyses, add to column replacement costs and increase instrument downtime.

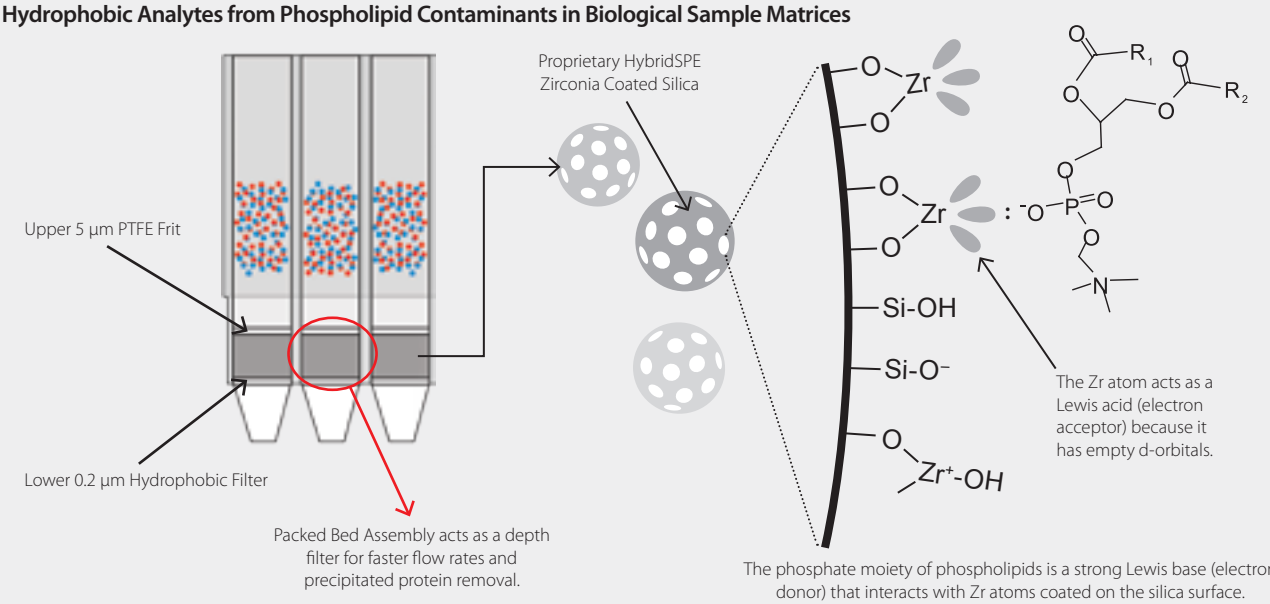
A variety of products designed specifically for the removal of both proteins and phospholipids are now commercially available, including HybridSPE plates and cartridges. Most of these products are simple, fast and easy-to-use, offering fairly standardized methods with minimal method development. Most, however, use a hydrophobic retention mechanism to separate phospholipids from analytes of interest in the sample. This poses a problem if the analytes of interest are also hydrophobic because they will be retained and removed along with the hydrophobic phospholipids. This results in decreased analyte recovery and inaccurate results. HybridSPE-Phospholipid Technology is different in that it completely removes both proteins and phospholipids from the sample without retaining other hydrophobic compounds.

How Is HybridSPE-Phospholipid Technology Different Than Other Phospholipid Removal Products?

The first of its kind, HybridSPE-Phospholipid technology was introduced in 2008. It fuses the simple, standardized methodology of traditional protein precipitation with the specificity of solid phase extraction (SPE) for the simultaneous removal of proteins and phospholipids from biological samples prior to LC-MS analysis. Unlike other phospholipid removal products that use a hydrophobic retention mechanism to remove phospholipids from biological samples, HybridSPE-Phospholipid technology uses a unique retention mechanism (**Figure 3**). This allows it to separate phospholipids from even very hydrophobic analytes, such as vitamins which are often retained along with phospholipids on competitive products.

To learn more about HybridSPE-Phospholipid technology and view a video of the product in action, visit
sigma-aldrich.com/hybridspe-pl

Figure 3. Unique Retention Mechanism of HybridSPE®-Phospholipid Technology Allows for Separation of Even Very Hydrophobic Analytes from Phospholipid Contaminants in Biological Sample Matrices



A Newer, Better "Go-to" Sample Prep Method for Phospholipid Removal

Labs working with biological samples often choose to perform protein precipitation prior to LC-MS analysis, using it as their "go-to" sample prep method. They view phospholipid removal as more costly, more time-consuming and unnecessary if the specific analytes of interest do not co-elute with the phospholipids in their sample. This is not the case.

Labs can actually reduce overall costs and increase overall throughput while generating more accurate data by using HybridSPE-Phospholipid technology to remove both proteins and phospholipids from all biological samples prior to small molecule LC-MS analysis.

Simple and Fast Phospholipid Removal

The 96-well plate protocol involves just a few simple steps (**Figures 4 and 5**), and plates can be used right out of the package with no pre-conditioning step required. The sample (containing internal standards if desired) and a precipitation solvent are first added to the well plate, followed by a mixing step and vacuum application to collect the sample. Collected samples are then ready to be analyzed.

Figure 4. Depiction of Basic HybridSPE-Phospholipid Sample Prep Workflow

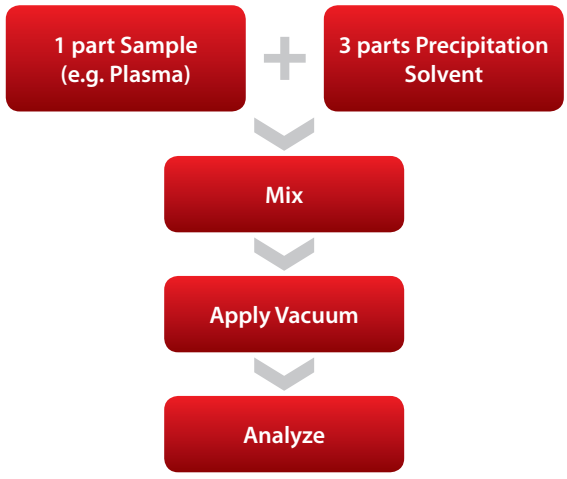
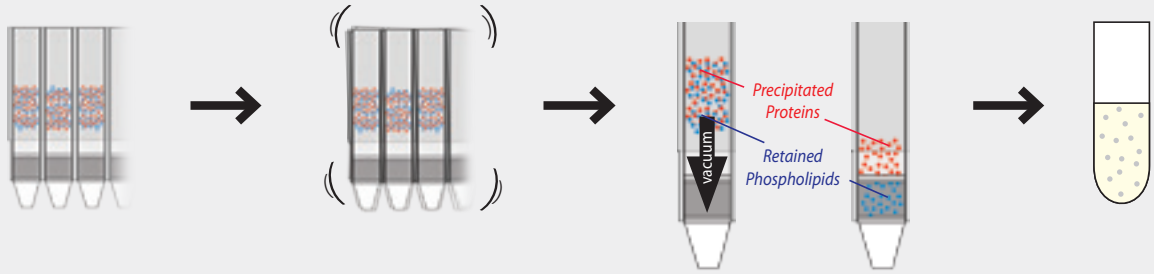


Figure 5. HybridSPE 96-well Plate Protocol

Featuring an "In-well" Precipitation Procedure for both proteins and phospholipids

1. Add Sample
   Pipette 100 µL plasma or serum to the HybridSPE plate followed by 300 µL precipitation solvent. Add internal standards as necessary.
2. Mix
   by vortexing/shaking HybridSPE plate or by aspirating/dispensing with 0.5–1 mL pipette tip.
3. Apply vacuum.
   The packed-bed filter/frit assembly acts as a depth filter for the concurrent physical removal of precipitated proteins and chemical removal of phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.
4. Collect Sample
   Resulting filtrate/eluate is free of proteins and phospholipids and ready for immediate LC-MS/MS analysis.



Produce More Accurate Data, Save Time and Reduce Overall Costs

With advances in LC-MS technology, many analysts seek to decrease LC run times by incorporating ballistic HPLC gradients and columns with sub-2 µm particles. Ballistic gradients are often inadequate at purging the column of the phospholipids that remain after standard protein precipitation techniques and sub-2 µm HPLC columns are more prone to clogging than larger particle size columns. In addition, because contaminating phospholipids are often very strongly retained on the analytical column, they can take more than 10 minutes to elute. When using short run times, phospholipids are more likely to accumulate on the column unless the analyst takes the time to allow the phospholipids to elute before beginning the next injection. This can dramatically decrease laboratory throughput (Figures 6 and 7).

Figure 6. Phospholipid Contamination from Standard Protein PPT Requires Increased Gradient Run Time (>10 min.)

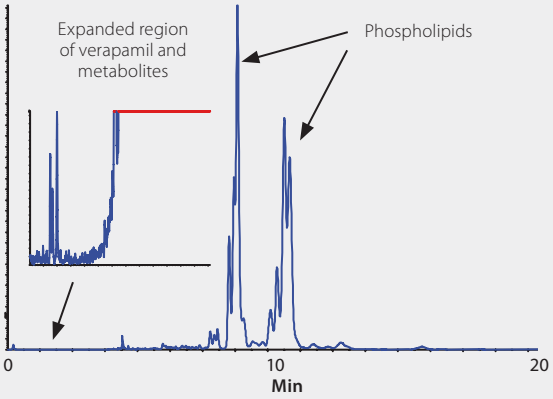
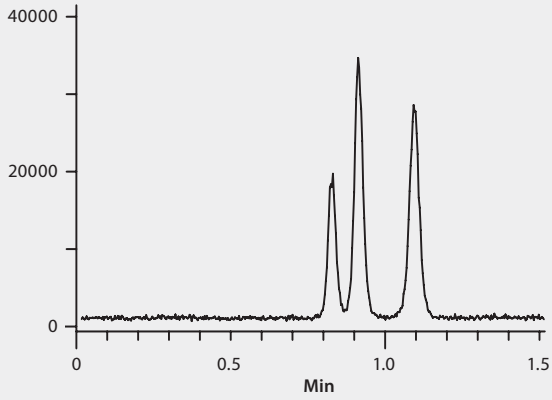


Figure 7. Less than 90 Second Run Time Achieved Using HybridSPE-Phospholipid and Ascentis® Express C18 (isocratic) for Verapamil and Metabolites in Rat Plasma



By removing phospholipids as part of the standard sample prep process, analysts can avoid issues with phospholipids building up on the analytical column, eluting unexpectedly and causing unpredictable ion suppression and poor reproducibility in later runs. They can also reduce the frequency of column replacement, thereby reducing consumable costs and avoiding downtime associated with column replacement. Finally, phospholipid removal can help analysts to achieve the increased throughput they are seeking to get answers as quickly as possible.

To learn more about HybridSPE-Phospholipid technology and view a video of the product in action, visit
sigma-aldrich.com/hybridspe-pl

Great Gets Better – Introducing, HybridSPE®-PLus, the Next Generation in HybridSPE-Phospholipid Technology

Figure 8. NEW – HybridSPE-PLus 96-well Plate

Our new HybridSPE-PLus plates offer the same time-proven chemistry of our traditional HybridSPE-Phospholipid plates coupled with plate manufacturing enhancements that stem from years of production experience. These manufacturing enhancements have allowed us to increase well-to-well flow consistency and reduce sample hold-up volumes, further improving analyte recoveries and assay reproducibility. We will continue to offer and support our traditional square-well HybridSPE-Phospholipid plates for those customers who have developed methods on them, but we invite those customers developing new methods to take advantage of the newest technology: HybridSPE-PLus.

All Your LC-MS Needs In One Place

In addition to our HybridSPE-Phospholipid technology, we provide the following premier selection of proven tools and consumables for your entire sample prep and LC-MS workflows.

- **Ascentis® Express HPLC/UHPLC Columns** improve throughput and sensitivity, allowing you to process more samples
- **Cerilliant® Certified Spiking Solutions® and Certified Reference Materials** manufactured and tested specifically for use as reference standards for laboratories performing bioanalysis, therapeutic drug monitoring, diagnostic and toxicology testing
- **Biocompatible SPME fibers and probes** for LC analysis of difficult or precious samples in biological matrices
- **Supel™-Select SPE** cartridges and well-plates for sample prep needs
- **ASTEC® CHIROBIOTIC® CSPs** for enantiomer separations under RP and LC-MS conditions
- **Low adsorption vials** for LC-MS applications

From Sample Prep to LC-MS Analysis…

The perfect complement of speed, selectivity and sensitivity for pharma bioanalysis

- Increase sample prep and LC-MS speed
- Decrease sample prep method development time
- Increase sensitivity by reducing ion-suppression and increasing LC efficiency

HybridSPE-Phospholipid Technology

- Simple 2–3 step protocol
- Reduce ion-suppression through phospholipid and protein removal
- Minimal to no method development
- Available in 96-well plates and 1 mL cartridges

Ascentis® Express HPLC with Fused-Core® Technology

- Half the back pressure of sub-2 µm particles
- Twice the efficiency of 3 µm particles
- Increased column ruggedness
- Available in six phases for small molecules and peptides

Ordering Information

Standard Sample Volume (100–300 µL) Phospholipid Removal Plates and Cartridges:

HybridSPE-Phospholipid technology is available in both a 96-well plate format (**Figure 8**) and a cartridge format (**Figure 9**). 96-well plates are sold individually and in 20 plate packs. Cartridges are sold in various pack sizes, depending on cartridge size (see ordering information).

Figure 9. HybridSPE-Phospholipid ULTRA Cartridge

Like the 96-well plate, our HybridSPE-Phospholipid Ultra cartridge is capable of removing both proteins and phospholipids in an online format. If an offline protein precipitation is desired, the 1 mL or 6 mL HybridSPE-Phospholipid cartridges, which do not contain a protein precipitation filter, can be used for phospholipid removal following a separate offline protein precipitation (using a product such as Cat. No. 55263-U, 96-well protein precipitation filter plate).

Small Sample Volume (20–40 µL) Phospholipid Removal Plates:

The HybridSPE-Phospholipid Small Volume 96-well Plate is designed for processing plasma/serum volumes between 20-40 µL. It is available in both single and 20 plate pack sizes.

Description	Qty.	Cat. No.
HybridSPE-PLus Plate Essentials Kit		
Includes HybridSPE-PLus 96-well plate (575659-U), plate cap mat (as in 575680-U), sealing film (as in Z721581) and collection plate (as in Z717266)	1	52818-U
HybridSPE-PLus 96-Well Plates		
50 mg/well	1	575659-U
	20	575673-U
HybridSPE-Phospholipid Small Volume 96-Well Plates		
15 mg/well	1	52794-U
	20	52798-U
HybridSPE-Phospholipid Cartridges		
HybridSPE-Phospholipid Ultra Cartridge, 30 mg/1 mL	100	55269-U
HybridSPE-Phospholipid Cartridge, 500 mg/6 mL	30	55267-U
HybridSPE-Phospholipid Cartridge, 30 mg/1 mL	100	55261-U
HybridSPE-Phospholipid Cartridge, 30 mg/1 mL	200	55276-U
Plate Accessories		
Round Well Cap Mat, Pierceable for HybridSPE-PLus	50	575680-U
96 Round/Deep Well Collection Plate, PP for HybridSPE-PLus	60	Z717266
96 Well-Plate Pre-cut Sealing Films	100	Z721581
Supelco PlatePrep Vacuum Manifold	1	57192-U
96-well Protein Precipitation Filter Plate (for offline protein precipitation)	1	55263-U
Cartridge Accessories		
Visiprep™ DL Solid Phase Extraction Cartridge Manifold		
12 Port Model	1	57044
24 Port Model	1	57265
Visiprep Solid Phase Extraction Cartridge Manifold		
12 Port Model	1	57030-U
24 Port Model	1	57250-U
Disposable Valve Liners, PTFE (for Visiprep DL Manifold)	100	57059
Equipment		
KNF Laboport® Vacuum Pumps	1	Inquire
SPE Vacuum Pump Trap Kit	1	57120-U
SPE Manifold Gauge/Bleed Valve, Remote In-Line Design	1	57161-U
IKA® VORTEX 3, vortex mixer (230 V)	1	Z654779
	1	Z654760

Sigma-Aldrich® Worldwide Offices

Argentina
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada
Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile
Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

People's Republic of China
Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark
Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1169

Hungary
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9068

India
Telephone
Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 4087 2364
Pune: (+91) 20 4146 4700
Hyderabad: (+91) 40 3067 7450
Kolkata: (+91) 33 4013 8000

Fax
Bangalore: (+91) 80 6621 9550
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Pune: (+91) 20 4146 4777
Hyderabad: (+91) 40 3067 7451
Kolkata: (+91) 33 4013 8016

Ireland
Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: +353 (0) 402 20370
Fax: + 353 (0) 402 20375

Israel
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4222
Fax: (+972) 8 948 4200

Italy
Free Tel: 800 827 018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan
Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea
Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Luxembourg
Tel: (+32) 3 899 1301
Fax: (+32) 3 899 1311

Malaysia
Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico
Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway
Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia
Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2511
Fax: (+41) 81 756 5449

Thailand
Tel: (+66) 2 126 8141
Fax: (+66) 2 126 8080

United Kingdom
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 01747 833 000
Fax: (+44) 01747 833 574

United States
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam
Tel: (+84) 8 3516 2810
Fax: (+84) 8 6258 4238

Internet
sigma-aldrich.com

Enabling Science to
Improve the Quality of Life

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173
Safety-related Information sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

LOP
11832 / T409095B
1053