One Quick Method, Three Big Advantages

1. Remove Proteins that can clog HPLC columns

2. Eliminate Phospholipids that cause ion suppression and increase your column lifetime!

3. No Method Development; one method for acids, bases, and neutrals

Remove Proteins
Solvent Shielding Technology™ prevents dripping of organic solvent, allowing for protein precipitation within the Phree Phospholipid Removal Product.

Eliminate Phospholipids
The Phree sorbent selectively removes phospholipids from precipitated plasma samples.

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“We have demonstrated that Phree Phospholipid Removal Plates resulted in >98% decrease in the peak area of the six phospholipids we monitored in rat plasma compared to traditional protein crash. We highly recommend the use of these plates for high throughput LC-MS/MS bioanalytical sample preparation.”

Nina Khoshaba
WIL Research

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1. Protein Precipitation Does Not Remove Phospholipids

Endogenous phospholipids are a primary source of ion suppression and resulting matrix effects in bioanalytical LC/MS work. Ion suppression caused by the presence of phospholipids can result in:

- irreproducible results
- quantitation issues
- loss in method sensitivity
- matrix to matrix bias

**Total Phospholipid Profile**

Protein Precipitation vs. Phree™ Phospholipid Removal Products

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**Plasma Cleanup:**
100 µL plasma plus 300 µL acetonitrile with 1% formic acid

**Column:**
Kinetex® 2.6 µm C18 100 Å

**Dimensions:**
50 x 2.1 mm

**Part No.:** 00B-4462-AN

**Mobile Phase:**
A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol

**Gradient:**
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>95</td>
</tr>
<tr>
<td>15.5</td>
<td>60</td>
</tr>
<tr>
<td>15.51</td>
<td>60</td>
</tr>
<tr>
<td>19.5</td>
<td>60</td>
</tr>
</tbody>
</table>

**Flow Rate:**
400 µL/min

**Detection:**
Mass Spectrometer (MS) @ 425 °C; 184 amu

**Temperature:**
22 °C
2. Reduce Ion Suppression

The presence of phospholipids in plasma samples produces zones of ion suppression that correlate exactly with the phospholipid elution profile when analyzed via mass spectrometer (MS).

![Graph showing ion suppression and suppression zone elimination]

- Ion suppression is observed in Protein Precipitated Plasma.
- Suppression zone is eliminated in Phree Extracted Plasma.

Amoxapine was infused post-column to establish an ion suppression/enhancement profile with both protein precipitated plasma (left) and Phree extracted plasma (right), showing that Phree can successfully reduce ion suppression.

3. Maximize Sensitivity and Column Lifetime

Phospholipids reduce the sensitivity of the MS signal and shorten column lifetime when they build up over time.

**Column Sensitivity after 250 Injections**

![Graph showing column sensitivity over 250 injections]

- Reduced sensitivity is immediately observed in Phree Extracted Plasma.
- Phospholipid build up results in significant loss of sensitivity in Protein Precipitated Plasma.

~5,000 µL plasma injected without significant loss of sensitivity.

To assess the effect of phospholipid build up, repetitive 20 µL injections of diclofenac in protein precipitated plasma versus diclofenac in Phree extracted plasma were made.
An Improved Phospholipid Removal Solution

Remove All Classes of Phospholipids

Lysophosphatidylcholines and phosphatidylcholines both contribute to matrix effects. Remove all classes of phospholipids using Phree™ Phospholipid Removal Products.

Phospholipid profile monitored using m/z 184-184

Phree is most effective at removing all classes of phospholipids
Extended Phospholipid Capacity

The Phree sorbent has an extended capacity for phospholipids, allowing you to load up to 400 µL of plasma without significant breakthrough of phospholipids.

Phospholipid profile monitored using m/z 184-184

Plasma Cleanup: 400µL plasma plus 1.2 mL acetonitrile with 1% formic acid
Column: Kinetex® 2.6 µm C18 100 Å
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol
Gradient: Time (min) % B
0  60
0.5  95
15.5  95
15.51  60
19.5  60
Flow Rate: 400 µL/min
Detection: Mass Spectrometer (MS) @ 425 °C; 184 amu
Temperature: 22 °C

Waters Corporation states that the true maximum recommended plasma volume that can be processed using the Ostro plate is 350 µL due to well volume limitations. Ostro is a trademark of Waters Corporation. Comparative separations may not be representative of all applications.
Phree Yourself of Method Development

One Method for Acids, Bases, and Neutrals

1. Dispense
   Plasma into the Phree tube or 96-well plate.

2. Add
   Organic solvent directly into the plasma sample.

3. Mix

4. Filter
   Using a centrifuge, vacuum manifold, or positive pressure system.

For complete method details and optimization tips, visit www.phenomenex.com/Phree

Recommended Organic Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solvent Volume</th>
<th>Plasma Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile with 1% Formic acid</td>
<td>300 µL</td>
<td>100 µL</td>
</tr>
<tr>
<td>Methanol with 1% Formic acid</td>
<td>400 µL</td>
<td>100 µL</td>
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</tbody>
</table>

Recommended Plasma Loading Capacities

<table>
<thead>
<tr>
<th>Format</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well plates</td>
<td>25 - 400 µL</td>
</tr>
<tr>
<td>Tubes</td>
<td>25 - 200 µL</td>
</tr>
</tbody>
</table>

One easy method for tubes and 96-well plates! Easily transfer methods between the two formats.

Tip

Phenomenex accessories pair perfectly with Phree Phospholipid Removal Products. Go to page 11 for a complete list of accessories.
High Recoveries of Target Analytes

The Phree general protocol has been developed to produce high recoveries of acids, bases, and neutrals while simultaneously removing all classes of phospholipids.

Absolute Recoveries of Acids, Bases, and Neutrals

Recovery data was obtained by calculating the average absolute recoveries of analytes extracted from 3 Phree plates.
Sample Preparation Specialists are Ready to Assist You

Two Levels of Method Development Support

Level 1
Contact one of our dedicated sample preparation specialists for immediate method development assistance.

Level 2
Send your sample to our analytical services group for custom method development. Visit www.phenomenex.com/PhenoLogix for more information.

Get Started
Contact your Sample Preparation Specialist:
By phone: 310-212-0555
By email: Support@phenomenex.com

“Phenomenex’s prompt support is very important for us to achieve our work. I thank them for their persistent support and innovative products.”

Allena Ji
Wyeth
Order Phree Now!

Tip
For more information about Phenomenex sample preparation products, visit www.phenomenex.com/sampleprepinfo

Ordering Information
Phree Phospholipid Removal Products

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>8B-S133-TAK</td>
<td>Phree Phospholipid Removal 1 mL Tube</td>
<td>100/box</td>
<td>$184</td>
</tr>
<tr>
<td>8E-S133-TGB</td>
<td>Phree Phospholipid Removal 96-Well Plates</td>
<td>2/box</td>
<td>366</td>
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</tbody>
</table>

Accessories
Collection Plates (deep well, polypropylene)

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHO-7192</td>
<td>Strata® 96-Well Collection Plate 350 µL/well</td>
<td>50/pk</td>
<td>$250</td>
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<tr>
<td>AHO-7193</td>
<td>Strata 96-Well Collection Plate 1 mL/well</td>
<td>50/pk</td>
<td>250</td>
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<tr>
<td>AHO-7194</td>
<td>Strata 96-Well Collection Plate 2 mL/well</td>
<td>50/pk</td>
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<tr>
<td>AHO-8635</td>
<td>Strata 96-Well Collection Plate, 2 mL Square/Round-Conical</td>
<td>50/pk</td>
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<tr>
<td>AHO-8636</td>
<td>Strata 96-Well Collection Plate, 2 mL Round/Round, 8 mm</td>
<td>50/pk</td>
<td>321</td>
</tr>
<tr>
<td>AHO-7279</td>
<td>Strata 96-Well Collection Plate, 1 mL/well Round, 7 mm</td>
<td>50/pk</td>
<td>210</td>
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Sealing Mats

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHO-8597</td>
<td>Sealing Mats, Pierceable, 96-Square Well, Silicone</td>
<td>50/pk</td>
<td>$255</td>
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<tr>
<td>AHO-8598</td>
<td>Sealing Mats, Pre-Slit, 96-Square Well, Silicone</td>
<td>50/pk</td>
<td>280</td>
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<tr>
<td>AHO-8631</td>
<td>Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone</td>
<td>50/pk</td>
<td>255</td>
</tr>
<tr>
<td>AHO-8632</td>
<td>Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone</td>
<td>50/pk</td>
<td>280</td>
</tr>
<tr>
<td>AHO-8633</td>
<td>Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone</td>
<td>50/pk</td>
<td>255</td>
</tr>
<tr>
<td>AHO-8634</td>
<td>Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone</td>
<td>50/pk</td>
<td>280</td>
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<tr>
<td>AHO-7362</td>
<td>Sealing Tape Pad</td>
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<td>109</td>
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</tbody>
</table>

Vacuum Manifolds

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHO-6023*</td>
<td>SPE 12-Position Vacuum Manifold Set, for tubes</td>
<td>ea</td>
<td>$634</td>
</tr>
<tr>
<td>AHO-6024*</td>
<td>SPE 24-Position Vacuum Manifold Set, for tubes</td>
<td>ea</td>
<td>874</td>
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<tr>
<td>AHO-8950</td>
<td>Strata 96-Well Plate Manifold, Universal with Vacuum Gauge</td>
<td>ea</td>
<td>1,109</td>
</tr>
</tbody>
</table>

*Manifolds include: Vacuum-tight glass chamber, vacuum gauge assembly, polypropylene lid with gasket, male and female luers and yellow end plugs, stopcock valves, collection rack assemblies, polypropylene needles, lid support legs. Waste container included with 12-positive manifold.

If Phree Phospholipid Removal products do not perform as well or better than your current phospholipid removal product, send in your comparative data within 45 days and keep the Phree Phospholipid Removal product for FREE.

Terms and Conditions
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