Thank you for choosing Phree™ Phospholipid Removal Solutions

In order to get the best results from your Phree 96-Well Plates or 1 mL Tubes, follow the General Protocols provided. For your convenience, optimization tips are also included in this User Manual.

Have questions?
Contact your local Phenomenex office or email info@phenomenex.com

If Phree Phospholipid Removal products do not perform as well or better than your current phospholipid removal product, return the product with your comparative data within 45 days for a FULL REFUND.
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96-Well Plate General Protocol

1. Dispense
Dispense plasma into the Phree™ 96-Well Plate.
See page 9 for recommended plasma volumes.

2. Add
Add organic solvent into the Phree 96-Well Plate in a volume of 3-4x the volume of the plasma sample. Be careful not to add solvent along the wall or sides of the well (see corresponding images), organic solvent must be added directly into the plasma to ensure complete precipitation. Aspirate 2x to mix plasma and solvent. Maximum loading volumes and recommended organic solvents are listed on page 9.

If solvent is added to the plasma incorrectly, mixing will not occur and a bilayer may form between the plasma and organic solvent which will prevent complete precipitation.

For more information about direct addition of solvent to plasma, view our online tutorial at www.phenomenex.com/DirectAddition.

3. Vortex†
2 minutes at a maximum speed of 1300 rpm, taking care not to spill the solvent. Sample can stand for up to 25 minutes.

† When used with a liquid-handling instrument or automation, aspirate/dispense cycles may be used to promote in-tip mixing and precipitation. This will ensure complete precipitation and filtration. Vortexing is not necessary when in-tip mixing is performed.
4. Filter
When properly precipitated, the resulting filtrate should be clear and free of proteins and phospholipids.

Centrifuge
Place the Phree™ 96-Well Plate on top of a collection plate and centrifuge at 500 g for 5 minutes or until filtrate is collected.

Vacuum
Place the Phree 96-Well Plate onto a suitable 96-well sample manifold or robot. Ensure that a 96-well collection plate is positioned inside the manifold or under the Phree 96-Well Plate. Vacuum at 2-7 inches Hg for up to 5 minutes or until filtrate is collected.

Positive Pressure
Place the Phree 96-Well Plate on top of a collection plate and apply 2-5 psi using a positive pressure manifold.

View of filtrate inside a collection plate after using Phree.

Left: Complete precipitation, resulting in clear filtrate.
Right: Incomplete precipitation. Incomplete precipitation is most likely due to improper mixing during the solvent addition step.

View of the inside of a Phree 96-Well Plate after filtration.

Left: Complete precipitation. Note the webbing of the yellow precipitate left behind in the Phree 96-Well Plate.
Right: Incomplete precipitation. Note the localized white precipitate. Incomplete precipitation is most likely due to improper mixing during the solvent addition step.

For more information, visit www.phenomenex.com/phree
For questions and technical support email support@phenomenex.com
**1 mL Tube General Protocol**

1. **Dispense**
   Dispense plasma into the Phree™ Tube.
   See page 9 for recommended plasma volumes.

2. **Add‡**
   Add organic solvent into the Phree Tube in a volume of 3-4x the volume of the plasma sample. Be careful not to add solvent along the wall of the tube (see below), organic solvent must be added directly into the plasma to ensure complete precipitation.

   If solvent is added to the plasma incorrectly, mixing will not occur and a bilayer may form between the plasma and organic solvent which will prevent complete precipitation.

   ![Incorrect Method](image1)
   ![Correct Method](image2)

   For more information about direct addition of solvent to plasma, view our online tutorial at [www.phenomenex.com/DirectAddition](http://www.phenomenex.com/DirectAddition).

‡ When used with a liquid-handling instrument or automation, aspirate/dispense cycles may be used to promote in-tip mixing and precipitation. This will ensure complete precipitation and filtration.
3. Filter

When properly precipitated, the resulting filtrate should be clear and free of proteins and phospholipids.

**Centrifuge**

Place the Phree™ Tube inside a collection tube (ensure that the diameter of the tabbed portion of the Phree Tube is larger than the diameter of the collection tube in order to keep the Phree tube suspended inside the collection tube, the diameter of the tab is approximately 17 mm). Centrifuge the Phree Tube and collection tube at 500 g until filtrate is collected inside the collection tube. If elution is not complete, centrifugal force can be increased up to 1500 g.

**Vacuum**

Place the Phree Tube on a suitable vacuum manifold or robot. Ensure that a collection tube is positioned inside the manifold or under the Phree Tube. Vacuum at 5-10 inches Hg (when precipitating with Acetonitrile) or 15-20 inches Hg (when precipitating with Methanol) for up to 5 minutes or until filtrate is collected.

**Positive Pressure**

Place the Phree Tube on a suitable positive pressure manifold. Ensure that a collection tube is positioned underneath the Phree Tube and apply 40 psi using a positive pressure manifold.

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Filtrate should be clear and free of proteins and phospholipids.

**Left:** Filtrate after complete protein precipitation using Phree.

**Right:** Filtrate after incomplete precipitation using Phree. Incomplete precipitation is most likely due to improper mixing during the solvent addition step.

View of the Phree frit after filtration.

**Left:** Complete precipitation. Note the webbing of the yellow precipitate left behind on the Phree frit.

**Right:** Incomplete precipitation. Note the localized white precipitate. Incomplete precipitation is most likely due to improper mixing during the solvent addition step.

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For more information, visit [www.phenomenex.com/phree](http://www.phenomenex.com/phree)

For questions and technical support email [support@phenomenex.com](mailto:support@phenomenex.com)
Optimization Tips

1. A 3:1 v/v ratio of organic solvent:plasma is the minimum ratio of solvent required for acetonitrile precipitation. A 4:1 v/v is the recommended minimum ratio of solvent:plasma required for methanol precipitation.

2. Increasing the ratio of organic solvent can provide a more complete protein precipitation.

3. To enhance analyte recovery, higher ratios of organic can be employed. This is especially recommended when working with plasma volumes of less than 100 µL. Keep in mind that increasing the percentage of organic also increases your overall dilution factor. A minimum total elution volume (plasma plus organic solvent) of 400 µL is recommended to maximize recovery when working with plasma volumes of less than 100 µL.

4. For some precipitations, higher levels of vacuum may be required for complete elution. In these cases you can apply 5-10 inches Hg for a longer period or use higher levels of vacuum (i.e. 15 inches Hg) after the initial vacuum step is applied.

5. Although adding plasma before solvent is recommended, solvent can be added to the Phree™ Phospholipid Removal products before plasma if desired. It is important to avoid adding plasma along the side of the wall of the Phree Phospholipid Removal product, plasma must be added directly into the solvent to ensure proper mixing and complete precipitation.

6. It is important to add solvent directly into the plasma or vice versa. When solvent or plasma is added alongside the wall of the Phree 96-Well Plate or Tube a bilayer will form making it difficult to properly mix the plasma and solvent. Improper mixing will result in incomplete precipitation.

Left: Bilayer formed when solvent is added to the wall of the 96-Well Plate or Tube.
Right: Precipitate immediately forms when solvent is added DIRECTLY into the plasma.
7. If resulting filtrate is not clear this may indicate that precipitation is not complete and mixing of solvent and plasma was not ideal. To achieve better mixing of solvent and plasma, an aspiration step can be performed. We recommend aspirating 2x if an aspiration step is performed. Please note that a long pipette tip may be necessary when performing an aspiration step in the Phree 1 mL tubes.

### Maximum Organic Solvent Volumes

<table>
<thead>
<tr>
<th>Format</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well plates</td>
<td>1.2 mL</td>
</tr>
<tr>
<td>1mL tubes</td>
<td>0.8 mL</td>
</tr>
</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>1mL tubes</td>
<td>25-200 µL</td>
</tr>
</tbody>
</table>

### Recommended Organic Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solvent Volume</th>
<th>Plasma Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile plus 1 % Formic acid</td>
<td>300 µL</td>
<td>100 µL</td>
</tr>
<tr>
<td>Methanol plus 1 % Formic acid</td>
<td>400 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>
May we also suggest...

Ordering Information

Phree™ Phospholipid Removal Products

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

**Accessories**

**Collection Plates (deep well, polypropylene)**

| AH0-7192       | Strata® 96-Well Collection Plate 350 µL/well             | 50/pk |
| AH0-7193       | Strata 96-Well Collection Plate 1 mL/well                | 50/pk  |
| AH0-7194       | Strata 96-Well Collection Plate 2 mL/well                | 50/pk  |
| AH0-8635       | Strata 96-Well Collection Plate, 2 mL Square/Round-Conical| 50/pk  |
| AH0-8636       | Strata 96-Well Collection Plate, 2 mL Round/Round, 8 mm  | 50/pk  |
| AH0-7279       | Strata 96-Well Collection Plate, 1 mL/well Round, 7 mm   | 50/pk  |

**Sealing Mats**

| AH0-8597       | Sealing Mats, Pierceable, 96-Square Well, Silicone       | 50/pk  |
| AH0-8598       | Sealing Mats, Pre-Slit, 96-Square Well, Silicone         | 50/pk  |
| AH0-8631       | Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone   | 50/pk  |
| AH0-8632       | Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone     | 50/pk  |
| AH0-8633       | Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone   | 50/pk  |
| AH0-8634       | Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone     | 50/pk  |
| AH0-7362       | Sealing Tape Pad                                         | 10/pk  |

**Vacuum Manifolds**

| AH0-6023*      | SPE 12-Position Vacuum Manifold Set, for tubes           | ea    |
| AH0-6024*      | SPE 24-Position Vacuum Manifold Set, for tubes           | ea    |
| AH0-8950       | Strata 96-Well Plate Manifold, Universal with Vacuum Gauge| ea    |

*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.
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THANK YOU

for choosing Phree™

Phospholipid Removal Solutions