

User Guide

 **Phree**TM
Phospholipid Removal Solutions



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...breaking with tradition[™]



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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 Guide.

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96-Well Plate General Protocol

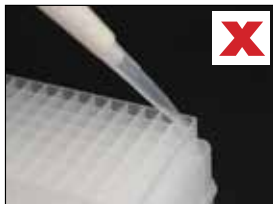
1. Dispense

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, most likely due to improper mixing during the solvent addition step.



Complete Precipitation



Incomplete Precipitation

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.



Complete Precipitation



Incomplete Precipitation

For more information, visit www.phenomenex.com/phree

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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.



For more information about direct addition of solvent to plasma, view our online tutorial at 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.

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.



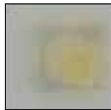
View of the Phree frit after filtration.

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.



Complete Precipitation



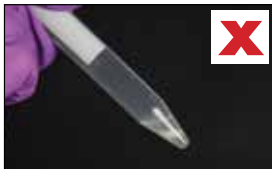
Incomplete Precipitation

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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

Format	Volume
96-well plates	1.2 mL
1 mL tubes	0.8 mL

Recommended Plasma Loading Capacities

Format	Volume
96-well plates	25-400 μ L
1 mL tubes	25-200 μ L

Recommended Organic Solvents

Solvent	Solvent Volume	Plasma Volume
Acetonitrile plus 1 % Formic acid	300 μ L	100 μ L
Methanol plus 1 % Formic acid	400 μ L	100 μ L

Ordering Information

Phree Phospholipid Removal Products

Part No.	Description	Unit
8B-S133-TAK	Phree Phospholipid Removal 1 mL Tube	100/box
8E-S133-TGB	Phree Phospholipid Removal 96-Well Plates	2/box

Accessories

Collection Plates (deep well, polypropylene)

AH0-7192	96-Well Collection Plate 350 μ L/well	50/pk
AH0-7193	96-Well Collection Plate 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	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	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.

May we also suggest...



Ordering Information

Presston 1000 Positive Pressure Manifold

Part No.	Description	Unit
AH1-7033	Presston 1000 Positive Pressure Manifold, 96 Well Plate Complete Assembly	ea



Phenomenex warrants the Presston 1000 Positive Pressure Manifold against defects in materials and workmanship under normal installation, use, and maintenance for a period of 12 months following delivery.

Please visit www.phenomenex.com/presstonwarranty for complete warranty information.



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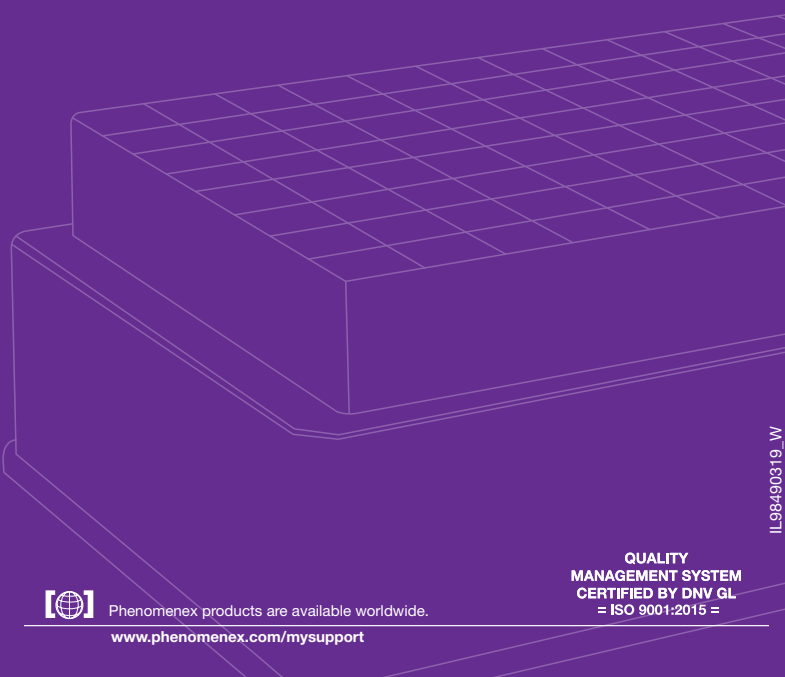
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