Phree™
Phospholipid Removal Solutions

FAQs
for Phospholipid Removal Plates

To learn more about Phree go to:

www.phenomenex.com/Phree

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GENERAL QUESTIONS

How does Phree remove proteins from my sample?

The Phree plate contains a specialized frit system that allows for the sample and precipitating solvents (such as Acetonitrile and Methanol) to interact without penetrating the frits. The frit system has a wide solvent compatibility including Acetonitrile and Methanol and can withhold sample and precipitating solvents above the frits for up to 20 minutes, allowing for complete in-well precipitation to occur. Precipitated proteins are then filtered and left above the frits when the sample and precipitating solvents are pulled through the Phree sorbent (via positive pressure, centrifugation, or vacuum) and into a collection plate.

SAMPLE and LOADING:

How much sample can I process per well on the Phree plate?

You can process 50 µL – 400 µL of plasma or tissue homogenate within each well of the Phree plate. However, the maximum total volume (organic and sample) should not exceed 1,350 µL. A total volume above 1,350 µL may lead to cross well contamination during the vortexing step.

What type of samples could I use with the Phree plates?

Plasma, serum, and tissue homogenates can be processed using Phree.

Do I have to use all 96-wells of the Phree plate?

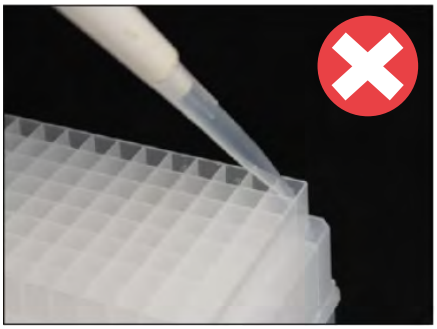
You can process a partial Phree plate by using vacuum and covering the unused wells with a sealing mat or sealing tape. This will ensure that the wells that are being used receive the proper amount of vacuum for the extraction.

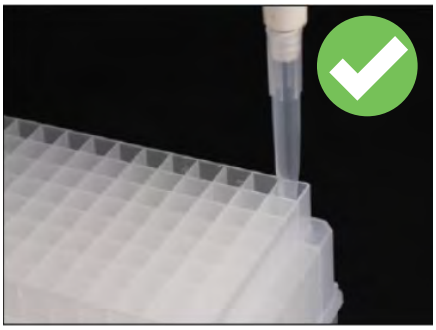
What solvents are compatible with the Phree plate?

Phree has a wide solvent compatibility however Acetonitrile and Methanol are recommended.

Should I add sample first or solvent first?

It is possible to add either sample first or solvent first. It is important to add solvent directly into the sample or vice versa. When solvent or sample is added alongside the wall of the Phree plate, a bilayer will form making it difficult to properly mix the plasma and solvent. Improper mixing will result in incomplete precipitation.





What is the purpose of adding 1% formic acid in the Phree method? Can I use any other acids besides formic acid?

Formic acid helps to release analytes from protein or matrix binding and also helps to facilitate protein precipitation. Other acids such as phosphoric acid or acetic acid can be used to acidify the sample.

Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

What should I do if I experience slow flow through the sorbent?

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.

What should I do if am working with a viscous sample?

Using proper technique to achieve thorough mixing is crucial to achieving the best results, especially with viscous samples. Refer to FAQ Question "Should I add sample first or solvent first" for mixing tips. Other tips to remedy slow flow due to viscous samples are as follows:

i. Increase vortex intensity and/or time
ii. Mix via pipette aspiration
iii. Increase volume of precipitation solvent to plasma ratio
iv. 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

EXTRACTION and RECOVERY:

How much volume should I expect to recover from the Phree plate?

In any protein precipitation, a loss of volume due to the precipitation will occur. The Phree plate also has a small dead volume which will reduce the recovered volume of sample due to solvent being retained on the sorbent. This can vary based on sample and solvent used for precipitation. If elution volumes are too low or analyte recoveries are not as high as preferred, refer to FAQ Question "What can I do to improve analyte recoveries?"

Q. What can I do to improve analyte recoveries?

The interaction of the analyte with the plate is primarily a function of the volume of solvent used with the plate. Simply put, the more solvent that is used for elution the higher your analyte recoveries will be. There are two approaches that can be used to increase recoveries.

1. Increase the ratio of organic to sample during the precipitation (i.e. use an 8:1 ratio instead of 3:1). In this case, make sure that the total volume in the well is not greater than 1,350 µL.
2. Perform a second elution. When using this approach, ensure that the ratio of organic to aqueous (sample) is the same for both elutions. For example, if a 3:1 ratio of organic:aqueous (sample) was used for the first elution then the second elution should also be a 3:1 ratio of organic:aqueous (use water for the aqueous during the second elution). If the organic strength of the second elution is higher than the first, there is a risk that additional protein will precipitate within the collection plate leaving a cloudy eluent.

The affinity of the sorbent for phospholipids is extremely high, phospholipids will not elute from the plate if the above approaches are employed.

If I see a cloudy extract what should I do?

If resulting filtrate is not clear this may indicate that precipitation is not complete and mixing of solvent and sample was not ideal. To achieve better mixing of solvent and sample, an aspiration step can be performed. We recommend aspirating 2x if an aspiration step is performed.

I'm performing a methanol extraction on the Phree plate and I see a decrease in flow.

Methanol produces a very fine precipitant that can sometimes slow down flow as the sample moves through the frit system. 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.

How can I confirm that phospholipids are being removed from my sample?

Phospholipids can be monitored using the 184-184 m/z transition (ion-source fragmentation) as a qualitative approach. This transition measures the polar head group fragment characteristic of phosphatidylcholines. A quantitative approach uses specific MRM's for specific phospholipids. These MRMS are widely available in peer reviewed literature.

Can Phree be automated?

Yes, the Phree 96-wel plate format conforms to SBS standard dimensions and works well with most automated liquid handlers and workstations.

Have more questions about Phree?
Chat live with a technical expert now!
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