

Phree Frequently Asked Questions

GENERAL QUESTIONS:

Q: How does Phree remove proteins from my sample?

A: Phree 96-well plates and 1 mL tubes contain 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:

Q: How much sample can I process with Phree?

A: You can process 25 μ L – 400 μ L of plasma or tissue homogenate within each well of the Phree plate or 25 μ L – 200 μ L of plasma or tissue homogenate in each Phree 1 mL tube. However, the maximum total volume (organic and sample) should not exceed 1,350 μ L for 96-well plates or 800 μ L for 1 mL tubes. Larger volumes may lead to cross well contamination during the vortexing step in the 96-well plates or spillage during the mixing step in the 1 mL tubes.

Q: What type of samples could I use with Phree?

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

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

A: 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

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

A: 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.

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

A: You can process a partial Phree plate by using vacuum and covering the un-used 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.

Q: What solvents are compatible with Phree?

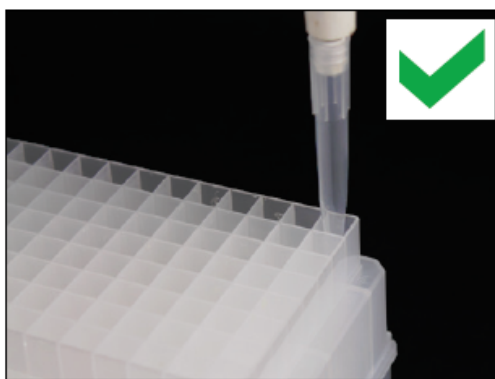
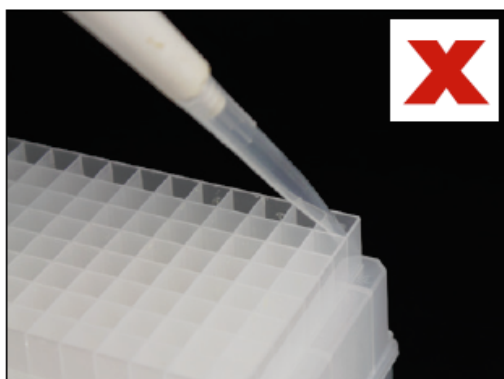
A: Phree has a wide solvent compatibility however Acetonitrile and Methanol are recommended.

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

A: 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.

Q: Should I add sample first or solvent first?

A: It is possible to add either sample first or solvent first, however we have found that the best mixing occurs when adding plasma first followed by solvent. 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.



EXTRACTION and RECOVERY:

Q: How much volume should I expect to recover from Phree?

A: In any protein precipitation, a loss of volume due to the precipitation will occur. Phree 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?

A: The interaction of the analyte with the sorbent is primarily a function of the volume of precipitation solvent used with Phree. 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 μ L when using Phree 96-well plates or 800 μ L when using Phree 1 mL tubes.
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.

Q: If I see a cloudy extract what should I do?

A: 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.

Q: I'm performing a methanol extraction using Phree and I see a decrease in flow.

A: 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.

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

A: 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.

Q: Can Phree be automated?

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

Please contact support@phenomenex.com if you would learn more about Phree and our other sample preparation products.