Resprep PPT^3 96-Well Plate

cat.# 26489-2 and 26489-5

Instructions

1. Place an empty Resprep 96-well collection plate (cat.# 26494 or 26495) on a flat surface.
2. Place a Resprep PPT^3 96-well plate (cat.# 26489-2 or 26489-5) on top of the collection plate. Make sure the tips point downward so that the square wells are open at the top. The collection plate will be on the bottom, and the Resprep PPT^3 96-well plate will be on the top, as shown in the figure.
3. Dispense three sample volumes of organic solvent (typically acetonitrile) using a pipettor or a robotic liquid-handling device.

   For example, dispense 300 µL of acetonitrile for a 100 µL plasma sample.
4. Add 25–250 µL of biological sample (e.g., human plasma) forcefully and directly into the organic solvent you dispensed in Step 3.
5. Cover the Resprep PPT^3 96-well plate with a sealing mat.
6. To mix, vortex the entire Resprep PPT^3 96-well plate and collection plate for 0.5–2 min at 2,000 rpm. Complete protein precipitation is critical before you proceed to Step 7. If protein precipitation is incomplete, proteins may break through the dual-layer membrane resulting in a cloudy final extract that contains proteins. Sample volume, solvent selection, and vortexing time and speed may need to be optimized during method development if you experience cloudiness in the final extract.
7. Remove precipitated proteins in your sample by filtration, using any of the three methods below.

   a. Vacuum manifold: 2–9 inches Hg for 5 min or until filtration is complete.

   b. Positive pressure manifold: 2–5 psi for 5 min or until filtration is complete.

   c. Centrifugation: 3,800 g for 2 min or until filtration is complete.
8. Remove the Resprep PPT^3 96-well plate and collection plate from the filtration device and detach the Resprep PPT^3 96-well plate (top) from the collection plate (bottom).
9. The collection plate wells now contain the filtrate. The filtrate may be further diluted if needed to improve chromatography (e.g., peak shape of the early eluting compounds). If further dilution is not possible due to limited instrument sensitivity, evaporation followed by reconstitution can also be performed.
10. Load the collection plate onto the autosampler for LC-MS analysis.

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Frequently Asked Questions

1. **Can I use solvents other than acetonitrile?** Yes, you can use a variety of solvents. Resprep PPT^3 96-well plates are made of high-quality polypropylene housing and solvent-resistant membrane materials. Many standard solvents such as methanol, dichloromethane, etc., can be used, but different solvents will produce different protein precipitation results. Acetonitrile is recommended because it is readily available in most laboratories, and it provides strong protein precipitation capability for samples that contain high levels of protein (e.g., plasma).
2. **Can I use acidified organic solvents?** Yes. Acetonitrile with 0.1–1% formic acid often is used to improve protein precipitation because it disrupts binding between the protein and the protein-bound analytes. Acidified acetonitrile also is widely used in LC-MS/MS analysis with an electrospray ionization (ESI) source.
3. **What should the organic solvent:sample ratio be for effective precipitation?** Typically, a 3:1 ratio of organic solvent to sample is used. Adding more organic solvent may produce better protein precipitation, but the additional solvent should be calculated into the dilution factor. The analyte concentration in the final filtrate will be diluted by the actual solvent:sample ratio that is used.
4. **How long should I vortex to mix in Step 6?** To ensure proper mixing, we recommend vortexing for 0.5–2 min at a minimum speed of 2,000 rpm. The mixing time will depend on solvent volume, sample volume, and sample type. If protein removal is incomplete, increase the mixing time and/or the vortex speed (up to 3,000 rpm).
5. **Is dilution necessary in Step 9?** Theoretically, if a 3:1 solvent:sample ratio is used for protein precipitation during the filtration steps, then the final filtrate will be approximately 75% acetonitrile. If the initial liquid chromatography mobile phase composition is not close to 75% organic solvent or if poor chromatographic peak shapes are observed for early eluting compounds, we recommend diluting the filtrate in the collection plate with water or aqueous mobile phase to more closely match the initial mobile phase composition. If further dilution is not suitable for your application due to limited instrument sensitivity, evaporation followed by reconstitution with your initial mobile phase can be performed. This approach can be used to obtain a suitable concentration while avoiding peak shape issues that can be caused by unmatched sample solvent and initial mobile phase.
6. **Does it matter what filtration device I use?** No. Resprep PPT^3 96-well plates are compatible with vacuum manifolds, positive pressure manifolds, and centrifuges. You may choose whichever method is available in your laboratory.
7. **Do I have to use all the wells on a single plate at the same time?** No. You may use some of wells on the plate and then use the unused wells later.
8. **Does this product have an expiration date?** Resprep PPT^3 96-well plates do not have an expiration date. Customers usually store them for a few months to a few years for inventory purposes.
9. **How long can Resprep PPT^3 96-well plates hold the solvent and sample without dripping?** No dripping for a minimum of 12 hours is guaranteed as shown on the certificate of analysis. Our experiments demonstrate 24 hours without dripping, using both acetonitrile and dichloromethane.

Resprep PPT^3 96-Well Plates

Catalog No.	Product Name	Units	Volume
26489-2	Resprep PPT3 96-Well Plate, Square Well, 2 mL, 2-pk.	2-pk.	2 mL
26489-5	Resprep PPT3 96-Well Plate, Square Well, 2 mL, 5-pk.	5-pk.	2 mL

26489-2

Well Plates

Catalog No.	Product Name	Units	Volume
26494	96-Well Plates, 1.3 mL, Non-Sterile, Round Well, Round Bottom, 50-pk.	50-pk.	1.3 mL
26495	96-Well Plates, 1.3 mL, Non-Sterile, Round Well, Round Bottom, 5-pk.	5-pk.	1.3 mL

26489-5


Well Plate

Questions about this or any other Restek product?
Contact us or your local Restek representative (www.restek.com/contact-us).

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