

Resprep PLR 96-Well Plates and SPE Cartridges

cat.# 28301 and 28300

Resprep PLR Products

Resprep PLR 96-well plates and SPE cartridges easily and effectively remove proteins and phospholipids from biological samples prior to analysis, which reduces the chance of matrix-related interferences and premature instrument maintenance. Proteins are precipitated and filtered while phospholipids are retained by a selective composite material. The 96-well plate format meets American National Standards Institute/Society for Laboratory Automation and Screening (ANSI SLAS 1-2004) dimensional requirements, making them compatible with most automation platforms and ideal for high throughput testing. The SPE cartridge format is suitable for labs with fewer samples or infrequent testing.

General Guidelines

While acetonitrile is recommended, other organic solvents, such as methanol, can be used to precipitate proteins. However, different solvents will produce different protein precipitation results (e.g., methanol produces fine precipitates that may require more time, vacuum, or pressure to effectively filter). 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). Acidification is recommended because it further helps with protein precipitation and is also commonly used in LC-MS/MS analyses with an electrospray ionization (ESI).

After step 6, the collection vessel will contain the filtrate. The final filtrate will contain a high percentage of the organic solvent, such as acetonitrile with 1% formic acid. For example, with a 3:1 solvent:sample ratio, the final filtrate will be approximately 75% organic solvent. If the initial liquid chromatography mobile phase composition is not close to the composition of the final filtrate, or if poor chromatography peak shapes are observed for early eluting compounds, we recommend diluting the filtrate in the collection vessel with water or an aqueous solution to more closely match the initial mobile phase composition and then vortexing briefly. If further dilution is not suitable for your application due to limited instrument sensitivity, evaporation followed by reconstitution with the initial mobile phase and a brief vortexing step to ensure proper mixing 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.

Resprep PLR 96-Well Plate Instructions

Note: All 96 wells do not have to be used at once. If using a vacuum manifold in Step 5 below, we recommend sealing unused wells (e.g., with sealing tape) to ensure that the wells in use receive the proper vacuum.

1. Start with a clean 1.3 mL Resprep 96-well collection plate (cat. # 26494 or 26495), which will be used to collect the sample after it passes through the Resprep PLR product.
2. Position the Resprep PLR 96-well plate (cat.# 28301) on top of the collection plate.
3. Dispense 300-400 μ L of acetonitrile with 1% formic acid using a pipettor or a robotic liquid-handling device.
4. Dispense 100 μ L of biological sample (e.g., human plasma) directly into the organic solvent (do not dispense with tip against well wall). To ensure efficient protein precipitation occurs, use one of the following methods to thoroughly mix the biological sample and the organic solvent:
 - a. Aspirate/dispense the sample 3-4 times.
 - b. Cover with a sealing mat and vortex the collection plate/Resprep PLR stack for 0.5-2 minutes at 2000 rpm.
 - c. Dispense the samples directly into the well(s) with force and allow 2-5 minutes for full precipitation.

Note: Complete protein precipitation is critical before proceeding further. If protein precipitation is incomplete, proteins may break through the Resprep PLR bed, resulting in a cloudy extract that contains proteins. Solvent selection and mixing method should be optimized during method development if you experience cloudiness in the final extract.



28301

5. Remove precipitated proteins from your sample by filtration. If using positive pressure or vacuum manifolds, full elution should occur within 2-5 minutes for best results. Example settings are provided below, but may need to be adjusted based on your specific equipment.
 - a. Vacuum manifold setting: 2-4" Hg.
 - b. Positive pressure manifold setting: 2-5 psi.
 - c. Centrifugation: 3800 g for 2 minutes or until filtration is complete.
6. Remove the Resprep PLR 96-well plate and collection plate from the filtration device and then remove the Resprep PLR 96-well plate from the collection plate. If dilution or reconstitution is necessary, see General Guidelines.
7. Cap the collection plate and load it onto the autosampler for LC-MS analysis.

Resprep PLR SPE Cartridge Instructions

1. Place a clean collection vessel (e.g., a 2 mL autosampler vial) for each sample in an empty SPE manifold.
2. Using an SPE manifold, attach a Resprep PLR SPE cartridge (cat.# 28300) above each empty collection vessel.
3. Dispense 100 µL of biological sample (e.g., human plasma) into the Resprep PLR SPE cartridge.
4. Dispense 300-400 µL of acetonitrile with 1% formic acid into the cartridge with force. To ensure efficient protein precipitation, allow 2-5 minutes for full precipitation.

Note: Complete protein precipitation is critical before proceeding further. If protein precipitation is incomplete, proteins may break through the Resprep PLR bed, resulting in a cloudy extract that contains proteins. Solvent selection and mixing method should be optimized during method development if you experience cloudiness in the final extract.

5. Remove precipitated proteins from your sample by filtration. Whether using vacuum or positive pressure manifolds, adjust to establish a drip rate of 1-2 drops per second (e.g., vacuum manifold set to 5-10" Hg).
6. Remove the Resprep PLR SPE cartridges and collection vessels from the filtration device. If dilution or reconstitution is necessary, see General Guidelines.
7. Cap the collection vessel and load it onto the autosampler for LC-MS analysis.

Verifying Phospholipid Removal

Phosphatidylcholines (PC) are a common class of phospholipids found in blood, plasma, or serum samples, and they all have choline as a part of their head group. Under commonly used LC-MS/MS ESI conditions, PC head groups generate a characteristic ion fragment that can be monitored with a 184→184 m/z transition. Using this transition, you can perform a qualitative assessment of phospholipid removal. Alternatively, you can monitor specific phospholipids using MRM transitions found in the literature for a more quantitative analysis.



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

Restek patents and trademarks are the property of Restek Corporation. (See www.restek.com/Patents-Trademarks for full list.) Other trademarks in Restek literature or on its website are the property of their respective owners. Restek registered trademarks are registered in the U.S. and may also be registered in other countries.

© 2019 Restek Corporation. All rights reserved. Printed in the U.S.A.

www.restek.com

#804-04-001 Rev. date: 10/19



RESTEK
Pure Chromatography