Introduction

The Protein Precipitation Plates provide an easy format for simple, rapid and automatable protein precipitation and filtration. Plates are suitable for processing plasma or serum samples in a standard 96-well format. The non-wetting frit design allows drip-free solvent and sample dispensing and mixing and protein precipitation. Filtrates are easily collected by vacuum, positive pressure or centrifugation. The leach-free graded hydrophobic filter frit has decreasing pore sizes that prevent clogging, ultimately filtering eluates through a 0.2 \( \mu \)m cutoff membrane. The plate is compatible with organic solvents commonly used for protein precipitation, including acetonitrile and methanol.

The study of small molecules and their behavior in complex biofluids (e.g., serum, plasma, blood, urine) is an essential part of pharmaceutical research. Before analysis by HPLC and MS, the target molecules must be separated and recovered from the protein matrix so they can be monitored for clearance rates, serum binding equilibrium and \textit{in vivo} modifications. Protein separation and recovery is accomplished by precipitating proteins with acetonitrile and recovering them by centrifugation or filtration (Figure 1). Although centrifugation is inexpensive and reliable, protein precipitation plates are becoming common because they increase the throughput, enable fully automated workflows, and improve the overall protein and particulate removal. Precipitated samples clarified by the Protein Precipitation Plates can be processed in standard downstream methods (e.g., solid-phase extraction) or by direct injection for LC-MS/MS analysis.

The design of the Protein Precipitation Plates provides many advantages over typical protein precipitation and centrifugation methods. The standard 96-well format requires no specialized equipment for automation, the solvent compatibility and leak-free design provide simple protein precipitation, and sample microfiltration improves downstream processing. The 2 ml 96-well Protein Precipitation Plates can process 15-600 \( \mu \)l of serum or plasma samples.

Additional Materials Required

- Acetonitrile (Product No. 51101)
- Plate shaker (e.g., Thermo Scientific Titer Plate Shaker, Product No. 14-271-9)
- Vacuum manifold, positive-pressure processor, or 96-well plate centrifuge
- 96-well collection plates for filtrates (Thermo Scientific ABGene 1.2 ml Square Well Storage Plate, Low Profile, Product No. AB-1127 or 2.2 ml Storage Plate Mark II, Product No. AB-0932)
Procedure for Protein Precipitation from Serum or Plasma

1. Spike serum or plasma sample with a quantitation standard, if desired.

2. Dispense three sample volumes of acetonitrile into Protein Precipitation Plate. The recommended final ratio is 3:1 (v/v) acetonitrile to serum or plasma. For example, dispense 60 µl of acetonitrile to precipitate protein from 20 µl of serum. Acetonitrile will not drip or leak for up to 4 hours.

3. Add serum or plasma sample (15-600 µl). Although the leak-free design prevents fluid loss, dispense within 5 minutes to minimize acetonitrile evaporation.

4. Cover plate with sealing cover. Shake at room temperature on a platform shaker for 1-3 minutes at medium speed.

5. Filter samples into a clean 96-well collection plate by vacuum or positive pressure manifold for 3 minutes (15” Hg pressure) or centrifugation for 3 minutes at 500 × g.

Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
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<tbody>
<tr>
<td>Variable protein removal</td>
<td>Insufficient mixing of acetonitrile and sample</td>
<td>Mix longer if necessary</td>
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<td></td>
<td>Insufficient pressure (vacuum or positive) to fully elute all wells</td>
<td>Increase vacuum, positive pressure or centrifugal force – centrifugal force depends on rotor radius and speed (see the Tech Tip section of our website for a conversion table)</td>
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<tr>
<td>Incomplete recovery of filtrate volume</td>
<td>Insufficient pressure (vacuum or positive) to fully elute all wells</td>
<td>Increase vacuum, positive pressure or centrifugal force – centrifugal force depends on rotor radius and speed (see the Tech Tip section of our website for a conversion table)</td>
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Related Products

90006 Single-Use RED Plate with Inserts, 1 each
90007 Single-Use RED Plate with Inserts, 5 each
89809 RED Device Inserts, 50 each
89810 RED Device Inserts, 250 each (5 × 50 packs)
89811 Resuable Base Plate made of PTFE, 1 plate
89812 RED Device Insert Removal Tool
51101 Acetonitrile, 1 L
28904 Trifluoroacetic Acid, Sequanal grade, 10 × 1 ml

General References


This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”). No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s). There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products. Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor. © 2008 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.