INSTRUCTIONS

Protein Precipitation Plates

90036 90037

2071.0

Number	Description
90036	**Protein Precipitation Plates,** 2 ml × 96-well, 2 plates
90037	**Protein Precipitation Plates,** 2 ml × 96-well, 10 plates

Note: Plate sealing covers are included with each product.

Storage: Upon receipt store plates at room temperature. Product shipped at ambient temperature.

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 µm 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 *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 µl of serum or plasma samples.

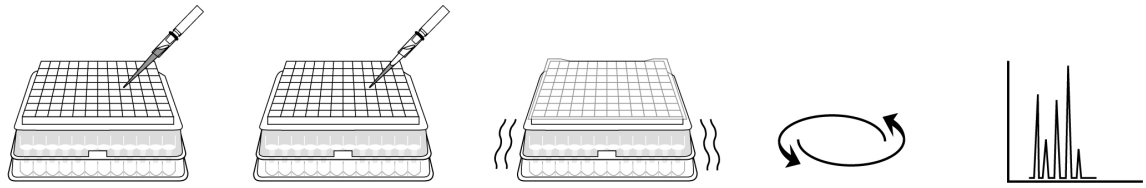

Figure 1. Schematic of the protocol for using the Protein Precipitation Plates.

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)

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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

Problem	Possible Cause	Solution
Variable protein removal	Insufficient mixing of acetonitrile and sample	Mix longer if necessary
	Insufficient pressure (vacuum or positive) to fully elute all wells	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)
Incomplete recovery of filtrate volume	Insufficient pressure (vacuum or positive) to fully elute all wells	

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

Lin, J.H. and Lu, A.Y.H. (1997). Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev.* **49**:403-49.

Pacifici, G.M. and Viani, A. (1992). Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. *Clin. Pharmacokinet.* **23(6)**:449-68.

Piafsky, K.M., *et al.* (1978). Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma alpha1 acid glycoprotein. *N. Engl. J. Med.* **299**:1435-9.

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