

HyperSep Protein Precipitation Plates 60304-201

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Introduction

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 analyzing 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.

The Thermo Scientific™ HyperSep™ Protein Precipitation Plates provide an easy format for simple, rapid, and automatable protein precipitation and filtration. The plates are suitable for processing plasma or serum samples in a standard 96-well format.

Important notes

The Protein Precipitation Plates can process between 15–1600 μL of serum or plasma samples.

Materials required

- Acetonitrile
- Pipette and tips
- Plate cover
- Plate shaker
- Vacuum manifold, positive pressure manifold, or 96-well plate centrifuge
- 96-well collection plates for filtrates

Protocol

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 one sample volume of 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, or thoroughly mix each sample by pipette aspiration action.
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 X 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

Description	Part Number
Thermo Scientific™ HyperSep™ 96-well Vacuum Manifold	60103-351
Thermo Scientific™ Vacuum Pump, North American Version	60104-243
Thermo Scientific™ WebSeal™ Mat (Solvent Resistant) Square Well	60180-M120

Current versions of product instructions are available at separatedbyexperience.com/chromexpert

Learn more about HyperSep dispersive SPE extraction and clean-up products at separatedbyexperience.com/chromexpert