Resprep

Protein and Particulate Removal That's Fast, Painless, and Effective

Resprep PPT^3 96-Well Plates

Restek's line of Resprep sample preparation products has expanded to include new 96-well protein precipitation (PPT) plates. Prepare serum, plasma, and other biological samples using the name you trust for quality, cleanliness, and performance.

RESTEK Pure Chromatography

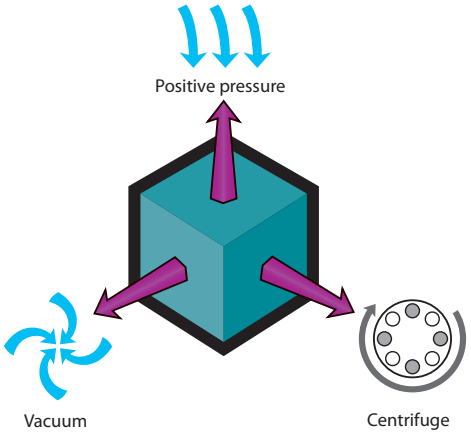
www.restek.com/PPT3

Resprep PPT^3 96-Well Plates

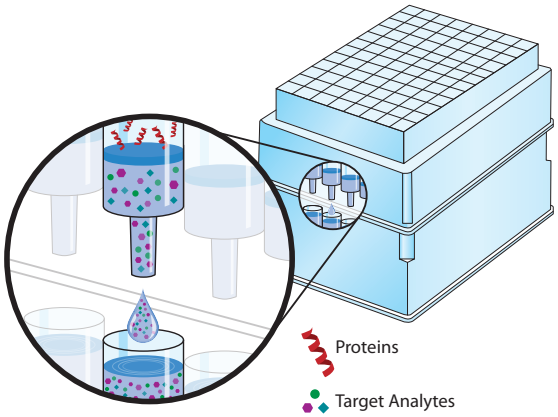
- Polypropylene protein precipitation (PPT) 96-well plates offer highly efficient >99% protein removal.
- Minimum 24-hour drip-free feature for easy in-well protein precipitation—no fear of backflushing or contamination.
- Built-in, dual-layer membrane with different porosities in each layer prohibits clogging and speeds filtration.
- Solvent-first method streamlines sample preparation.
- 2.0 mL deep well—suitable for mixing by vortex or pipette.
- 3-way versatility for filtration—compatible with all common devices:
  - Vacuum manifold
  - Positive pressure manifold
  - Centrifugation
- Can also be used to increase throughput in general filtration applications.

Order today at **www.restek.com/PPT3**

Pick your filtration method, then pick up a Resprep PPT^3 96-well plate and get to work.



Recover your target analytes and leave the protein behind.



Reliably Drip-Free, Even with Aggressive Solvents

While some competitors start to drip almost immediately, Resprep PPT^3 96-well plates will not drip until vacuum, positive pressure, or centrifuge is applied—whether you are using acetonitrile or more aggressive solvents like methylene chloride (Table I). In fact, our testing has shown drip-free performance for over 24 hours!

Table I: Drip test comparing commonly available protein precipitation (PPT) plates using methylene chloride solvent.

	# of wells dripped			
	Resprep PPT^3	**Competitor A**	**Competitor B**	**Competitor C**
5 minutes	**0**	5	0	2
10 minutes	**0**	9	1	6
15 minutes	**0**	9	4	7
30 minutes	**0**	9	8	9

Highly Efficient Protein Removal, Consistently Greater than 99%

Drips or no drips, it is paramount that your protein precipitation plate also performs its primary job—protein removal—exceptionally well. Resprep PPT[3] 96-well plates remove over 99% of protein from biological samples whether your method calls for the solvent or the matrix to be added first (Table II).

Table II: Protein removal efficiency for Resprep PPT[3] plates as measured by the Bradford assay using acetonitrile solvent and plasma sample matrix.

	% Protein Removal	
	Solvent-First	Matrix-First
Analysis 1	99.1	99.2
Analysis 2	99.4	99.2
Analysis 3	99.5	99.2
Analysis 4	99.6	99.2
Analysis 5	99.5	99.5
Analysis 6	99.5	99.5
Average	99.4	99.3

Excellent Recovery of Target Analytes

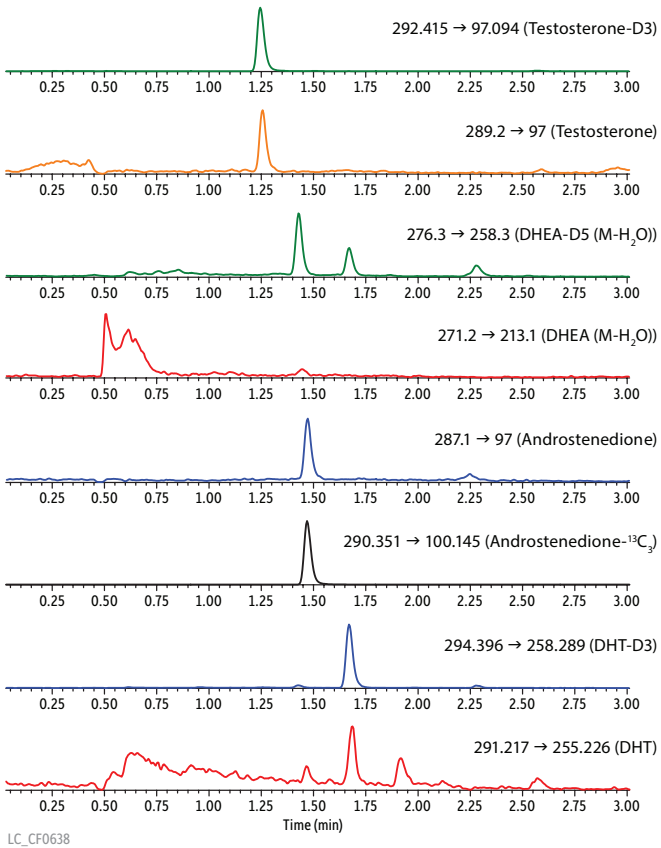
In addition to highly efficient protein removal, Resprep PPT[3] 96-well plates offer superior precision and accuracy, with mean recovery and relative standard deviation (RSD) values all falling well within acceptable limits (Table III).

When paired with Restek's Raptor SPP LC columns, Resprep PPT[3] 96-well plates offer outstanding chromatographic results (Figure 1).

Table III: Mean recovery and RSD values for steroids in serum.

	Conc. (ng/mL)		Intraday (n=6)	Interday (n=3, three days)
DHT	low	3	88.7 ± 2.1	93.6 ± 5.8
	mid	60	97.9 ± 0.7	96.2 ± 8.1
	high	120	107.2 ± 0.8	102.1 ± 8.5
Androstenedione	low	3	94.8 ± 1.0	98.8 ± 4.4
	mid	60	94.8 ± 0.8	94.4 ± 3.2
	high	120	94.4 ± 1.0	97.8 ± 4.2
DHEA	low	3	100.5 ± 10.5	97.2 ± 10.4
	mid	60	101.2 ± 1.6	97.9 ± 8.0
	high	120	103.4 ± 0.7	101.1 ± 7.2
Testosterone	low	3	95.1 ± 1.5	99.3 ± 5.1
	mid	60	92.8 ± 1.3	95.0 ± 2.6
	high	120	97.6 ± 2.2	98.9 ± 2.3

Figure 1: Androgen Hormones in Beagle Serum (LOQ) on Raptor C18 by LC-MS/MS.



Peaks	tR (min)	Precursor Ion	Product Ion 1	Product Ion 2
1. Testosterone-D3*	1.25	292.4	97.0	-
2. Testosterone	1.25	289.2	97.0	109.0
3. DHEA-D5*	1.42	276.3	258.3	-
4. DHEA	1.44	271.2	213.1	253.2
5. Androstenedione	1.47	287.1	97.0	109.0
6. Androstenedione-$^{13}C_3$*	1.47	290.4	100.1	-
7. DHT-D3*	1.67	294.4	258.3	-
8. DHT	1.68	291.2	159.2	255.2

*Internal standard

Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor C18 EXP guard column cartridge 2.7 µm (cat.# 9304A0252)
Temp.: 40 °C

Sample
Diluent: See extraction procedure
Conc.: 1 ng/mL
Inj. Vol.: 25 µL

Mobile Phase
A: Water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	50	50
2.00	0.4	35	65
2.01	0.4	50	50
4.00	0.4	50	50

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes Extraction Procedure:

1. A 300 µL aliquot of a 5 ng/mL internal standard mix prepared in acetonitrile + 0.1% formic acid was transferred to a Resprep PPT[3] 96-well plate (cat.# 26489-2).
2. A 100 µL aliquot of double charcoal stripped beagle serum fortified at 1 ng/mL was added.
3. The Resprep PPT[3] 96-well plate was placed on top of a 2.0 mL 96-well plate reservoir (cat.# 26493) and capped with a sealing mat.
4. The sample was vortexed for 30 seconds at ~2,000 rpm, then allowed to precipitate for 3 minutes.
5. The sealing mat was removed and the sample was filtered using a vacuum manifold set at 0.02 mPa for 5 minutes, followed by 0.04 mPa vacuum for an additional 5 minutes.
6. The sample extract was diluted 1:1 in water prior to injection.

Streamlined Sample Preparation with Solvent-First Method

By following our recommended sample preparation steps (Figure 2), you can speed up and simplify your workflow. For full instructions, visit **www.restek.com/PPT3** and click on the instruction sheet.

Figure 2: Overview of recommended sample preparation steps for Resprep PPT^3 plates.

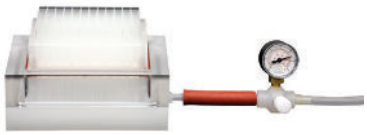
1) Add solvent to Resprep PPT^3 plate.

2) Introduce sample to Resprep PPT^3 plate.

3) Vortex.

4) Place collection plate on vacuum manifold.

5) Place Resprep PPT^3 plate and spacer (if needed) on collection plate. Apply vacuum.



6) Dilute sample to match initial mobile phase (if needed); transport collection plate to LC-MS/MS autosampler.

Resprep PPT^3 96-Well Plates

Description	Well Shape	qty.	cat.#
Resprep PPT^3 96-Well Plate	2 mL square	2-pk.	26489-2
Resprep PPT^3 96-Well Plate	2 mL square	5-pk.	26489-5

Well Plates

Ideal for use as collection plates when paired with Resprep PPT^3 96-well plates.

Description	Well Shape	Well Bottom	qty.	cat.#
0.45 mL 96-Well Plates	round	conical	20-pk.	26497
0.45 mL 96-Well Plates	round	conical	case of 120	26496
1.3 mL 96-Well Plates	round	round	5-pk.	26495
1.3 mL 96-Well Plates	round	round	case of 50	26494
2.0 mL 96-Well Plates	round	round	5-pk.	26493
2.0 mL 96-Well Plates	round	round	case of 60	26492

Also be sure to visit **www.restek.com/LC** for our full selection of Ultra, Pinnacle DB, and Raptor LC columns.

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

Lit. Cat.# BASS2301C-UNV