

Instructions for HybridSPE®-PLus 96-Well Plates

Product Description

Phospholipids are a major component of all cell membranes and it is well-known that phospholipid contamination is one of the principal causes of ion-suppression when analyzing small molecules by LC-MS in biological sample matrices. In addition, phospholipids can build up on analytical columns and elute unpredictably. This can greatly impact the accuracy of downstream LC-MS analyses. Buildup of phospholipids on analytical columns also increases the frequency with which columns need to be replaced, increasing instrument downtime and column replacement costs while negatively impacting laboratory throughput and turnaround time.

Due to the inherent amphiphilic nature of phospholipids (hydrophobic tail and polar head group), they are often co-extracted with a broad range of analytes of interest during sample preparation. HybridSPE-Phospholipid technology (available in both plate and cartridge formats) is a simple sample cleanup platform designed for the gross level removal of endogenous protein and phospholipid interference from biological sample matrices prior to LC-MS or LC-MS/MS analysis. Proteins are removed via precipitation/filtration and phospholipids are removed using a unique and highly selective Lewis acid-base interaction between the proprietary zirconia modified silica sorbent and the phosphate moiety present on all phospholipids. This unique retention mechanism allows HybridSPE-Phospholipid technology to separate phospholipids from analytes of interest that competitive products may not be able to address due to their use of a standard hydrophobic retention mechanism. Samples processed via HybridSPE-Phospholipid technology are suitable for use with both ultra high pressure liquid chromatography (UHPLC) and traditional HPLC.

Standard Protocol for In-Well Sample Preparation on the HybridSPE-PLus Well Plate

1. Add 100 µL of each plasma/serum sample (containing internal standards if required) to individual wells on the HybridSPE-PLus plate. Add 300 µL of precipitation solvent to each well containing sample (see Procedural Note #6 for solvent selection).
2. Place a sheet of sealing film securely over the plate and agitate on an oscillating table for 4 minutes at a setting of 1,000 oscillations per minute. (Agitation may also be performed via a robotic liquid handler through repeated draw and dispense cycles).
3. Place a collection plate in the PlatePrep vacuum manifold and position the HybridSPE-PLus plate on top of it. Remove the sealing film, engage and apply vacuum (10" Hg) until samples have fully passed through the HybridSPE-PLus packed bed (a minimum of 4 minutes).
4. Remove the collection plate from the vacuum manifold, cover with a pierceable sealing film or cap mat to minimize evaporative losses and analyze directly via LC-MS. No further processing of the sample is necessary unless concentration of the eluent is desired.

Procedural Notes to Maximize Recovery and Reproducibility

1. Minimize the distance between the collection plate and the bottom of the HybridSPE-PLus plate to reduce the potential for well-to-well cross-contamination. Spacers (such as empty collection plates) may be used to properly space the HybridSPE-PLus plate and collection plate.
2. Any unused wells on the HybridSPE-PLus plate should be covered with sealing tape or film prior to engaging the vacuum to help maintain consistent vacuum pressure and minimize flow variability across the plate.
3. Leaving the vacuum engaged after the precipitated sample has completely eluted from the HybridSPE-PLus packed bed may result in apparent analyte recoveries >100%, due to solvent evaporation. Internal standards can be used to correct for this.
4. Samples should be analyzed directly after processing. Storing processed samples in an improperly sealed collection plate for an extended time may result in evaporation and misleadingly elevated recoveries.
5. When agitating samples with a robotic liquid handler, consider using wide bore tips, such as Biohit® wide bore pipette tips (Sigma-Aldrich #Z709980-960EA), to minimize clogging and improve precipitation efficiency.

6. Precipitation Solvents:

The standard precipitation solvent recommended for use with the HybridSPE-PLus plate is LC-MS grade acetonitrile with 1% formic acid. If recoveries are less than desired for a specific analyte when using the standard precipitation solvent, consider alternatives in the table below:

Precipitation Solvent	Use	Compound Type
Acetonitrile with 1% formic acid	Standard precipitation solvent. Works well for most applications.	Most compounds - (especially acidic compounds)
Methanol with 1% ammonium formate	Applications with lower than desired absolute recovery using standard precipitation solvent.	Some basic compounds and acid-labile analytes
Acetonitrile with 0.5% citric acid	Applications with lower than desired absolute recovery using standard precipitation solvent.	Some basic compounds and mild to moderate chelators

NOTE: When acetonitrile with 0.5% citric acid is used as the precipitation solvent, it is recommended that the HybridSPE-PLus plate be pre-conditioned with 400 µL of precipitation solvent prior to following the standard protocol for in-well sample preparation on the HybridSPE-PLus well plate.

After the initial method is developed, further optimization may be desired to enhance recoveries for specific analytes or applications. For assistance with sample prep optimization, please contact Technical Service at 800-325-5832 or 314-286-8032.

7. When working with fresh serum or plasma samples, an offline protein precipitation method prior to phospholipid removal may facilitate more efficient protein precipitation.

For Offline Precipitation:

- Add 100 µL of each plasma/serum sample (containing internal standards if required) to each centrifugation tube or individual wells of a 96-deep well collection plate.
- Add 300 µL of precipitation solvent to each tube or well containing sample.
- Facilitate precipitation by agitating/vortexing for 1-3 minutes.
- Remove precipitated protein by centrifugation (3,000 rpm for 2-5 minutes) or by passing through a 96-well protein precipitation filter plate.
- Using a pipette, transfer sample supernatants to the HybridSPE-PLus plate and follow the standard protocol starting at Step 3.

In addition to HybridSPE-Phospholipid technology, we provide the following premier selection of proven tools and consumables for your entire sample prep and LC-MS workflows.

- Ascentis® Express HPLC/UHPLC Columns improve throughput and sensitivity allowing you to process more samples
- LC-MS Mobile Phase Solvents and Additives are pre-tested for LC-MS applicability
- LC-MS Ultra CHROMASOLV® Solvents, Blends and Additives are suitable for UHPLC-MS
- Cerilliant® Certified Spiking Solutions® and Certified Reference Materials are manufactured and tested specifically for use as reference standards for laboratories performing bioanalysis, therapeutic drug monitoring, diagnostic and toxicology testing
- Biocompatible SPME probes are for LC analysis of difficult or precious samples in biological matrices
- Supel™-Select SPE tubes and well plates for sample prep needs
- Astec CHIROBIOTIC® CSPs for enantiomer separations under RP and LC-MS conditions
- Low adsorption vials for LC-MS applications

Frequently Asked Questions

1. Can I use HybridSPE-PLUS plates with smaller volumes (e.g., 20-40 µL) of plasma?

HybridSPE-PLUS plates were designed for use with sample volumes between 100 and 300 µL; however our HybridSPE-Phospholipid Small Volume plate is available for processing sample volumes between 20-40 µL.

2. Can I increase assay sensitivity by either increasing sample volume and/or concentrating (evaporation and reconstitution) the HybridSPE-PLUS eluent?

Larger plasma/serum sample volumes (up to 300 µL) may be applied to the HybridSPE-PLUS plate, however some phospholipid breakthrough may occur (see table).

Plasma Volume	% Phospholipid Removal
100 µL	99.5%
200 µL	94.9%
300 µL	88.0%

When increasing sample volume, it is important to increase the volume of the precipitation solvent accordingly in order to maintain a 1:3 volume/volume ratio of plasma to precipitation solvent.

Evaporation of the sample eluent and reconstitution of the sample in a smaller volume of mobile phase is another option to increase sensitivity.

3. Why might LC-MS ion-suppression still be evident after processing samples with HybridSPE-PLUS?

HybridSPE technology removes gross levels of precipitated proteins and phospholipids from biological samples, however other chemical entities that can lead to ion-suppression may also be present in the sample. If present, it may be necessary to adjust chromatographic conditions to separate analytes of interest from interfering matrix components. Examples of non-phospholipid interferences found in biological samples that can lead to ion-suppression include:

- Anti-coagulants used to prepare plasma from blood
- Phthalates, plasticizers and other extractables – found in plastic-ware and seals used to collect/store samples
- Polyethylene glycol – common dosing vehicle for many drugs

4. Why is the resulting eluent lower in volume than what was applied to the HybridSPE packed bed?

Eluent volume is reduced due to the dead volume of the packed bed as well as evaporative losses during processing. Typical volume loss is 100-120 µL. Addition of an internal standard is recommended prior to processing to account for any losses.

5. What are the effects of conditioning the phase prior to processing?

Conditioning could result in a dilution effect resulting in lower absolute recoveries. See Question #2 for recommendations to improve sensitivity.

6. What if my analyte of interest is not soluble in acetonitrile?

If the analyte of interest is not soluble in acetonitrile, other precipitation solvents may be used. See "Precipitation Solvents" in the Procedura Notes section of this data sheet.

Featured Products

Description	Qty.	Cat. No.
HybridSPE-PLUS Plates		
HybridSPE-PLUS 96-well Plate, 50 mg/well	1	575659-U
HybridSPE-PLUS 96-well Plate, 50 mg/well	20	575673-U
HybridSPE-Phospholipid Small Volume Plates		
HybridSPE-Phospholipid Small Volume 96-well Plate, 15 mg/well	1	52794-U
HybridSPE-Phospholipid Small Volume 96-well Plate, 15 mg/well	20	52798-U
Plate Accessories		
Collection Plates:		
96 Round/Deep Well Collection Plates, PP for HybridSPE-PLUS	60	Z717266-60EA
Sealing Mats and Films:		
Round Well Cap Mat, Pierceable for HybridSPE-PLUS	50	575680-U
96 Well-Plate Pre-cut Sealing Films	100	Z721581-100EA
Supelco PlatePrep Vacuum Manifold	1	57192-U
Filter Accessories		
96-well Protein Precipitation Filter Plate (for offline protein precipitation)	1	55263-U

Related Products

Description	Qty.	Cat. No.
Equipment		
KNF Laboport® Vacuum Pumps	1	Inquire
SPE Vacuum Pump Trap Kit	1	57120-U
Supelco PlatePrep Vacuum Manifold	1	57192-U
SPE Manifold Gauge/Bleed Valve, Remote In-Line Design	1	57161-U
IKA® VORTEX 3, mixer (230V)	1	Z654779
IKA® VORTEX 3, mixer (115V)	1	Z654760
Precipitation Solvents, Blends and Additives		
LC-MS CHROMASOLV Acetonitrile ≥99.9%	250 mL, 1L, 2.5L	34967
LC-MS CHROMASOLV Methanol ≥99.9%	1L, 2.5L	34966
LC-MS CHROMASOLV Acetonitrile with 0.1% formic acid	2.5L	34668
Formic acid puriss p.a., eluent additive for LC-MS	10 x 1mL, 50 mL	56302
Ammonium formate, puriss p.a., eluent additive for LC-MS	50 g	55674
Citric Acid, ACS Reagent Grade, ≥99.5%	5 g, 100 g, 500 g, 2.5 kg, 12 kg	251275
Cartridges		
HybridSPE-Phospholipid Ultra Cartridge 30 mg/1 mL	100	55269-U
HybridSPE-Phospholipid Cartridge 500 mg/6 mL	30	55267-U
HybridSPE-Phospholipid Cartridge 30 mg/1 mL	100	55261-U
HybridSPE-Phospholipid Cartridge 30 mg/1 mL	200	55276-U
Cartridge Accessories		
Visiprep™ DL Solid Phase Extraction Cartridge Manifold:		
12-Port Model	1	57044
24-Port Model	1	57265
Disposable Valve Liners, PTFE (for Visiprep DL Manifold)	100	57059
Visiprep Solid Phase Extraction Cartridge Manifold:		
12-Port Model	1	57030-U
24-Port Model	1	57250-U

Trademarks

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 IKA — IKA Works, Inc.