Empore™
96 Well Solid Phase Extraction Plate
MPC – Mixed Phase Cation
(Standard and Deep Well Plates)

Empore™ Solid Phase Extraction Plates are designed for the solid phase extraction (SPE) of analytes from 96 samples. The MPC (Mixed Phase Cation) bonded silica plates contain both hydrophobic (C8) and strong cation exchange (benzene sulfonic acid) functionalities. This combination of functionalities offers the potential to perform selective extractions of amine-containing analytes or the ability to extract acidic and neutral analytes separately from basic analytes.

Note: Empore Sample Preparation Products are intended for solid phase extraction during scientific research only. These products are not intended for use in medical devices or in assessment and treatment of clinical patients.

Section:
1. Applications of Mixed Phase Cation (MPC) Sorbent
2. Selective Extraction of Amine-Containing Analytes
3. Reversed Phase Method for Acidic/Neutral Analytes
4. Empore™ Manifold Assembly and Instructions for Use
5. Product Characteristics
Section 1: Applications of Mixed Phase Cation (MPC) Sorbent

The successful use of mixed phase chemistry relies on adsorbing an analyte by reversed phase and/or cation exchange interactions. The optimal analyte typically contains a hydrophobic region and at least one amine group that can be protonated. The pH of the sample load solution is very important to ensure that the amine group is fully protonated (sample pH should be at least 2 pH units below the pKa of the targeted amine group).

When MPC sorbent is used in the reversed phase mode for a neutral analyte, it may be substituted into an existing C8 or C18 bonded silica method. Acidic analytes can be retained using reversed phase methodology if the pH can be lowered to make the analyte neutral.
Section 2: Selected Extraction of Amine-Containing Analyte
Cation Exchange Method

Step 1: Sample Pretreatment
- Adjust sample pH to at least two pH units below the pKa of basic analytes to ensure the amine group and sorbent are charged. This is needed for retention of cationic isolates.
- A low ionic strength buffer, e.g., 0.05 to 0.1 M buffer (pH 6 or lower) is recommended for sample pH adjustment. Low ionic strength minimizes competition for available ion exchange sites.

Step 2: Condition
- Insert a waste collection tray in the vacuum manifold. Place the manifold collar and Empore™ plate onto the manifold.
- Add 100 µL of methanol to each well and wait 30 seconds before proceeding to Step 3.

Step 3: Rinse/Sorbet Equilibration
- Add 200 µL of water to each well. Apply vacuum until all wells have drained but are not dry.
- Add 200 µL buffer (pH 6.0 or lower) to charge the sorbent. Apply vacuum until all wells have drained. Do not allow wells to dry.
Section 2: Selected Extraction of Amine-Containing Analyte
Cation Exchange Method (continued)

Step 4: Load

- Add a minimum of 100 µL of prepared sample to each well. (1:2 sample:buffer ratio recommended). Apply vacuum until all wells have drained.

Step 5: Wash

- Add 500 µL or twice the sample load solution volume of water to thoroughly rinse the extraction disk, prefilter and well. Apply vacuum until all wells have drained.
- Add 500 µL 0.1 M acetic acid or 0.1 N HCl. Apply vacuum until all wells have drained and dry for about 30 seconds.

Step 6: Elute Acidic and Neutral Analytes/Wash

- If analyzing filtrate: Remove the waste collection tray and replace it with a collection plate. Place the manifold collar and Empore plate onto the manifold.
  - Add 150 to 300 µL of organic elution solvent to each well.
  - Wait 30 seconds for the eluent to soak into the membrane and initiate elution before applying the vacuum. Then proceed to step 7.

- If not analyzing filtrate: Keep waste collection tray in place and proceed to Step 7.
Section 2: Selected Extraction of Amine-Containing Analyte
Cation Exchange Method (continued)

**Step 7: Wash**

- Replace the collection plate with a waste collection tray.
- Add 500 µL of hexane to each well to remove nonpolar interferences and apply vacuum to drain wells.
- Add 500 µL methanol to each well to remove polar interferences and apply vacuum to drain wells.

**Step 8: Elute Basic Analytes**

- Replace the waste collection tray with a collection plate.
- Add 150 to 300 µL CH₂Cl₂/isopropanol/NH₄OH (78:20:2, v/v/v), (Important: prepare fresh solution daily).
- Wait 30 seconds for the eluent to soak into the membrane and initiate elution before applying the vacuum.
Section 3: Reversed Phase Method for Acidic/Neutral Analytes

Reversed Phase Method for Acidic/Neutral Analytes

- Adjust sample pH to at least two pH units below the pKa of analytes.
- A low ionic strength buffer, e.g., 0.1 M buffer (pH 6 or lower) is recommended for sample pH adjustment. (Lowering the pH ensures the acidic analyte is protonated for better retention by the sorbent.)

- Insert a waste tray in the vacuum manifold. Place the manifold collar and Empore™ plate onto the manifold.
- Add 100 µL of methanol to each well and wait 30 seconds before proceeding to Step 3.
- Add 200 µL of water to each well. Apply vacuum until all wells have drained but are not dry.
- Add a minimum of 100 µL of prepared sample to each well. Apply vacuum until all wells have drained.
Section 3: Reversed Phase Method for Acidic/Neutral Analytes
Reversed Phase Method for Acidic/Neutral Analytes (continued)

Step 5: Wash
- Add 500 µL or twice the sample load solution volume of water or methanol/water (10/90, v/v) to thoroughly rinse the extraction disk, prefilter and well. Apply vacuum until all wells have drained.

Step 6: Elute
- Add 150 to 300 µL methanol, acetonitrile, or hexane/ethyl acetate (50:50, v/v)
- Wait 30 seconds for the eluent to soak into the membrane and initiate elution before applying the vacuum.
Section 4: Empore™ Manifold Assembly and Instructions for Use
96 Well Plate Vacuum Manifold – Catalog #610

A. Waste Collection

B. Elution
### Manifold Use

- Seal any unused wells in the extraction disk plate with sheets of Empore™ Cat. 660 Sealing Tape to maintain uniform vacuum.
- For conditioning, loading and wash steps, place waste collection tray inside manifold base.
- Place manifold collar on top of manifold base.
- Replace waste tray with collection plate for elution of analytes. Check collection plate compatibility to assure the nozzle and collar of the Empore plate fit inside the collection well.
- Use the spacers provided with the Empore manifold to adjust collection plate height.
- Fit the nozzles and collars of the Empore plate into the top of the well of the collection plate. Vacuum will lower the nozzle into the collection plate wells during elution, minimizing the potential for cross contamination between wells.
Section 5: Product Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Composition</td>
<td>90% or greater sorbent particle (nominal)</td>
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<tr>
<td></td>
<td>10% or less PTFE</td>
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<tr>
<td>Well Volume</td>
<td>1.2 mL for standard well plates</td>
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<tr>
<td></td>
<td>2.5 mL for deep well plates</td>
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<tr>
<td>Bed Volume</td>
<td>18 µL/well</td>
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<tr>
<td>Particle Size</td>
<td>32 µm (nominal)</td>
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<tr>
<td>Sorbent Mass</td>
<td>15 mg/well (nominal)</td>
</tr>
<tr>
<td>pH Range</td>
<td>Stable between 2 and 12 under normal use conditions</td>
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<tr>
<td>Prefilter</td>
<td>Graded density polypropylene</td>
</tr>
</tbody>
</table>

Reading the Plate Label

![Empore Extraction Disk Plate](image)

Membrane lot number  Sorbent chemistry

Refer to the label on the Empore Extraction Disk Plate (sample shown above) to identify the sorbent chemistry and membrane lot number. MPC = Mixed Phase Cation.
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