



Empore™

96-Well Solid Phase Extraction Plate

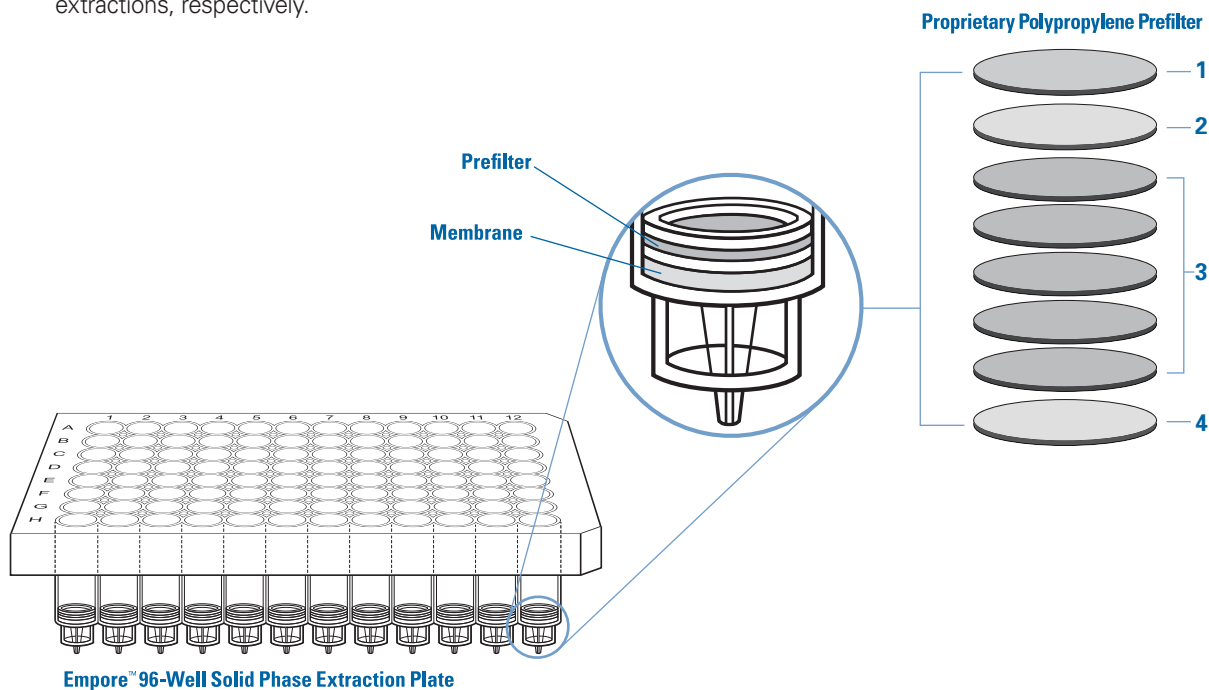
C8 (Octyl) and C18 (Octadecyl)

General Information

Empore™ Solid Phase Extraction Plates are designed for the simultaneous solid phase extraction of analytes from 96 samples. The C8 and C18 bonded silica sorbents combine good retention capacity with high recoveries. These bonded silicas are endcapped to minimize polar interactions. Empore™ C8 and C18 96-Well Solid Phase Extraction Plates are recommended for moderately non-polar and strongly non-polar analyte extractions, respectively.

Product Information

The Empore C8 and C18 plates are available in a standard well, 1.2 mL volume size (C8 - Cat. 6014SD, C18 - Cat. 6015SD) and a deep well, 2.5 mL volume size (C8 - Cat. 6314SD, C18 - Cat. 6315SD). If sample or reagent volumes exceed the volume of the well, multiple aliquots of solution may be used.



Instructions For Use – Generic Reversed Phase SPE Method

Step 1: Condition

Insert a waste tray in the vacuum manifold. Place the manifold collar and Empore™ plate onto the manifold. Add 100 μ L of methanol to each well and wait 30 seconds before proceeding to Step 2.

Step 2: Rinse

Add 200 μ L or more of water or buffer to each well. Apply vacuum until all wells have drained. Turn off vacuum as soon as wells have emptied. Drawing vacuum longer may dry out extraction disk and negatively impact recoveries.

Step 3: Load

Add a minimum of 100 μ L of prepared sample to each well. Apply vacuum until all wells have drained.*

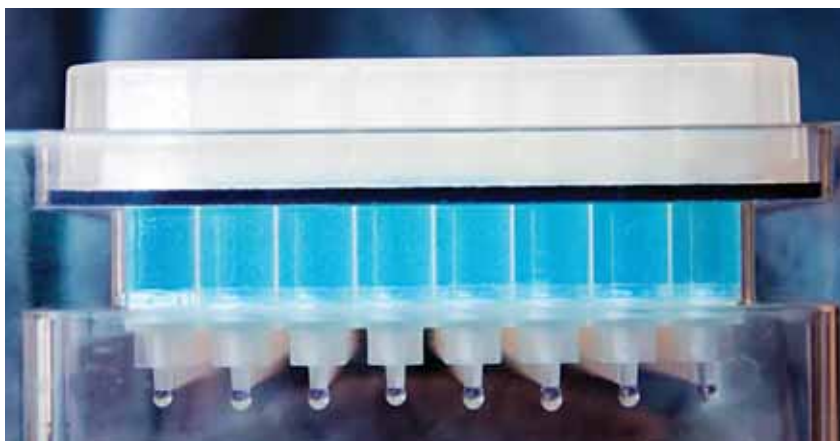
Step 4: Wash

Add at least 500 μ L of water to thoroughly rinse the extraction disk, prefilter, and well. Apply vacuum until all wells have drained. Repeat with a second aliquot of water or buffer.

Step 5: Elute

Replace the waste tray with a collection plate. Align collar and nozzle with collection plate wells. Add 150 μ L of organic solvent to each well. **Wait 30 seconds.** Apply vacuum until all wells have drained.

* Suggested flow rate for the load and elution steps is 2 mL/minute. Adjust vacuum settings as needed to obtain adequate flow rates. Viscous samples may require greater than 8 inHg (0.3 bar) to obtain a 2 mL/minute flow rate during the loading step.



Suggestions for Method Optimization

Sample Preparation

- Add acid to samples if analytes are highly bound to serum or plasma proteins.
- Adjust the sample pH two units above the pKa of basic analytes or two units below the pKa of acidic analytes to suppress ionization and enhance the recovery of acidic and basic analytes.
- If sample flow problems are encountered when adding samples directly to extraction disk plate:
 - Dilute sample up to 1:4 with water or buffer.
 - Centrifuge samples and add the supernatant to the extraction disk plate.

Conditioning

- Discontinue vacuum after the wells have drained. If the wells dry out, repeat the conditioning steps.
- A vacuum setting of 8 inHg (0.3 bar) or greater is recommended for the rinse step.

Sample Loading

- Flow rates of 2 mL/min generally provide a good starting point.
- Evaluate sample loading at both low (5-7 inHg/0.17-0.24 bar) and high vacuum (15-20 inHg/0.5-0.68 bar) and examine the effect on analyte recovery. If an analyte has low affinity for the sorbent, it may need to pass more slowly through the sorbent bed for sufficient attraction to occur.
- For samples less than 100 μ L in volume, dilute with water or buffer to 100 μ L or more.

Wash

- Water is suggested as a first wash to remove proteins that may precipitate and occlude the membrane.
- For cleaner eluates/improved chromatography, evaluate the following:
 - Keep wash composition constant and evaluate varying wash volume (at least twice the load volume).
 - Keep the wash volume constant and increase the organic concentration in increments of 5% to determine the amount of organic that results in the cleanest chromatography without loss of analyte.
 - Compare multiple consecutive washes to a single aliquot.

- To increase selectivity
 - Use an acidic organic wash of 1.0% organic acid (v/v) for acidic analytes.
 - Use a basic organic wash of 1.0% ammonium hydroxide (v/v) for basic analytes.

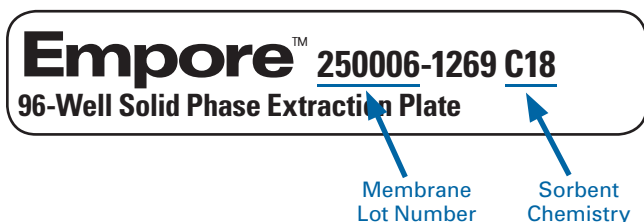
Elution

- Elution solvent composition should be optimized for individual analytes and LC mobile phase compatibility.
- Wait 30 seconds for the elution solvent to soak into the extraction disk and begin desorbing analyte before applying the vacuum.
- A flow rate of 2 mL/min generally provides a good starting point.
 - Evaluate eluting at both low (5-7 inHg/0.17-0.24 bar) and high vacuum (15-20 inHg/0.5-0.68 bar) and examine the effect on analyte recovery. If an analyte has a strong affinity for the sorbent, elution may need to occur more slowly to allow adequate desorption.
- Determine the minimum effective elution volume.
 - Increase elution volume in 25 μ L aliquots. For example, compare single 25, 50, 75, 100 μ L aliquots.
 - Compare a single larger volume aliquot of eluting solvent to two smaller volume aliquots.
- To increase sensitivity for dissociable analytes:
 - Compare 100% organic to 70 – 90% organic with 1% acid (v/v) as the elution solvent for basic analytes.
 - Compare 100% organic to 70 – 90% organic with 1% ammonium hydroxide (v/v) as the elution solvent for acidic analytes.
- Use minimum volume of elution solvent that gives optimal recoveries.
 - If additional dilution is necessary, add water or buffer directly to the collection plate.

Product Characteristics

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| Composition | 90% or greater sorbent particle 10% or less PTFE |
| Well Volume | 1.2 mL for standard well plates 2.5 mL for deep well plates |
| Bed Volume | 18 µL/well |
| Particle Size | 50 µm (nominal) |
| Sorbent Mass | 15 mg/well (nominal) |
| pH Range | Stable between 2 and 12 under normal use conditions |
| Prefilter | Graded density polypropylene |

Identifying C8 and C18 96-Well Solid Phase Extraction Plate Lot Numbers



Refer to the label on the Empore™ Extraction Disk Plate (sample shown above) to identify the sorbent chemistry and membrane lot number.

Note: Empore Solid Phase Extraction 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.

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