# **Empore**<sup>TM</sup> 96-Well Solid Phase Extraction Plates

# C8 (Octyl) and C18 (Octadecyl)

# **General Information**

Empore<sup>™</sup> 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<sup>™</sup> C8 and C18 96-Well Solid Phase Extraction Plates are recommended for moderately non-polar and strongly non-polar analyte extractions, respectively.

Empore<sup>™</sup> 96-Well Solid Phase Extraction Plate

# **Product Information**

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

# Colar Colar Membrane Proprietary Polypropylene Profilter



# 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 <sup>™</sup> plate onto the manifold. Add 100 µl of methanol to each well and wait 30 seconds before proceeding to Step 2.
Step 2: Rinse	Add 200 µl 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 extrac- tion disk and negatively impact recoveries.
Step 3: Load	Add a minimum of 100 $\mu I$ of prepared sample to each well. Apply vacuum until all wells have drained.*
Step 4: Wash	Add at least 500 $\mu l$ 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: Elution	Replace the waste tray with a collection plate. Align collar and nozzle with collection plate wells. Add 150 $\mu$ l of organic solvent to each well. <b>Wait 30 seconds.</b> 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 30 kPa (0.3 bar) to obtain a 2 ml/minute flow rate during the loading step.

Note: When using solvents or other chemicals, be sure to read and follow the manufacturer's precautions and directions for use.





# **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 30 kPa (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 (15 25 kPa/0.15 0.25 bar) and high vacuum (50 - 70 kPa/0.5 - 0.7 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 μl in volume, dilute with water or buffer to 100 μl 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 (15 25 kPa/0.15 0.25 bar) and high vacuum (50 70 kPa/0.5 0.7 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 μl aliquots. For example, compare single 25, 50, 75, 100 μl 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.



### Empore<sup>™</sup> 96-Well Solid Phase Extraction Plates – C8 (Octyl) and C18 (Octadecyl)

## **Product Characteristics**

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	10 mg/well (nominal)
pH Range	Stable between 2 and 12 under normal use conditions
Prefilter	Graded density polypropylene

#### **Disclaimer:**

All statements, technical information and recommendations herein are based on our tests we believe to be reliable, but the accuracy of completeness thereof is not guaranteed. Before using or specifying the product, user shall determine the suitability of the product for intended use. All questions of warranty and liability relating to this product are governed by the terms of the sale subject where applicable to the prevailing law.

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



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



#### AUTHORIZED DISTRIBUTOR

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