



WARNING: To reduce the risks associated with improper disposal and/or handling of contaminants in used plates: Take appropriate steps to assess the disposal required for any altered product or materials added to the product. (Alteration of the product or the addition of other materials to the product may require different disposal methods.)

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If Products are proven not to meet CDS specifications, the sole and exclusive remedy available and CDS's only obligation shall be, at CDS's option, to replace such quantity of Products which are proven out of specification or to refund the purchase price paid for Product.

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Empore™

High Performance Extraction Disk Plate

C2, C8, C18 Standard Density (SD)

Empore™ Extraction Disk Plates are designed for the solid phase extraction of analytes from biological samples. The bonded silica plates provide good retention and capacity, resulting in high recoveries. Empore bonded silicas are endcapped to minimize polar interactions and simplify reversed phase adsorption and elution.

Section:

1. *General Reversed Phase Solid Phase Extraction Method*
2. *Suggestions for Optimizing Recoveries*
3. *Empore™ Manifold Assembly and Instructions for Use*
4. *Product Characteristics*

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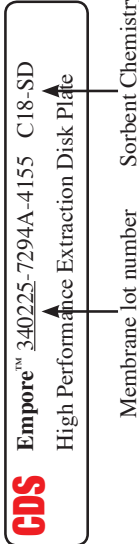


Section 4: Product Characteristics

Composition:	92% sorbent particle (nominal) 8% PTFE
Well Volume:	1.2 mL for standard well plates 2.5 mL for deep well plates
Bed Volume:	18 μ m/well
Particle Size:	50 μ m (nominal)
Sorbent Mass:	10 mg/well (nominal)
pH Range:	Stable between 2 and 12 under normal use conditions
Prefilter:	Graded density polypropylene



Reading the Plate Label

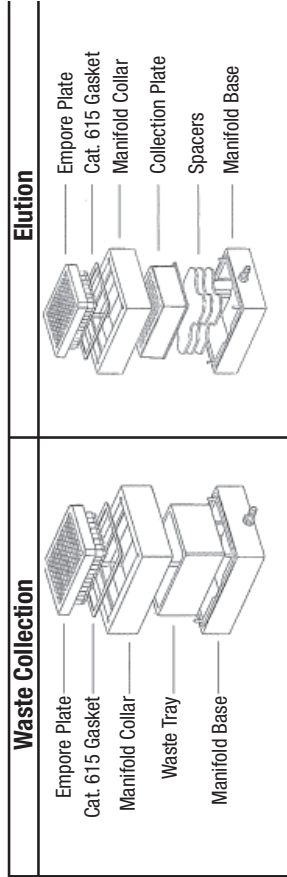


Refer to the label on the Empore™ Extraction Disk Plate (sample shown above) to identify the sorbent chemistry and membrane lot number.





Section 3: Empore™ Cat. 610 Vacuum Manifold



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 1: General Reversed Phase SPE Method

(Method optimization is discussed in Section 2)

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.

Add 200 μ L 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 impact recoveries.

Add a minimum of 100 μ L of prepared sample to each well. Apply vacuum until all wells have drained. An approximate flow rate of 2 mL/min is suggested.*

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

Replace the waste tray with a collection plate. Align collar and nozzle with collection plate wells. Add 150 μ L of eluting 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 inches Hg (0.3 bar) to obtain a 2 mL/minute flow rate during the loading step.*





Section 2: Suggestions for Method Optimization

Sample Preparation

- Adjust the sample pH two units above the pKa of the analyte for basic analytes or two units below the pKa of the analyte for 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.
- A vacuum setting of 8 inches Hg (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 inches Hg [0.17-0.24 bar]) and high vacuum (15-20 inches Hg [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 eliminate proteins that may precipitate and occlude the membrane.
- For cleaner eluates/improved chromatography, evaluate the following:
 - ♦ Keep 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 2% acetic acid (v/v) for acidic analytes
 - ♦ Use a basic organic wash of 2% ammonium hydroxide (v/v) for basic analytes

Elution

- Acetonitrile followed by water or buffer is suggested as the elution solvent to obtain maximum analyte recoveries. Elution solvent composition should be optimized by the user 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 inches Hg [0.17-0.24 bar]) and high vacuum (15-20 inches [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% acetonitrile with 2% acetic acid (v/v) as the elution solvent for basic analytes
 - ♦ Compare 100% organic to 70-90% acetonitrile with 2% ammonium hydroxide (v/v) as the elution solvent for acidic analytes
- To eliminate evaporation and reconstitution of the eluate (for direct injection of the eluate onto the LC/MS System):
 - ♦ Follow 100% organic elution with a second aqueous aliquot for enhanced mobile phase compatibility.
 - ♦ If additional dilution is necessary, add water or buffer directly to the collection plate.

