Empore™

Solid Phase Extraction Cartridges

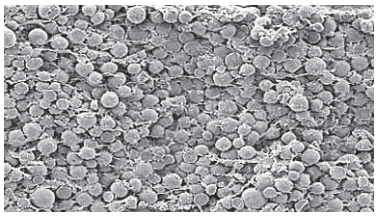
UR (Universal Resin)

General Information

Empore™ Solid Phase Extraction (SPE) Cartridges are designed for sample pretreatment to remove or minimize sample matrix and other interferences to "cleanup" a sample prior to analysis.

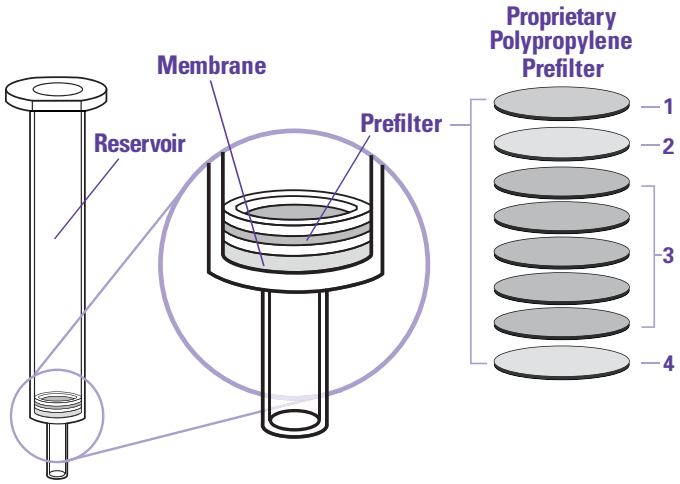
This procedure can also concentrate an analyte to achieve the desired sensitivity range of an analytical method. Compounds are isolated from complex mixtures by proper selection of a variety of sorbent chemistries.

The cartridge is molded from a polypropylene resin. An Empore™ Solid Phase Extraction Disk is secured in place at the bottom of each cartridge with a sealing ring. A proprietary prefilter is placed above the Empore disk. This prefilter aids in preventing particulates and macromolecules from reaching the underlying membrane and improves the flow of biological samples, such as serum and plasma, through the cartridge.



Empore™ Standard Density (SD) Resin Membrane

The prefilter is composed of polypropylene microfiber layers of graded densities. Three different densities are used, with the coarsest one on top and the finest at the bottom. The top two microfiber layers are individual layers of material. The third microfiber layer, having the smallest effective pore size, is on the bottom of the prefilter and contains five individual layers of material. A porous polypropylene support membrane comprises the final layer.



Product Information

Polymeric sorbents are commonly used for the solid phase extraction of analytes from complex sample matrices.

The Universal Resin (UR) sorbent is a terpolymer based on styrenedivinylbenzene and designed to provide good retention of a wide range of analytes. A single reversed phase sorbent can often be used to isolate and concentrate a variety of acidic, basic and neutral compounds. Method development time is saved by eliminating the need to screen a variety of sorbents.

The Empore™ Universal Resin Solid Phase Extraction Cartridge is available in a 3 mL volume which has an effective SPE membrane diameter of 7 mm. The Empore Universal Resin cartridge contains a Standard Density (SD) membrane which is composed of chromatographic particles commonly referred to as from 40-60 µm in size (actual mean size is about 44 µm). The standard density membrane has been optimized for improved flow rates for samples processed in most bioanalytical applications.

CDS Analytical

Extraction Method with Universal Resin Cartridge

The following method contains suggestions for 3.0 mL Empore™ Universal Resin Solid Phase Extraction Cartridges. Refer to the "Volume Guidelines" on the next page for volume suggestions.

Step 1: Condition

Insert a collection tube in the vacuum manifold, replace manifold cover and place an Empore universal resin cartridge in the appropriate position. Add 250 µL methanol to the cartridge and wait 30 seconds before proceeding to the next step.

Step 2: Rinse

Add 500 µL of water or buffer to the cartridge. Apply vacuum until the cartridge has drained. Turn off the vacuum as soon as the cartridge has drained to avoid drying the extraction disk.

Step 3: Load

Add prepared sample to the cartridge. Apply vacuum until the cartridge has drained.*

See "Suggestions for Method Optimization" below for sample preparation and sample volumes.

Step 4: Wash

Add 500 µL of water to thoroughly rinse proteins and salts from the extraction disk, prefilter and cartridge. Apply vacuum until the cartridge has drained. Repeat with a second 500 µL aliquot of water or organic/aqueous mixture. Dry the cartridge for 30 seconds to remove excess aqueous solution.

Step 5: Load

Replace the collection tube in the vacuum manifold. Add 300 µL eluting solvent to the cartridge. **Wait 30 seconds.** Apply vacuum until the cartridge has drained.

* Adjust vacuum as needed to start and maintain adequate flow rate. Viscous samples may require vacuum of 15-20 inHg (0.5-0.7 bar).

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

- 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.
- Use at least enough sample volume to cover the pre-filter. Recommendations for minimum sample volumes are found on the next page. If this minimum amount of sample is not available use a smaller extraction disk cartridge (e.g. 1ml). In the event that this is not possible, proceed as follows: Add prepared sample to the cartridge. Apply vacuum until the cartridge has drained. Determine the minimum recommended sample volume from the Volume Guidelines chart. Add this amount of sample preparation buffer to the cartridge. Apply vacuum until cartridge has drained.
- If sample flow problems are encountered when adding samples directly to the extraction disk cartridge:
  - Dilute sample up to 1:4 with water or buffer, maintaining appropriate pH
  - Centrifuge samples and add the supernatant to the extraction disk cartridge

Conditioning/Rinse

- Discontinue vacuum after the cartridges have drained.
- A minimum vacuum setting of 15-20 inches Hg (0.5-0.7 bar) is recommended for the rinse step.

Sample Loading

- Compare loading the sample at both low (5-10 inHg/0.17-0.24 bar) and high vacuum (15-20 inHg/(0.5-0.7 bar). An analyte with a low affinity for the sorbent may need to pass through the sorbent more slowly. A slower flow rate may improve retention of the analyte of interest.

Wash

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

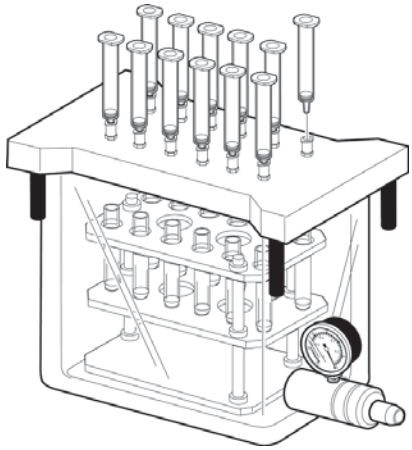
Elution

- Wait 30 seconds for the elution solvent to soak into the extraction disk to begin desorbing analytes before applying vacuum.
- Follow organic with 500 µL of water or buffer to maximize recoveries and enhance mobile phase compatibility.
- Compare eluting at both low (5-10 inHg/0.17-0.24 bar) and high vacuum (15-20 inHg/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 250 µL increments. For example, compare single 250 µL, 500 µL, 750 µL and higher aliquots.
  - Compare a single larger volume of elution solvent to two smaller elution 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 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 tube.

Cartridge Vacuum Manifold System

Vacuum Processing

Vacuum manifolds are commonly used to draw liquids through the cartridge. Manifolds accommodating 10 to 24 cartridges or more are available from several suppliers. Collection tubes are placed below each cartridge position to collect liquids.



Reversed Phase Extractions

The small bed mass of sorbent in the disk cartridge allows for the use of small solvent volumes. A general guide to solvent volumes for a universal resin cartridge SPE method is listed in the table below. Each assay will need further optimization in terms of selecting the best wash solvent composition (10% methanol as shown in the example will not be optimal for all assays) and the particular elution solvent (commonly methanol or acetonitrile).

Important Notes: It is recommended to optimize the volume of elution solvent to ensure that the minimum volume is used to elute the analyte reproducibly from the sorbent phase.

Volume Guidelines: Universal Resin Cartridge

Step	Solvent	3mL/7mm
Condition	Methanol	250 µL
Rinse	Water	500 µL
Load	Sample	≥250 µL
Wash	Water or Organic/Aqueous (10:90 v/v)	500 µL/500 µL
Elute	Organic solvent 100%	300 µL

Note: The volumes shown above are representative examples only. Methods may be optimized to accommodate smaller volume samples as long as the sample completely covers the disk and prefilter. Volumes may also be optimized to accommodate differing physical-chemical characteristics of the analyte, affinities of the analyte for the sorbent, strengths of eluting solvents or to meet a particular laboratory need.

Suggested Product Applications

Sorbent	Cartridge Size	Empore Cat #	Suggested Applications
Universal Resin Standard Density	7 mm/3 mL	4245SD	A range of basic, neutral, and acid compounds.

Product Characteristics

	Standard Density
Membrane Composition	90% or greater sorbent 10% or less PTFE
Prefilter Composition	Graded density polypropylene
Membrane Thickness	0.75 mm (nominal)
Particle Size	44 µ (nominal)
Pore Size	65 Å (nominal)
pH Range	Stable between 1 and 14 under normal use conditions

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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CDS Analytical, LLC Headquarter:
465 Limestone Road
Oxford, PA 19363-0277 USA
Tel.: 1-800-541-5963 (US); 1-610-932-3636 (International)
Website: www.cdsanalytical.com
Email: info@cdsanalytical.com

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