Empore™ Solid Phase Extraction (SPE) Cartridges are designed for sample pretreatment to remove or minimize sample matrix and other interferences to clean up 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™ 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.

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.

The Empore™ C8 cartridge is available in both standard density (SD) and high density (HD) membranes. The Empore™ C18 cartridge is available with an SD membrane. Standard density (SD) membranes are composed of chromatographic particles commonly referred to as from 40 - 60 μm in size (actual mean size is about 55 μm). The standard density membrane has been optimized for improved flow rates for samples processed in most bioanalytical applications. High density (HD) membranes are composed of chromatographic particles commonly referred to as from 10 - 12 μm in size. The high density membranes are designed for maximum extraction efficiency with minimal elution volumes of samples that have less matrix interference.
Empore™ Solid Phase Extraction Cartridges – C8 (Octyl) and C18 (Octadecyl)

Extraction Method with C8 or C18 Cartridges

The following method contains suggestions for 3.0 ml Empore™ C8 SD or C18 SD Solid Phase Extraction Cartridges. Refer to the “Volume Guidelines” on the next page for volume suggestions for 1 and 6 ml disk cartridges.

Step 1: Condition
Insert a collection tube in the vacuum manifold, replace manifold cover and place an Empore 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.*

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 buffer. Dry the cartridge for 30 seconds to remove excess aqueous solution.

Step 5: Elution
Replace the collection tube in the vacuum manifold. Add 250 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. SD cartridges generally require 15 - 35 kPa (0.15 - 0.35 bar) during the loading step. Viscous samples and HD cartridges may require vacuum of 50 - 70 kPa (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. 1 ml). 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 - 35 kPa (0.15 - 0.35 bar) is recommended for the rinse step.

Sample Loading
Compare loading the sample at both low (15 - 25 kPa/0.15 - 0.25 bar) and high vacuum (50 - 70 kPa/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 25 to 100 µl of water or buffer to maximize recoveries and enhance mobile phase compatibility.
- Compare 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 increments. For example, compare single 50, 75, 100 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.

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 disk cartridge SPE method using reversed phase sorbents (C8 and C18) is listed in the table below. Each assay will need some 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 that will elute the analyte reproducibly from the sorbent phase.

### Volume Guidelines: Empore™ C8 and C18 Cartridge

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>1 ml/4mm</th>
<th>3 ml/7mm</th>
<th>6 ml/10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>Methanol</td>
<td>150 µl</td>
<td>250 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Rinse</td>
<td>Water</td>
<td>300 µl</td>
<td>500 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Load</td>
<td>Sample</td>
<td>100 µl</td>
<td>250 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Wash</td>
<td>Water or Organic/ Aqueous (10:90, v:v)</td>
<td>300 µl</td>
<td>500 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Elute</td>
<td>Organic solvent 100 %</td>
<td>125 - 150 µl</td>
<td>150 - 275 µl</td>
<td>400 - 500 µl</td>
</tr>
</tbody>
</table>

Note: The volumes shown above are representative examples only. Volumes may differ between HD and SD membranes. 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

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Cartridge Size</th>
<th>Product Number</th>
<th>Suggested Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8-SD (Standard Density)</td>
<td>4 mm/1 ml</td>
<td>4114SD</td>
<td>Nonpolar compounds from water or biological fluid that are too highly bound to C18</td>
</tr>
<tr>
<td></td>
<td>7 mm/3 ml</td>
<td>4214SD</td>
<td></td>
</tr>
<tr>
<td>C8-HD (High Density)</td>
<td>4 mm/1 ml</td>
<td>4114HD</td>
<td></td>
</tr>
<tr>
<td>C18-SD (Standard Density)</td>
<td>4 mm/1 ml</td>
<td>4115SD</td>
<td>Nonpolar compounds for water or biological fluids</td>
</tr>
<tr>
<td></td>
<td>7 mm/3 ml</td>
<td>4215SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mm/6 ml</td>
<td>4315SD</td>
<td></td>
</tr>
</tbody>
</table>

Product Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Standard Density</th>
<th>High Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Composition</td>
<td>90 % or greater sorbent 10 % or less PTFE</td>
<td>90 % or greater sorbent 10 % or less PTFE</td>
</tr>
<tr>
<td>Prefilter Composition</td>
<td>Graded density polypropylene</td>
<td>Graded density polypropylene</td>
</tr>
<tr>
<td>Membrane Thickness</td>
<td>0.75 mm (nominal)</td>
<td>0.50 mm (nominal)</td>
</tr>
<tr>
<td>Particle Size</td>
<td>50 μ (nominal)</td>
<td>12 μ (nominal)</td>
</tr>
<tr>
<td>pH Range</td>
<td>Stable between 2 and 12 under normal use conditions</td>
<td>Stable between 2 and 12 under normal use conditions</td>
</tr>
</tbody>
</table>