PoraPak Rxn Cartridges
for post synthesis cleanup
Waters now offers PoraPak™ Rxn, a family of polymer-based chromatography products for superior cleanup of synthetic reactions. PoraPak Rxn products are available in two chemistries:

- PoraPak Rxn CX, a strong cation-exchange sorbent
- PoraPak Rxn RP, a reversed-phase sorbent.

PoraPak Rxn sorbents are available in fritted syringe-barrel devices in 6, 20 and 60 cc volumes. The resins are also sold in bulk units, and custom configurations are available on request.

NEW SOLUTIONS FOR FASTER RESULTS

PoraPak Rxn sorbents are based on copolymers that exhibit the following properties:

- Hard material that does not develop increasing back pressure with flow.
- Little swelling or shrinking across a range of solvents and pH extremes.
- Low hydraulic resistance enables flow by gravity.
- pH extreme tolerance without dissolution or hydrolysis, both limitations of silica-based sorbents.

This combination of physical and chemical properties makes PoraPak Rxn cartridges ideal for synthesis cleanup. The polymers characteristics and particle size maintain gravity, pressure - or vacuum - assisted flow; even when reaction mixtures contain precipitate that may contribute additional resistance to flow. The sample will still pass through the cartridge.

The polymer used in PoraPak Rxn products is resistant to shrinking or swelling in the organic solvents typically used in synthetic reactions. Tests with the following solvents demonstrate that the packed bed maintains good flow properties:

- DCE
- DMF
- DCM
- THF
- DMSO
- Acetone

Some Medicinal Chemists are familiar with silica-based chromatographic products for reaction cleanup. One of the limitations of these silica-based ion-exchange materials is pH. Silica will dissolve at high pH, while bonded phases are hydrolyzed at low pH; both conditions result in loss of sample and/or impurities (silica and bonded phase) collected in product fractions. PoraPak Rxn polymer-based chromatographic phases are stable at extreme pH. This feature permits using pH as a very powerful tool to create a separation, particularly in ion-exchange mode.

WATERS: A WORLD LEADER IN SEPARATIONS SCIENCE

PROVIDING SEPARATIONS SOLUTIONS

Waters is highly respected world wide for its expertise in chromatography. Coupled with our ability to seamlessly link critical instrumentation, chemistries, separation technologies, and software, this expertise puts us in a unique position to deliver value-added solutions to our customers.

MANUFACTURING

Our world-class manufacturing facilities are continuously expanded and upgraded to keep pace with market demand for our new and existing products. We manufacture under the highest quality standards in the industry, including ISO-9001:2000, ISO 13485:2003 and Current Good Manufacturing Practices (cGMP).
### CLEANUP CHALLENGES

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convert or remove high boiling solvent before next reaction step - product is a base.</td>
<td>Load reaction mix on PoraPak Rxn CX cartridge and pass through high boiling point solvent such as DMSO. Wash with methanol. Elute using 5% ammoniated methanol. Achieve sample enrichment and save time by evaporative removal of a low boiling point solvent before the next step.</td>
</tr>
<tr>
<td>Separate product (bases) from reactants and reaction solvent.</td>
<td>Load on PoraPak Rxn CX cartridge using a catch-and-elute strategy. Wash with methanol, and then elute with ammoniated methanol.</td>
</tr>
<tr>
<td>Remove TFA used in the reaction from bases.</td>
<td>Load reaction mixture on PoraPak Rxn CX cartridge using a catch-and-elute strategy. TFA passes through unretained. Wash with methanol and elute with ammoniated methanol.</td>
</tr>
<tr>
<td>Convert from a solvent used in the last synthesis before Prep LC to get sample in the starting mobile phase conditions.</td>
<td>Load on PoraPak Rxn CX cartridge using a catch-and-elute strategy. Wash with methanol and elute with ammoniated methanol. Evaporate the fraction and reconstitute the product in the Prep LC starting solvent.</td>
</tr>
<tr>
<td>Remove water from fractions collected from Prep LC for faster dry down.</td>
<td>Load on PoraPak Rxn CX cartridges using a catch-and-elute strategy. Wash with methanol and elute with ammoniated methanol.</td>
</tr>
</tbody>
</table>
**CHOSE THE BEST PRODUCT AND TECHNIQUE FOR YOUR CLEANUP**

PoraPak Rxn products are available in two sorbents: **CX**, a strong **Cation-exchange** resin and **RP**, a **Reversed-Phase** resin. The illustrations below recommend the sorbent to use and the separation technique to apply during cleanup. The choice of the appropriate sorbent and technique depends upon the nature of the reaction products, reactants, solvents, and/or preparative columns.

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### Reaction Products

**Bases**
- Technique: **Catch and Elute**
- Recommended Product: PoraPak Rxn CX

**Acids**
- Technique: **Pass Through**
- Recommended Product: PoraPak Rxn CX

**Neutrals**
- Technique: **Adsorption**
- Recommended Product: PoraPak Rxn RP

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### Reaction Components

**TFA removal**
- Technique: **Catch and Elute**
- Recommended Product: PoraPak Rxn CX

**Convert from high boiling point solvent for bases**
- Technique: **Catch and Elute**
- Recommended Product: PoraPak Rxn CX

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### Pre-and-Post Preparative Column

**Fractionate sample before Prep for bases**
- Technique: **Catch and Elute**
- Recommended Product: PoraPak Rxn CX

**Remove water from post column fraction for bases**
- Technique: **Catch and Elute**
- Recommended Product: PoraPak Rxn CX

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**Catch and Elute**

In the “catch and elute” technique, the reaction mixture is loaded onto the cartridge; the analytes of interest are retained by the sorbent. A wash step follows to remove additional reaction components from the cartridge. A strong solvent is used to elute the analytes from the cartridge. Sample concentration results when the final elution volume is smaller than the load volume.

**Pass Through**

In the pass-through technique, the sorbent does not retain the compounds of interest, but does retain some class(es) of compounds and impurities, that can be discarded with the column. Fractionation is achieved by the interaction between the impurities in the reaction mixture and the sorbent. No sample enrichment occurs during the solid-phase extraction (SPE) step.

**Adsorption**

In the adsorption chromatography technique on a reversed-phase sorbent, the neutral compounds are moving down the chromatographic column in the presence of organic solvent, albeit slowly, for small-percent organic washes. Load the reaction mixture in high water content (75% water for example), wash to remove polar compounds, and then increase the organic content in large increments to elute neutrals. The increase is determined by the hydrophobicity and MW of the compounds.

**Catch and Elute**

**Condition Step**
100% methanol

**Load Step**
Fraction collected contains acids, neutrals and reaction solvent

**Wash Step**
100%-methanol fraction contains more impurities, acids and neutrals

**Elution Step**
5% ammoniated methanol – fraction contains bases

**Pass Through**

**Condition Step**
100% methanol

**Load Step**
Fraction collected contains acids, neutrals and reaction solvent

**Wash Step**
100%-methanol fraction contains acids and neutrals

**Recombine Load and Wash Steps**

**For PoraPak Rxn RP: Reversed-Phase Protocol**

For a compound to be retained, a reaction, carried out in a polar organic solvent will have to be concentrated by evaporation and/or diluted with water (3-to-1 or greater). This dilution brings the organic content down to 25% or less so as to enable reversed-phase retention. The generic protocol, shown to the right, can be used as a starting point to clean up reaction mixture.
CLEANUP OF A REDUCTIVE AMINATION MIXTURE

Reductive amination is a common reaction carried out in medicinal chemistry laboratories. In this example, a PoraPak Rxn CX cartridge is used to fractionate the reaction mixture.

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\[ \text{RCHO} + \text{H}_2 \text{N} + \text{H}_2 \text{O} \rightarrow \text{RNHR'} + \text{H}_2 \]

Crude reaction mixture. MBA, the starting amine, is present in small concentration with the product and an excess of the aldehyde.

Load Step

Collected fraction from the loading step. The fraction that passes through the cartridge contains aldehyde which is not retained on the cation exchanger.

Wash Step

When the cartridge is washed with methanol, additional aldehyde elutes in this fraction.

Elution Step

The cartridge is washed with ammoniated methanol, eluting all the bases. This fraction contains the product.

Catch-and-Elute Procedure

Using the ‘catch-and-elute’ procedure to clean up this synthesis mixture enables a quick collection of a limited number of fractions, increasing the certainty of finding the product rapidly. Assay the product fraction for confirmation, then begin the evaporation of the more volatile elution solvent.

UPLC Conditions

Instrument: ACQUITY UPLC® System
Column: ACQUITY UPLC BEH Shield RP18, 2.1 X 100 mm, 1.7 µm
Column part number: 186004049
Mobile phase A: 0.01% formic acid
Mobile phase B: 100% acetonitrile
Gradient and flow rate:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow Rate (mL/min)</th>
<th>A(%)</th>
<th>B(%)</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.42</td>
<td>95</td>
<td>5</td>
<td>Initial</td>
</tr>
<tr>
<td>7</td>
<td>0.42</td>
<td>1</td>
<td>99</td>
<td>6</td>
</tr>
</tbody>
</table>

Injection volume: 2 µL
Column temperature: 30 °C
Detector: ACQUITY UPLC PDA
UV wavelength: 250-340 nm (total absorbance mode)

ORDERING INFORMATION

<table>
<thead>
<tr>
<th></th>
<th>PoraPak Rxn CX</th>
<th>PoraPak Rxn RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 cc Flanged cartridges, 400 mg, 30/pkg</td>
<td>186004541</td>
<td>186004545</td>
</tr>
<tr>
<td>6 cc Flangeless cartridges, 400 mg, 30/pkg</td>
<td>186004542</td>
<td>186004546</td>
</tr>
<tr>
<td>20 cc cartridges, 2 g, 20/pkg</td>
<td>186004543</td>
<td>186004547</td>
</tr>
<tr>
<td>60 cc cartridges, 5 g, 10/pkg</td>
<td>186004544</td>
<td>186004548</td>
</tr>
<tr>
<td>Bulk, 200 mL/container</td>
<td>186004569</td>
<td>186004570</td>
</tr>
</tbody>
</table>
PRODUCT GUIDELINES

The following tables should be used as guidelines for loading capacity, flow rates, and volumes of wash and elution solvents. These tables take into consideration the dimension of the device and the bed volumes suggest appropriate flow rates and volumes for the condition, wash and elute steps.

- **Maximum Capacity Guidelines**

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Retained Compounds</th>
<th>Retained Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 cc-150 mg</td>
<td>15 mg/50 µmole</td>
<td>15 mg/50 µmole</td>
</tr>
<tr>
<td>6 cc-400 mg</td>
<td>40 mg/150 µmole</td>
<td>40 mg/150 µmole</td>
</tr>
<tr>
<td>20 cc-2 g</td>
<td>200 mg/350 µmole</td>
<td>200 mg/350 µmole</td>
</tr>
<tr>
<td>60 cc-5 g</td>
<td>500 mg/2000 µmole</td>
<td>500 mg/2000 µmole</td>
</tr>
</tbody>
</table>

The capacity is for the total of all compounds in the reaction that will be retained. For cation exchange, the capacity is for all bases in the reaction mixture including product and reactants. Consider smaller loads than maximum capacity guidelines or choose a larger device to avoid compound breakthrough.

- **Flow Rate Guideline (mL/min)**

<table>
<thead>
<tr>
<th>Device</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 cc cartridge</td>
<td>2 mL/min</td>
</tr>
<tr>
<td>20 cc cartridge</td>
<td>5 mL/min</td>
</tr>
<tr>
<td>60 cc cartridge</td>
<td>9 mL/min</td>
</tr>
</tbody>
</table>

Flow rate guideline is meant to provide a flow for reaction loading step.

- **Wash and Elution Volumes (mL)**

<table>
<thead>
<tr>
<th>Device</th>
<th>Condition</th>
<th>Wash</th>
<th>Elute</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 cc-150 mg</td>
<td>3 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>6 cc-400 mg</td>
<td>5 mL</td>
<td>10 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>20 cc-2 g</td>
<td>20 mL</td>
<td>20 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>60 cc-5 g</td>
<td>45 mL</td>
<td>45 mL</td>
<td>45 mL</td>
</tr>
</tbody>
</table>

This table provides guidelines for volumes used in the condition, wash and elution steps for the various sizes of PoraPak Rxn cartridges.
Sales Offices

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 43 1 877 18 07
Australia 61 2 9933 1777
Belgium 32 2 726 1000
Brazil 55 11 4134 3788
Canada 1 800 252 4752 x2205
China 86 21 6879 5888
CIS/Russia +7 495 3367000
Czech Republic 420 2 617 1 1384
Denmark 45 46 59 8080
Finland 09 5659 6288
France 33 1 30 48 72 00
Germany 49 6196 400600
Hong Kong 852 29 64 1800
Hungary 36 1 350 5086
India and India Subcontinent 91 80 2837 1900
Ireland 353 1 448 1500
Italy 39 02 265 0983
Japan 81 3 3471 7191
Korea 82 2 820 2700
Mexico 52 55 5200 1860

The Netherlands 31 76 508 7200
Norway 47 6 384 60 50
Poland 48 22 833 4400
Puerto Rico 1 787 747 8445
Singapore 65 6273 1221
Spain 34 93 600 9300
Sweden 46 8 555 11 500
Switzerland 41 56 676 70 00
Taiwan 886 2 2543 1898
United Kingdom 44 208 238 6100

All other countries:
Waters Corporation U.S.A.
1 508 478 2000
1 800 252 4752
www.waters.com