Mixed-Mode Chromatography: Hydrophobic Ion Exchange Resins

Introduction to Nuvia aPrime 4A and Nuvia cPrime Resins
Nuvia aPrime 4A and Nuvia cPrime are mixed-mode chromatography resins that allow both hydrophobic and electrostatic interactions to be utilized for the purification of a variety of biomolecules.

<table>
<thead>
<tr>
<th></th>
<th>Nuvia aPrime 4A Resin</th>
<th>Nuvia cPrime Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix material</td>
<td>Macroporous highly crosslinked polymer</td>
<td>Macroporous highly crosslinked polymer</td>
</tr>
<tr>
<td></td>
<td>Particle size: 50 μm</td>
<td>Particle size: 70 μm</td>
</tr>
<tr>
<td>Functional group</td>
<td>Aromatic hydrophobic anion exchanger</td>
<td>Aromatic hydrophobic cation exchanger</td>
</tr>
<tr>
<td>Benefits</td>
<td>✪ Straightforward method development</td>
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<tr>
<td></td>
<td>✪ Effective across a wide range of salt concentrations and pH; suitable for easy integration into multistep processes</td>
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<tr>
<td></td>
<td>✪ Effective purification of salt- and pH-sensitive proteins</td>
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<tr>
<td></td>
<td>✪ Mechanical and chemical stability</td>
<td></td>
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<tr>
<td>Most popular applications</td>
<td>✪ Monoclonal antibody purification</td>
<td></td>
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<tr>
<td></td>
<td>✪ Recombinant protein purification</td>
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<tr>
<td></td>
<td>✪ Aggregate/host cell protein/process impurities removal</td>
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<tr>
<td></td>
<td>✪ Variant separation</td>
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</tbody>
</table>
**Nuvia aPrime 4A and Nuvia cPrime Resin Ligands**

Ligand for Nuvia aPrime 4A Hydrophobic Anion Exchange Resin.  
Ligand for Nuvia cPrime Hydrophobic Cation Exchange Resin.

**Method Development — Bind/Elute or Flow-Through Mode**

This schematic illustrates how Nuvia aPrime 4A and Nuvia cPrime Resins can be used under either bind/elute or flow-through mode, depending on the buffer pH and the target biomolecule pI.

![Diagram of method development](image)

- **When pH < pI**
  - Target biomolecule has a net (+) charge under this condition
  - Screening Considerations
  - Nuvia aPrime 4A Resin — flow-through mode

- **When pH > pI**
  - Target biomolecule has a net (−) charge under this condition
  - Screening Considerations
  - Nuvia cPrime Resin — bind/elute mode

* See Elution Strategies with Nuvia cPrime Resin.  
** See Elution Strategies with Nuvia aPrime 4A Resin.
**Method Development — Elution**
Developing an effective and robust method with Nuvia aPrime 4A and Nuvia cPrime Resins is straightforward. In most cases, conducting a few simple design of experiments protocols to identify optimal elution conditions will yield an effective, robust, and scalable method.

### Elution Strategies with Nuvia aPrime 4A Resin

1. **Elution by pH decrease**
   - pH
   - Elution not achieved
   - Elution achieved

2. **Elution by varying conductivity**
   - [Salt]
   - Elution not achieved
   - Elution achieved

3. **Elution using modifiers**
   - Refine method using pH gradient
   - Convert gradient into step elution protocol
   - Refine method using salt gradient at a given pH
   - Convert gradient into step elution protocol
   - Refine method using modifier or different salt
   - Convert gradient into step elution protocol

   - Consider mobile phase modifiers
   - Consider using a different salt

* At optimum pH, determined from step 1.

### Elution Strategies with Nuvia cPrime Resin

1. **Elution by pH increase**
   - pH
   - Elution not achieved
   - Elution achieved

2. **Elution by varying conductivity**
   - [Salt]
   - Elution not achieved
   - Elution achieved

3. **Elution using modifiers**
   - Refine method using pH gradient
   - Convert gradient into step elution protocol
   - Refine method using salt gradient at a given pH
   - Convert gradient into step elution protocol
   - Refine method using modifier or different salt
   - Convert gradient into step elution protocol

   - Consider mobile phase modifiers
   - Consider using a different salt
   - Consider a different pH within the range

* At optimum pH as determined in step 1.
Bio-Rad Mixed-Mode Offering in Prepacked EconoFit Columns

Prepacked and disposable EconoFit Columns offer a convenient way to incorporate mixed-mode chromatography in your purification workflow, either at laboratory scale or just for early method development. In addition to Nuvia aPrime 4A and Nuvia cPrime Resins, EconoFit Columns are also available prepacked with ceramic apatite mixed-mode media.

Catalog numbers by column size and configuration are shown.

<table>
<thead>
<tr>
<th>Media</th>
<th>Catalog Numbers by Column Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 x 1 ml</td>
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<tr>
<td>Nuvia aPrime 4A</td>
<td>12009280</td>
</tr>
<tr>
<td>Nuvia cPrime</td>
<td>12009281</td>
</tr>
<tr>
<td>CHT Type I, 40 µm</td>
<td>12009255</td>
</tr>
<tr>
<td>CHT Type II, 40 µm</td>
<td>12009259</td>
</tr>
<tr>
<td>CHT Type I, 80 µm</td>
<td>12009256</td>
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<tr>
<td>CHT Type II, 80 µm</td>
<td>12009260</td>
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<tr>
<td>CHT XT, 40 µm</td>
<td>12009261</td>
</tr>
<tr>
<td>CFT Type II, 40 µm</td>
<td>12009252</td>
</tr>
<tr>
<td>MPC Type I, 40 µm</td>
<td>12009279</td>
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Additional Information

Visit bio-rad.com/NuviaaPrime4A and bio-rad.com/NuviacPrime for more details about these resins.

Visit bio-rad.com/CHTGuide to download our ceramic hydroxyapatite application guide.

Visit bio-rad.com/EconoFit to view the complete EconoFit product line offering.

Visit bio-rad.com/MixedMode to view other prepacked formats and bulk media offerings.

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EconoFit Columns: Prepacked, disposable, low-pressure columns that are compatible with commonly used chromatography systems.