Protein deamidation at neutral or alkaline pH is of significant concern to manufacturers of biotherapeutic proteins.

Deamidation at asparagine and glutamine residues:
- Caused by
  - Enzymatic or alkaline hydrolysis
- Affects
  - Immunogenicity, stability, and activity of proteins

Results in:
- Minimal changes in protein structure and charge
- Downstream purification challenges due to deamminated species
- Molecular heterogeneity

Refer to experimental details on page 2.
### Monoclonal Antibody Purification Strategy Details

#### Intermediate purification to resolve charge variants with Nuvia HR-S

<table>
<thead>
<tr>
<th>Step</th>
<th>Buffer Description</th>
<th>Column Volume</th>
<th>Flow Rate, cm/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equilibration</strong></td>
<td>10 mM sodium acetate, pH 4.5 (buffer A)</td>
<td>15</td>
<td>300</td>
</tr>
<tr>
<td><strong>Sample Loading</strong></td>
<td>Elute from the capture step diluted 1:4 in buffer A</td>
<td>–</td>
<td>300</td>
</tr>
<tr>
<td><strong>Wash 1</strong></td>
<td>Buffer A</td>
<td>15</td>
<td>300</td>
</tr>
<tr>
<td><strong>Wash 2</strong></td>
<td>10 mM sodium citrate, 10 mM sodium phosphate, pH 5.0 (buffer B)</td>
<td>15</td>
<td>300</td>
</tr>
<tr>
<td><strong>Elution</strong></td>
<td>Gradient of buffer B to 10 mM sodium citrate, 10 mM sodium phosphate, pH 8.0</td>
<td>15</td>
<td>300</td>
</tr>
</tbody>
</table>

**Capture Affinity/ion exchange chromatography**

- **Elution**: Gradient of buffer B to 10 mM sodium citrate, 10 mM sodium phosphate, pH 8.0

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**Shoulder fraction protein sequence**: Examination of the shoulder fraction protein sequence confirms deamidation events on a single mAb peptide.

**Resources**

Visit [bio-rad.com/web/NuviaHRS](http://bio-rad.com/web/NuviaHRS) to get more information.

Visit [bio-rad.com/web/ProcessResins](http://bio-rad.com/web/ProcessResins) to get technical details about Nuvia HR-S Resin.

Visit [bio-rad.com/web/ProcessApplications](http://bio-rad.com/web/ProcessApplications) to see other applications in which these resins can be used.

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**Purification of the mAb on Nuvia HR-S**: The distinct shoulder on the front side of the main peak is a modified mAb fragment. $A_r$ (−); conductivity (−); pH (−).

**Mass spectrometry (MS) analysis of the mAb**: MS analyses of the shouldered peak (top spectrum) and the main peak (lower spectrum) show a mass shift of about 2.5 atomic mass units between the two peaks, which is consistent with two or three deamidation events on a single peptide.