**Asp-N Digestion**

TCA-precipitated Proteins

1. Add 30µl of 100mM Tris-HCl, pH 8.5, 8M Urea, 5mM TCEP (made fresh). Vortex @ RT, 30min

2. Bring solution to 10mM CAM with 0.5M stock @ RT, 30min, in dark

3. Dilute to 2M Urea with 100mM Tris-HCl, pH 8.5

4. Add Asp-N at a concentration 0.25µg/µL to an enzyme-to-substrate ratio of 1:25 (wt/wt) @ 37°C, 5-6 hours

5. Add 90% Formic acid to 5%

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<th>④</th>
<th>⑤</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tris-Urea TCEP</td>
<td>CAM</td>
<td>100mM Tris</td>
<td>Asp-N</td>
<td>Formic</td>
</tr>
<tr>
<td>Sample</td>
<td>30µl</td>
<td>0.6µl</td>
<td>90µl</td>
<td>1.4µl</td>
<td>7µl</td>
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</tbody>
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*Pseudomonas fragi* protease

*EC 3.4.24.33*

**Inhibitors:** EDTA and α-phenanthroline

**Molecular weight:** $M_r = 27$ kD

**Optimum pH:** 7.0 - 8.0

**Specificity:** Metalloprotease that hydrolyzes peptide bonds at the amino side of Asp and cysteic acid. If cysteine is reduced or alkylated, only -j-Asp-X is cleaved.