**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 0.25ug/µL to an enzyme-to-substrate ratio of 1:25 (wt/wt) @ 37°C. 5-6 hours

**GLU-C DIGESTION**

- ⑤ Dilute to **1M Urea** with 100mM Tris-HCl, pH 7.8
- ⑥ Add Endoproteinase Glu-C (Roche) at 1µg/µl (1:20 to 1:100) @ 25°C, 6hrs
- ⑦ Add 90% Formic acid to 5%

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<tr>
<td></td>
<td>Tris-Urea TCEP</td>
<td>CAM</td>
<td>100mM Tris</td>
<td>Asp-N</td>
<td>100mM Tris, pH 7.8</td>
<td>Glu-C</td>
<td>Formic</td>
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<tr>
<td>Sample</td>
<td>30µl</td>
<td>0.6µl</td>
<td>90µl</td>
<td>1.4µl</td>
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