Rapid Digestion -Promega Trypsin/Lys-C Kit

Guidelines for sample preparation

(How to protect your samples from contamination with keratin)

1. Try to avoid any contact of samples and solutions with dust, skin or hair
2. Clean your bench
3. Wear gloves at all times
4. Trypsin/Lys-C Mix, MS store the Rapid Trypsin protease, Rapid Trypsin Lys-C protease, Resuspension Buffer and Rapid Digest Buffer at –10°C to –30°C. After reconstitution of the protease in Resuspension Buffer, store at –70°C for up to 1 month. Minimize freeze-thaw cycles of all reagents.
5. Use ultra-pure water for all solutions (MilliQ water)
6. Rapid Digestion Kit–Trypsin/Lys-C – Product number :VA1061 (The kit contains sufficient reagents to perform up to 100 reactions).
7. Protein samples (50µl–1ml) should be resuspended in pure water, mildly-buffered solutions (Tris buffers at a concentration of 25mM or lower, pH 7.0–8.0), or those that have been eluted following affinity purification (with organic acid solutions like 0.1% TFA) for best compatibility with the Rapid Trypsin Digestion workflow

Guidelines for sample submission

1. Provide 10ul of samples in no recovery vials* or vials with insert for small volumes for LC-MS/MS analysis

***Autosampler vials appropriate for analysis**

Waters Total Recovery (part number: 186000385C.)

Figure 1: Waters Total Recovery Vial

2. Label your tube with the sample ID.

3. Fill in online proteomic submission form to provide us with more information about your sample.

Solutions of reagents

50mM acetic acid

-Prepare 50 mM acetic acid solution by adding 287.36 µL of glacial acetic acid to 100 mL of ultrapure water.

200 mM ammonium bicarbonate (NH_4HCO_3, MW 79.06)

-1,58 g NH_4HCO_3 in 100 ml MilliQ water

-Store at -20°C in aliquots of 10ml

100M TCEP (**tris(2-carboxyethyl) phosphine** $C_9H_{15}O_6P$, MW 250,187)

- 0.25 g TCEP in 10 ml of 200 mM ammonium bicarbonate

- Store at -20°C in aliquots of 500 µl

100 mM IAA (Iodoacetamide, C_2H_4INO, MW184.96)

-Dissolve 68 mg of IAA in 3327 µl of 200 mM ammonium bicarbonate

- Store at -20°C in aliquots of 250 µl

100µg/µl of Rapid Trypsin/Lys-C Mix

- Resuspend 100µg of the protease (Rapid Trypsin or Rapid Trypsin/Lys-C Mix) in 100µl of resuspension Buffer to make a concentration of 1mg/ml.

- After reconstitution of the protease in resuspension Buffer, store at –70°C for up to 1 month. Minimize freeze-thaw cycles of all reagents.

Procedure

The following protocols are meant to produce protein digests, typically within 30–60 minutes. Some samples, such as complex mixtures or substrates, which might be more difficult to digest (i.e., disulfide rich or membrane proteins) may take longer (usually no longer than 3 hours). While reduction and alkylation is often needed for applications like peptide mapping, where all of the protein needs to be characterized, many applications like quantitative analysis of complex mixtures or biomarker studies don't always require reduction or alkylation. Because the samples are heated (and therefore denatured), the protocols do not require (or recommend) denaturants. Here we provide protocols for proteolysis with and without reducing/alkylating agents.

Proteolysis without Reduction and Alkylation

1. Set a heat block (such as an Eppendorf Thermomixer.) to 70°C
2. Add 3x volume of Rapid Digest Buffer. For example, to 50µl of protein substrate add 150µl of Rapid Digest Buffer.
3. Resuspend 100µg of the protease (Rapid Trypsin or Rapid Trypsin/Lys-C Mix) in 100µl of Resuspension Buffer to make a concentration of 1mg/ml. Store the resuspended material on ice until ready for use.
4. Add enzyme in a 1:10 E/S ratio. For example, add 4µl of the resuspended Rapid Trypsin/Lys-C Mix (4µg of the Rapid Trypsin/Lys-C for every 20µg of substrate).
5. Incubate for up to 60 minutes at 70°C. (Note: Increase digestion time to as long as 3 hours for more difficult-to-digest proteins or complex samples) Optional: Shake at 450–600rpm on a Thermomixer.
6. Terminate the reaction with 5µl of formic acid for every 200µl of digestion reaction. The final concentration of the organic acid should be 0.1–2%.
7. Freeze the samples at –20°C or below or proceed directly to LC-MS/MS analysis.
8. A Zip Tip to clean up the sample or any other off-line desalting procedure needs to be followed (please see separate protocol).

Proteolysis with Reduction and Alkylation

1. Set a heat block (such as an Eppendorf Thermomixer.) to 70°C
2. Add 3X volume of Rapid Digest Buffer. For example, to 50µl of protein substrate add 150µl of Rapid Digest Buffer.
3. Add TCEP to a final concentration of 2mM. For example, add 4µl of 100mM TCEP.
4. Incubate the reaction at 37C for 45 minutes

5. Allow the mixture to cool to room temperature, then add iodoacetamide to a concentration 2.5X above the TCEP concentration. For the example provided in Step 2, add 10µl of 100mM of iodoacetamide

6. Incubate for 60 minutes at room temperature and in the dark.

7. Add enzyme in a 1:10 E/S ratio. For example, add 4µl of the resuspended Rapid Trypsin/Lys-C Mix (4µg of the Rapid Trypsin/Lys-C for every 20µg of substrate).

8. Incubate for up to 60 minutes at 70°C. (Note: Increase digestion time to as long as 3 hours for more difficult-to-digest proteins or complex samples) Optional: Shake at 450–600rpm on a Thermomixer.

9. Terminate the reaction with 5µl of formic acid for every 200µl of digestion reaction. The final concentration of the organic acid should be 0.1–2%.

10. Freeze the samples at –20°C or below or proceed directly to LC-MS/MS analysis.

11. A Zip Tip to clean up the sample or any other off-line desalting procedure needs to be followed (please see separate protocol).