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Product Information

SOLu-Trypsin Rapid Digestion Kit

Catalog Number **MSKT0002**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

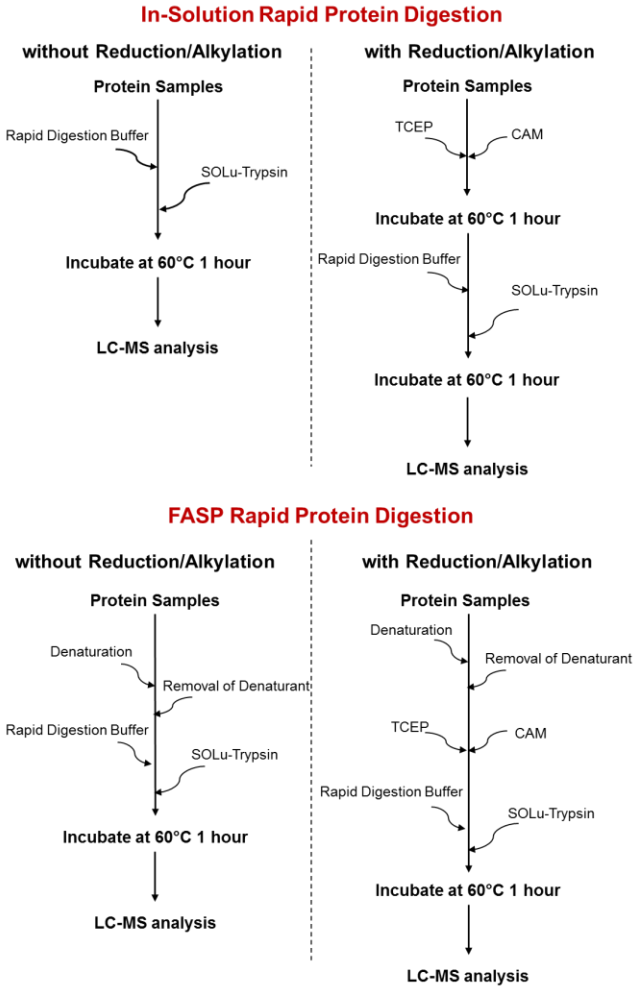
Protein digestion is performed in most proteomics studies. Current protein digestion workflows are often cumbersome and tedious, which involve chemical denaturation, reduction, alkylation, overnight protease digestion, and off-line desalting prior to LC-MS analysis. These protocols require 3–4 hours of lab work followed by 16–20 hours incubation, potentially introducing variability to results.

This Rapid Digestion Kit contains two components: Rapid Trypsin Digestion Buffer and SOLu-Trypsin. It provides a fast and efficient method for protein digestion. Using this kit, protein digestion can be done at 60 °C within 1 hour. Removal of denaturants by off-line desalting is not necessary, which further shortens the time for sample preparation. This kit is compatible with a variety of sample types, reduction and alkylation protocols, and downstream workflows (e.g., isobaric tag labeling with TMT or iTRAQ, and MRM quantitation). While this kit can be used for protein sequencing, artifactual deamidation and oxidation may be induced during protein digestion. To avoid such artifactual modifications, please refer to the Low Artifact Digestion Buffer (Catalog Number EMS0011).

Although reduction and alkylation are commonly performed in protein digestion protocols, it has been demonstrated that a comparable number of peptide and protein IDs in a complex mixture can be obtained without reduction and alkylation.[1,2] Therefore, in the protocols provided here, reduction and alkylation are optional, especially for complex samples (e.g., cell lysate) and non-disulfide bond containing proteins. When reduction and alkylation are desired, for example, in the characterization of disulfide bond containing proteins, 2-chloroacetamide (CAM) is recommended as the alkylation reagent instead of iodoacetamide (IAM), since artifacts related to over-alkylation using IAM have been reported.[1,3] In addition, concurrent reduction and alkylation reactions are suggested here to further reduce sample preparation time.[4]

Filter-aided sample preparation (FASP), an alternative protein digestion method, is recommended for samples with contaminants which interfere with LC-MS analysis, such as SDS, salts, etc. FASP also has been shown to allow in-depth proteome coverage.[5]

Figure 1.
Workflow Overview



Kit Components
- Rapid Trypsin Digestion Buffer (EMS0009)
- SOLu-Trypsin (EMS0004, 1 mg/mL trypsin solution)

Reagents and Equipment Required but Not Provided.
- Formic acid, such as Catalog Number 399388
- TCEP solution, 0.5 M, such as Catalog Number 646547 (if performing reduction and alkylation)
- 2-chloroacetamide (CAM), such as Catalog Number C0267 (if performing reduction and alkylation)
- 30 kDa filter, such as Catalog Number MRCF0R030, Microcon Unit with Ultracel-30 membrane or Catalog Number UFC503024, Amicon 30 kDa MWCO, (if performing FASP digestion)
- Urea, such as Catalog Number 51456 (if performing FASP digestion)
- Sodium deoxycholate (DOC), such as Catalog Number 30970 (if performing FASP digestion on <20 µg of protein)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Both Rapid Trypsin Digestion Buffer and SOLu-Trypsin are ready to use. Store unused portion in the cooler for up to 2 years.

SOLu-Trypsin may be diluted into Rapid Trypsin Digestion Buffer if desired. Long term stability may be compromised so it is best to dilute only the amount required for daily use.

Storage/Stability
The product is stable for at least 2 years at 2–8 °C.

Procedures
Two protein digestion protocols are provided in detail here: in-solution and FASP rapid digestion. In addition, Rapid Trypsin Digestion Buffer and SOLu-Trypsin can be substituted into users' existing workflows with SOLu-Trypsin being added at a 1:10 enzyme:substrate ratio and protein digestion being performed at 60 °C for 1 hour.

Note: Conventional protein denaturation using urea and DOC is not compatible with the in-solution rapid digestion workflow. Urea will decompose at 60 °C and may modify proteins/peptides. DOC is not compatible with Rapid Trypsin Digestion Buffer and should only be used with the FASP protocol.

A. In-solution Rapid Protein Digestion

Preparation of Reagents (only needed if performing protein reduction and alkylation)

100 mM TCEP – Pipette 200 µL of 500 mM TCEP solution into a centrifuge tube and add 800 µL of Rapid Trypsin Digestion Buffer. Mix well.

500 mM CAM – Dissolve CAM in Rapid Trypsin Digestion Buffer at 46–48 mg/mL

Digestion Procedure

1. Set a water bath or heat block to 60 °C.

2. If protein is in solution, dry by vacuum centrifugation.
   Note: If not performing reduction and alkylation, skip steps 3–4 and proceed to step 5. Reduction and alkylation is recommended for characterization of disulfide bond containing proteins. For proteins which do not have disulfide bond or complex protein samples (e.g., cell lysate), steps 3–4 can be skipped.

3. Add 10 µL of 100 mM TCEP and 10 µL of 500 mM CAM.

4. Vortex, wrap it in foil, and incubate the sample at 60 °C for 1 hour.

5. Add appropriate volume of Rapid Trypsin Digestion Buffer to a final concentration which can be directly injected for LC-MS analysis.

6. Add SOLu-Trypsin to the sample solution to have an enzyme:protein mass ratio of 1:10.

7. Incubate at 60 °C for 1hour.

8. Quench the digestion by adding 1.5 µL of formic acid to every 50 µL of digestion solution.

9. Freeze the sample at –20 °C or proceed directly to LC-MS analysis.

B. FASP Rapid Protein Digestion (15–400 μg protein)
Note: It is recommended to start with at least 15 μg protein, but ≥20 μg protein is ideal. Protein volume should be 10–100 μL.

Preparation of Reagents

50 mM TCEP (only needed if performing protein reduction and alkylation) – Pipette 100 μL of 500 mM TCEP solution into a centrifuge tube and add 900 μL of Rapid Trypsin Digestion Buffer. Mix well.

50 mM CAM (only needed if performing protein reduction and alkylation) – Dissolve CAM in Rapid Trypsin Digestion Buffer at 4.6–4.8 mg/mL

Denaturation solution:
Protein samples <20 μg – Prepare a 20% (w/v) stock solution of DOC by dissolving 200 mg of DOC in 1 mL of water. For 1 mL of denaturation solution, dissolve 0.75 g of urea in 850 μL of water and 150 μL of 20% DOC solution.
Protein samples ≥20 μg – Dissolving 0.75 g of urea in 1 mL of Rapid Trypsin Digestion Buffer.

Digestion Procedure
1. Set a water bath or heat block to 60 °C.

2. Add 200 μL of denaturation solution to sample and mix. Apply the mixture to a filter unit and centrifuge at 14,000 × *g* for 15 minutes.

3. Discard the flow-through fraction from the collection tube. Steps 2-3 should be repeated three times if SDS is present the protein samples.

4. Add 200 μL of water to sample and centrifuge at 14,000 × *g* for 15 minutes. This step should be repeated once more.
   Note: If not performing reduction and alkylation, skip steps 5-6 and proceed to step 7.

5. To the filter unit, add 20 μL of 50 mM TCEP and 100 μL of 50 mM CAM. Wrap it in foil and incubate at 60 °C for 1 hour. Centrifuge at 14,000 × *g* for 15 minutes.

6. Add 100 μL of Rapid Trypsin Digestion Buffer to the filter unit and centrifuge at 14,000 × *g* for 10 minutes. Repeat this step once more.

7. To the filter unit, add 100 μL of Rapid Trypsin Digestion Buffer and an appropriate amount of SOLu-Trypsin to have an enzyme:protein mass ratio of 1:10. Wrap the tube with Parafilm® sealing film.

8. Incubate the tube at 60 °C for 1 hour.

9. Centrifuge at 14,000 × *g* for 5-10 minutes or until all liquid passes through the filter.

10. Add 40 μL of Rapid Trypsin Digestion Buffer to the filter unit and centrifuge at 14,000 × *g* for 5–10 minutes. Repeat this step.

11. Add 2 μL of neat formic acid to the filtrate and vortex well.

12. Digest solution can be dried and reconstituted in appropriate volume of 0.1% formic acid or diluted to appropriate concentration using 0.1% formic acid for LC-MS analysis.

References
1. Muller, T. et al., *Molecular & Cellular Proteomics*, **16**, 1173-1187, (2017).
2. Cao, Z. et al., *ASMS poster*, (2018).
3. Nielsen, M.L. et al., *Nature Methods*, **5**, 459-460 (2008).
4. Kulak, N.A. et al., *Nature Methods*, **11**, 319-324, (2014).
5. Wisniewski, J.R. et al., *Nature Methods*, **6**, 359-362, (2009).

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