Capturem™ Trypsin Miniprep Kit (Mass Spectrometry Grade) Protocol-At-A-Glance

I. Introduction

This protocol is provided for **Capturem Trypsin Miniprep Kit (Mass Spectrometry Grade)** (Cat. No. 635740). This kit includes single-use disposable mini spin columns containing membrane-immobilized trypsin isolated from porcine pancreas that was stabilized by reductive methylation to increase resistance to autolytic digestion. Capturem trypsin's spin digest allows for easy, rapid, and complete digestion of protein samples in 2–3 min. To achieve good sequence coverage, we recommend digesting no more than 80 µg of protein or 25 µg of antibody per column. Each column can hold up to 850 µl of sample and requires a minimum elution volume of 250 µl. The columns are supplied together with an activation buffer.

NOTE: Capturem Trypsin digestion may generate longer and more unique peptides than in-solution trypsin digestion due to an increase in missed cleavage sites. Identification of these unique peptides may require adjustments to the minimum/maximum peptide mass and the maximum number of allowed missed cleavages in bioinformatics data analysis, to obtain complete sequence coverage of the target protein.

II. Materials and Reagents

A. Components

- 20 Capturem Trypsin Miniprep Columns (Cat. No. 635740)
  (mini spin columns containing a blue insert, supplied in 2-ml collection tubes)
- 5 ml Capturem Trypsin Activation Buffer (Cat. No. 635739)

B. Additional Materials Required

1. Digestion Buffer

This product is compatible with commonly used ammonium bicarbonate and Tris-based buffers, such as:

- 20–100 mM Tris, pH 8.0
- 10–50 mM ammonium bicarbonate, pH 8.0

2. 100% Trifluoroacetic acid (TFA) or acetic acid

3. Collection Tubes

Each sample will require one additional standard 2-ml collection tube, with or without a cap. These tubes should be used to collect peptides generated by digestion of protein samples, which will be used for downstream analyses such as SDS-PAGE, HPLC, mass spectrometry, etc.

III. Sample Preparation

NOTE: Some protein samples may require denaturation, reduction, and alkylation before enzymatic digestion. If your sample needs denaturation, follow instructions in Section III, otherwise proceed to Sample Digestion, Section IV.

Some protein samples (e.g., monoclonal antibodies) may require denaturation/reduction and alkylation before enzymatic digestion. An example of a denaturation/reduction/alkylation protocol is provided below. However, other protocols may be used if necessary.

1. Dissolve up to 80 µg of the target protein or protein extract or up to 25 µg of antibody in your digestion buffer (see Section II.B.1) containing 6–8 M urea, in a reaction volume of 25–100 µl.
2. Add either DTT or TCEP to a concentration of 5 mM, vortex to mix, and incubate at 65˚C for 45 min.
3. After denaturation, add 20 mM IAA and incubate at 37˚C for 30 min in the dark.

4. After alkylation, add a sufficient volume of digestion buffer (200–800 µl, as needed) to reduce the sample concentration to 0.1–0.2 mg/ml and the urea concentration to 1 M or below.

NOTE: If you wish to make a direct comparison with in-solution trypsin digestion, the volume of the denatured sample that you load onto the Capturem Trypsin column in Section IV, Step 4 will need to be 50 µl less than the volume of the in-solution trypsin digest. This is necessary because the Capturem Trypsin column is eluted with an additional 50 µl of digestion buffer in Section IV, Step 4 that is combined with the flowthrough from the denatured sample.

IV. Sample Digestion

Digest the protein samples, as-is or after denaturation (Section III), as follows:

1. Insert the Capturem Trypsin Column into the provided 2-ml collection tube.
2. Load 200 µl of Capturem Trypsin Activation Buffer onto the Capturem Trypsin Column to activate the column. Centrifuge at 500*g* for 1 min. Discard the flowthrough along with the collection tube and place the column in a new collection tube (supplied by the user—see Section II.B).

NOTE: When performing centrifugation, use the following formula to convert rpm to centrifugal force units (RCF or *g*) if the centrifuge does not automatically provide this information:

Centrifugal Force Conversion Formula: $RPM = \sqrt{[RCF/(R \times 1.118)]} \times 1 \times 10^5$
where R is the distance (in millimeters) from the center of the rotor to the end of the spin bucket when held horizontally and away from the center.

3. Load the protein sample from as-is or denatured onto the activated Capturem Trypsin Column. Centrifuge at 500*g* for 1 min and save the flowthrough, which contains the eluted peptides, keeping the column inserted into the collection tube.
4. Load 50 µl of digestion buffer containing 1 M urea onto the Capturem Trypsin Column. Centrifuge at 1,000*g* for 1 min to elute additional peptides that remain bound to the column into the tube containing the flowthrough from Step 3.

NOTE: Some samples may stick to the column because of their hydrophobicity. Depending on the nature of your sample, it may be necessary to repeat Step 4 (eluting into the same tube as in Steps 3 and 4) to ensure higher yield.

5. Acidify the combined eluates from Step 4 to pH 2.0–3.0 within 1 hr of digestion by adding TFA or acetic acid. For example, add 5 µl of glacial acetic acid (>99.5%) if the total volume of the combined eluates is 200 µl. The eluted peptides are now ready for downstream analysis.

NOTE: Depending on the downstream analysis, a clean-up step (e.g., Zip-Tip, Stage-Tip, ZEBA spin column) may be required to remove high salt content and/or other reagents like urea which may interfere with mass spec analysis.

Appendix A. Troubleshooting Guide

Table 1. Troubleshooting Guide

Problem	Possible Explanation	Solution
Protein not fully digested	Too much protein was loaded	Do not load more than 80 µg of protein or 25 µg of antibody.
Minimal sequence coverage	Peptide fragments are retained on the column	Apply a 2nd elution step using 50 µl of Digestion Buffer or re-load and re-spin the flowthrough.
Protein not fully digested or minimal sequence coverage	Protein was not fully denatured, reduced and alkylated	Verify the denaturation, reduction, and alkylation conditions are appropriate for your sample.
The spin column does not fully drain	Clogging due to particles or very viscous sample	• Pre-clarify the solution by centrifugation or using a 0.2, 0.45, or 0.8-micron filter. • Repeat spin at 500*g* for 2 min.

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