I. Introduction

This protocol is provided for **Capturem Trypsin** (Cat. No. 635722), single-use disposable mini spin columns containing membrane-immobilized trypsin for easy, rapid, and complete digestion of protein samples in 2–3 min. The columns are supplied together with an activation buffer.

II. Materials and Reagents

A. Components

- 20 Capturem Trypsin Columns
  (mini spin columns containing a blue insert, supplied in 2-ml collection tubes)
- 5 ml Capturem 1X Activation Buffer

B. Additional Materials Required

1. Digestion Buffer

This product is compatible with commonly used ammonium bicarbonate and Tris-based buffers. Examples are shown below, but other buffers may also be used if necessary.

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

2. Collection Tubes

Each sample will require 1 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 analysis such as SDS-PAGE, HPLC, mass spectrometry, etc.

III. Sample Preparation

Some protein samples may require denaturation and/or reduction before enzymatic digestion. An example of a denaturation/reduction protocol is provided below. However, other protocols may be used if necessary.

1. Dissolve up to 80 µg of target protein or protein extract or up to 20 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 37˚C for 30 minutes.
3. After denaturation, 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 3 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 prepared in Section III as follows:

1. Insert the Capturem Trypsin Column into the provided 2-ml collection tube.
2. Load 200 µl of Capturem 1X 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).

3. Load the protein sample from Section III, Step 3 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: Depending on the sample type, it may be necessary to repeat Step 4 (eluting into the same tube as in Steps 3 and 4).
5. Acidify the combined eluates from Step 4 to pH 2–3 within 1 hr of digestion by adding TFA or acetic acid. For example, add 5 µl of glacial acetic acid if the total volume of the combined eluates is 200 µl. The eluted peptides are now ready for downstream analysis.

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.

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This document has been reviewed and approved by the Quality Department.