I. Introduction

This protocol is provided for Capturem Pepsin (Cat. No. 635728) single-use, disposable mini spin columns containing membrane-immobilized pepsin for easy, rapid, and complete digestion of antibody samples in less than three minutes. The columns are supplied together with an activation buffer. Each column can process 10–50 µg of sample in a loading volume of up to 850 µl and requires a minimum elution volume of 200 µl.

II. Materials and Reagents

A. Components

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

B. Additional Materials Required

- Digestion buffer

NOTES:

- Capturem Pepsin Columns are compatible with 5% formic acid-, HCl-, and TFA-based buffers. If your digestion is done in 5% formic acid, you can use the provided 1X Activation Buffer for the activation step. Otherwise, use your digestion buffer to activate the column.
- Do not mix the supplied 1X Activation Buffer with other acids, as this could interfere with antibody digestion.

- 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 the 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

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

1. Dissolve 10–50 µg of target antibody in digestion buffer to a concentration of 1 mg/ml. Add HOAc (acetic acid) to a concentration of 10 mM.
2. Add TCEP to a concentration of 10 mM, mix, and incubate at 75°C for 15 min.
3. After denaturation/reduction, bring the volume up to 100–400 µl with digestion buffer.

IV. Sample Digestion

Digest the antibody samples (prepared in Section III) as described below.

1. Insert the Capturem Pepsin Column into the provided 2-ml collection tube.
2. Load 200 µl of activation buffer (use the supplied 1X Activation Buffer for formic acid digestions, otherwise use your digestion buffer) onto the Capturem Pepsin 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).
3. Load the antibody sample from Section III, Step 3 onto the activated column. Centrifuge at 500*g* for 1 min.

4. With the column still in the collection tube, load a second volume of digestion buffer equal to the volume of sample loaded in Step 3. Centrifuge at 500*g* for 1 min.
5. Neutralize to pH 6–8 within 15 min of digestion by adding NaOH to the eluate. For example, add 25–30 µl of 10N NaOH to the eluate if 200 µl total of 5% formic acid was used for digestion. The eluted peptides are now ready for downstream analysis.

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