# Capturem™ Trypsin 96-Well Plate (Mass Spectrometry Grade) Protocol-At-A-Glance

## I. Introduction

This protocol is provided for **Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade)** (Cat. Nos. 635737 and 635736). This kit includes single-use disposable 96-well plates 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 antibodies and other protein samples in ~4 min. To achieve good sequence coverage, we recommend digesting no more than 80 µg of protein or 25 µg of antibody per well. Each well can hold up to 1 ml of sample and requires a minimum elution volume of 100 µl.

**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

- Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) (Cat. Nos. 635737, 635738)
- Capturem Trypsin Activation Buffer (50 ml) (Cat. No. 635739; not included with 635737)

### 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. Capturem Trypsin Activation Buffer

Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) 4 x 96 (Cat. No. 635737) does **not** include the Capturem Trypsin Activation Buffer, and it must be purchased separately.

#### 4. Solubilizing Surfactant

The Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) is compatible with surfactants (e.g., RapiGest SF [Waters]) which may improve the digestion efficiency by increasing protein solubility without reducing enzymatic activity.

#### 5. Collection Plates

- **96-well collection plates:** Each sample will require two standard 96-well collection plates. These plates should be used to collect peptides generated by digestion of protein sample(s) that will be used for downstream analysis such as HPLC, Mass Spectrometry, etc. The Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) is compatible with Nunc 96-Well Polypropylene DeepWell Storage Plates (Thermo Fisher, Cat. No. 260252).
- **96-well plate filtration device:** The Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) is compatible with standard 96-well plate filtration devices, such as the NucleoVac™ 96 Vacuum Manifold (Cat. No. 740681).

- **96-well plate centrifuge:** The Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) is compatible with standard 96-well plate centrifuges (e.g., Eppendorf Centrifuge 5804R with Deepwell-plate rotor A-2-DWP).
- **Multi-channel pipette**

## 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 used in target quantitation workflow is provided below. However, other protocols may be used if necessary.

1. Dissolve your samples in 20 mM Tris-HCl (pH 8.0) in a reaction volume of ~100 µl.
2. Add 11 µl of 50 mM DTT in 1% RapiGest, vortex to mix, and then incubate at 60˚C for 45 min with shaking.
3. After denaturation, cool the samples to room temperature, add 5 µl of 500 mM IAM (iodoacetamide) and incubate for 30 min at room temperature in the dark.

## IV. Sample Digestion

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

1. Place the Capturem Trypsin 96-Well Plate (Mass Spectrometry Grade) securely on top of a 96-well collection plate (supplied by the user—see Section II.B). Load 200 µl Capturem Trypsin Activation Buffer to each well of the Capturem Trypsin 96-Well Plate using a multi-channel pipette to equilibrate the wells. Centrifuge at 500*g* for 2 min at room temperature. Make sure all the liquid has drained completely. Remove the flowthrough and discard it along with the collection plate. Place the Capturem Trypsin 96-Well Plate securely on top of a new 96-well collection plate.

   **NOTES**:
   - 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.

   - In case wells do not drain fully, spin your plate again at 500*g* for 2 min at room temperature.

2. Load the protein sample as-is or denatured onto the activated Capturem Trypsin 96-Well Plate. Centrifuge at 500*g* for 2 min and reload the flowthrough into the Capturem Trypsin 96-Well Plate using a multichannel pipette. You can use the same collection plate for the second loading. Spin once more and save the flowthrough, which contains the peptide fragments.

   **NOTE:** Instead of reloading the flowthrough, it is also possible to elute retained surrogate peptides by applying an elution step with 50 µl of 20 mM Tris buffer (pH 8.0) and centrifugation at 500*g* for 2 min. However, the elution step will dilute the final sample, which is not the case if the flowthrough is reloaded.

3. Add 1 µl of 100% TFA (Trifluoroacetic acid) to the flowthrough from the previous step, and your sample is ready to be applied to the mass spectrometry instrument. If you prefer to remove RapiGest, incubate the samples for 45 min at 37°C to precipitate the RapiGest.

4. Centrifuge at 2,000*g* for 10 min to remove the precipitated RapiGest or apply your sample directly to your mass spectrometry instrument. For further information regarding the usage of RapiGest, please refer to the RapiGest protocol.

**NOTE:** If you are applying a different solubilizing reagent such as chaotropic salts (e.g., urea, guanidine hydrochloride) you may have to perform a cleanup step or buffer exchange (e.g., Zip-Tip, Stage-Tip and/or ZEBA spin column) prior to injecting your sample into the mass spectrometry instrument.

## 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 reload 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. |
| **Wells do not fully drain** | Clogging due to particles or very viscous sample | • Pre-clarify the solution by centrifugation or using a 0.8-micron filter.<br>• Repeat spin at 500*g* for 2 min. |


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