

# In-Solution Digestion Using Trypsin-ultra™, Mass Spectrometry Grade (Recombinant) (NEB #P8119)

## Overview

In-solution digestion provides a flexible approach compatible with multiple sample-prep workflows. Trypsin-ultra enables digestion with minimal autolysis, ideal for high-quality peptide mapping workflows.

Pre-treatment steps (i.e. purification, denaturation, reduction, alkylation, and desalting) may be required and should be optimized for your workflow.

## Protocol

To reconstitute Trypsin-ultra, Mass Spectrometry Grade (Recombinant), add 1 mL high purity water (0.1 µg/µL). Aliquot and store at -20 °C for up to six months, avoid multiple freeze-thaw cycles.

Trypsin digestions are performed using a trypsin:protein ratio starting at 1:100 (or higher, as needed), incubate at 37 °C for 3 hours (longer times can be used as required). This autolysis-resistant enzyme can be used at a trypsin:protein ratio of 1:10 for 30 min without detectable autolytic artifacts. Common reaction buffers are: 50 mM ammonium bicarbonate (pH 7.8), or 50 mM Tris-HCl (pH 8.0) in the presence of 1 to 10 mM CaCl<sub>2</sub>.

Example of a Trypsin-ultra, Mass Spectrometry Grade (Recombinant) in-solution digestion for peptide mapping (trypsin:protein ratio of 1:40).

Components	20 µl Reaction
Protein sample	2 µg
2X Buffer (e.g.: 100 mM ammonium bicarbonate, 10mM CaCl <sub>2</sub> , pH 7.8)	10 µl
Trypsin-ultra, Mass Spectrometry Grade (Recombinant) 0.1 µg/µl	5 µl
Nuclease-free Water	to 20 µl

Incubate at 37 °C for 1 to 3 hours

### NOTES

1. Optional: after incubation, the reaction can be stopped by adding diluted trifluoroacetic acid (TFA) to a final concentration of ~0.5% TFA.
2. If needed, tryptic peptides can be cleaned up using a solid-phase extraction method of choice.