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# mP-Trypsin

**Catalog No:** mPT-9000

**Activity:** 1 µL of mP-Trypsin will digest 1 µg of protein in 1 hour

**Shipping:** 0.5 M ammonium bicarbonate

**Appearance:** Pink liquid

**Storage:** Upon receipt store at 4 °C

**Shelf life:** One year at 4 °

**About:**

mP-Trypsin is a modified version of trypsin that has been stabilized to prevent autodigestion and tagged with ProMTag for easy removal after digestion of a protein sample using the ProMTag Capture Resin (Catalog No. CSB-8000). Use it on its own for fast, clean digestion of proteins into peptides, or pair with ProMTag for complete sample preparation from start to finish.

ProMTag (Catalog No. T-6000) is a pH reversible protein labeling agent. At pH 8.0 or higher, ProMTag reacts with amines on the surface of proteins to covalently link an affinity tag to the surface. At pH 3.0 or less, this covalent link is broken, returning the protein amines to their original, unmodified state. This reversible tag can be used for fast affinity purification without the need for permanent covalent or genetic tags that could interfere with the native mass or isoelectric points.

Questions about compatibility, optimization, or troubleshooting? Email info@impactproteomics.com for help.

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## General ProMTag Protocol

1. Make sure your protein sample starts in a buffer pH 8 or greater that does not contain primary amines. Our favorite buffers are 100 mM HEPES, pH 8.0 and 100 mM Sodium Phosphate, pH 8.0.
2. Dilute your starting sample to 1 mg/mL. For every 10 µg of protein, add 25 µg of ProMTag. Vortex briefly to mix
    a. Ex: If you are labeling 100 µg of protein, you need to add 250 µg (8.3 µL) of ProMTag
3. Incubate for 30 minutes to label your protein sample.
4. Add sample to the appropriate amount of ProMTag Capture Resin to capture labeled proteins. Incubate for 30 minutes with end over end rotation to keep the beads suspended.
    a. For every 10 µg of labeled protein, you need 20 µL of ProMTag Capture Resin.
    b. If you are using a buffer that has SDS, you need at least 10 µL of ProMTag Capture Resin for every 10 µL of 1% SDS. The total amount of SDS is what is important here so 10 µL of resin will also tolerate 20 µL of 0.5% SDS or 5 µL of 2% SDS.
5. If you are washing away contaminants or doing a buffer exchange, wash the resin at least three times with a wash buffer that is at pH 8.0 or greater. Use a volume of wash buffer that is at least five times the volume of capture resin if possible. Ex: For 40 µL of beads, wash with 200 µL of wash buffer.
    a. If washing away nucleic acids, we recommend adding a wash step that uses 250 mM sodium chloride.
    b. If washing away SDS, we recommend adding a wash step that uses a wash buffer that contains 50-75% acetonitrile
    c. For the final wash, wash using just purified, deionized water
6. To elute proteins from the ProMTag Capture Resin, add a buffer with a pH of 3.0 or less at a volume equal to the volume of ProMTag Capture Resin

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used. Our favorite buffer uses 100 mM formic acid. Incubate for 15 minutes to release the protein from the resin

   a. Ex: If you used 40 µL of ProMTag Capture Resin, you need 40 µL of elution buffer.
   b. If you would like to digest proteins into peptides, do not centrifuge your sample to elute, only add the elution buffer and incubate for 15 minutes. Then continue on to the next step.
   c. If you are not digesting proteins, repeat the elution step once to ensure all protein is eluted.

7. If you would like to digest proteins into peptides using mP-trypsin, add 10 µg of mP-trypsin to the sample for every 10 µg of protein that needs to be digested. Incubate for 1 h to digest.
   a. During this time the mP-trypsin will also covalently attach irreversibly to the ProMTag Capture Resin to ensure a clean final sample
8. After digestion, spin to elute the final sample. Add the same volume of elution buffer and elute one more time to ensure capture of the entire protein sample.