

Protocol: Affinity-Removable Trypsin for Recombinant Protein Processing

This protocol provides a suggested starting workflow for proteolytic processing of an untagged target protein followed by selective removal of C-terminal His-tagged recombinant trypsin using Ni-NTA resin.

1. Prepare the Protein Substrate

- Prepare the target protein in a trypsin-compatible buffer.
- Recommended starting condition: 20-50 mM Tris-Cl, pH 7.5-8.0.
- Avoid EDTA, EGTA, excess free histidine, or high concentrations of imidazole if Ni-NTA removal will be performed directly after digestion.

2. Add Recombinant His-Tagged Trypsin

- Use a trypsin-to-substrate ratio of 1:20 to 1:100 (w/w) as a starting range.
- A ratio of 1:50 (w/w) is recommended for initial optimization.
- Example: for 1 mg target protein, add approximately 20 µg trypsin for a 1:50 enzyme-to-substrate ratio.

3. Perform Proteolysis

- Incubate at approximately 37°C for 1-4 hours.
- The optimal trypsin concentration and incubation time depend on the substrate and desired extent of cleavage and should be determined empirically.
- For limited proteolysis or precursor processing, shorter reaction times and lower trypsin concentrations may be appropriate.

4. Capture the His-Tagged Trypsin

- Equilibrate an appropriate amount of Ni-NTA resin with binding buffer.
- Add the completed digestion mixture to the equilibrated Ni-NTA resin and gently mix for approximately 15-30 minutes.

5. Recover the Processed Protein

- Separate the Ni-NTA resin by centrifugation, filtration, or magnetic separation, depending on the matrix used.
- Collect the flow-through.
- The His-tagged trypsin is retained by the Ni-NTA matrix, while compatible untagged target proteins remain primarily in the recovered fraction.

Optimization note: Ni-NTA capture efficiency and product recovery should be optimized for each target protein and reaction condition.

Storage and Handling

- Store the frozen trypsin at -20°C to -80°C.

- Keep the enzyme cold during handling when practical.
- Avoid repeated freeze-thaw cycles; single-use aliquots are recommended when appropriate.

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