Immunoprecipitation with on-bead digestion

This document describes a protocol for performing on-bead digestion of an immunoprecipitated sample.

Quick links

1. Protein Sample Preparation

For all the solutions described below and throughout this protocol in general, you should do your best to use sterile glassware and reagents. As the goal here is to measure proteins, make an effort to minimize potential keratin contamination (e.g. wear a lab coat and don't touch your tubes with un-gloved hands).

1.1 Reagents and materials

- 1.5mL or 2.0mL Safe-Lock tubes (Thermo Scientific, CAT#05-402-25 or CAT#05-402-7)
- Benchtop centrifuge with holder for microcentrifuge tubes (multiple vendors)
- 1M TrisCl pH 7.5 (Thermo Scientific, CAT#J60636.K2)
- Calcium chloride (Sigma, CAT#C4901)
- Acetic acid (Sigma, CAT#A6283)
- Clean water (Thermo Scientific, CAT#10977023)
- Trypsin/rLysC mix (Promega, CAT#V5071)
- Trifluoroacetic acid, HPLC grade (CAT#85183, Thermo Scientific)
- Thermomixer with 2mL tube block (Eppendorf)

1.2 Solution recipes

- 100mM TrisCl pH 7.5 - 10uL of 1M stock for every 90uL of water (stock, stable at room temperature indefinitely)
- 50mM acetic acid - 28.7uL of 17.4M acetic acid in 9.97mL of water
- 1M calcium chloride - 1.1g in 10mL of water
- Digestion solution (need 200uL per sample)
  - 2uL of 1M calcium chloride solution
  - 20uL of 1M TrisCl pH 7.5
  - 178uL of water
- Acidification solution - 10% trifluoroacetic acid (TFA) in water

1.3 Protocol

This protocol assumes you have already performed your immunoprecipitation (or other on-bead purification) and are prepared for digestion.

1. If you have been rinsing with detergent-based solutions, you should perform at least 3 rinses with 100mM TrisCl pH 7.5 (800uL per rinse) to remove any residual detergent that may negatively impact digestion.
2. If your beads are not magnetic, add 800uL of 100mM TrisCl pH 7.5 and pipette to suspend and mix the beads.
3. Centrifuge for 1-minute at 500g. Discard the supernatant.
4. Repeat Steps 1 & 2 an additional two times for a total of 3 rinses.
5. If your beads are magnetic, you can skip the centrifugation and just use a magnetic rack.
6. Prepare your trypsin mixture:
    1. Reconstitute a 20ug trypsin/rLysC vial using 100uL of the provided reconstitution solution (or the same volume of 50mM acetic acid).
    2. Transfer 1uL of trypsin per sample to a fresh tube, and add 200uL of Digestion solution per sample. Vortex mix and keep on ice.
7. Add 200uL of the trypsin mixture to the tubes and incubate overnight at +37C in a Thermomixer with shaking at 800rpm.
8. The next day, spin the tubes at 1,000g for 5-minutes and recover the supernatant to a fresh 1.5mL tube.
9. Acidify the digests by adding 2uL of 10% (v/v) TFA to each.
10. At this point, the samples can be stored in the -20C freezer indefinitely, or desalted prior to MS analysis.