# 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)
- Ammonium bicarbonate (Sigma, CAT#A6141-500G)
- Urea (Sigma, CAT#U5128)
- 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

- 2M urea (120mg for every 100uL of 100mM TrisCl pH 7.5, prepare just before use)
- Ammonium bicarbonate solution (need 400uL per sample, recipe for 5mL)
  - 395mg ammonium bicarbonate
  - water to 5mL
- 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. If you have been rinsing with detergent-based solutions, you should perform at least 3 rinses with a solution like 1M ammonium bicarbonate to remove any residual detergent that may negatively impact digestion.

1. Reconstitute urea powder with an appropriate amount of 1M ammonium bicarbonate solution. Vortex mix. NOTE - urea will increase the volume of the solution generally by the amount of powder you have

weighed out. For example, to 120mg of urea, I would add 880uL of 1M ammonium bicarbonate solution to make 1mL of solution.
2. Reconstitute a 20ug trypsin/rLysC vial using 100uL of the vendor-provided reconstitution solution.
3. Transfer 2uL of trypsin per sample to a fresh tube, and add 100uL of prepared urea per sample. Vortex mix and keep on ice.
4. Centrifuge the sample tubes at 1,000g for 5-minutes at room temperature. Discard the supernatant.
5. Add 100uL of the prepared trypsin/urea mix to each tube. Do not attempt to mix, vortex, or pipette.
6. Transfer the tubes to a shaking Thermomixer set at +30C and 1,000rpm, and digest for 2-4 hours.
7. Add 300uL of 1M ammonium bicarbonate solution to each tube and place back in the Thermomixer and incubate overnight at +30C and 1,000rpm mixing.
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 40uL 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.