

## Quick start guide for UPECK-Pep

For extraction, cleanup, and digestion of protein samples

Cat #: Pep-1000

**Prior to starting:** Resuspend the provided DTT in 100  $\mu$ L water (first use only) and resuspend a tube of IAA per sample in 20  $\mu$ L water.

1. Add up to 40  $\mu$ g of your protein sample in up to 36  $\mu$ L to a provided 1.5 mL tube.
  - a. Consult the full-length guide if your input volume is larger than 36  $\mu$ L.
2. Bring sample volume to 36  $\mu$ L with the provided lysis buffer.
  - a. If your sample is already at 36  $\mu$ L, move ahead to step 3
3. Add 2  $\mu$ L DTT and incubate at 56°C for 30 minutes.
4. Add 2  $\mu$ L IAA and incubate your sample at room temperature in the dark for 30 minutes.
5. Add 3.4  $\mu$ L of ProMTag to the sample and vortex to mix. Incubate 30 min at room temperature.
6. While waiting, prepare the capture resin.
  - a. Place a ProMTag capture tube into a provided 2 mL waste collection tube.
  - b. Spin briefly and discard flow through. Repeat if necessary to remove all liquid.
  - c. Add 200  $\mu$ L of WB3 and vortex. Spin briefly and discard flow through.
7. Add 16.3  $\mu$ L lysis buffer to tagged protein sample.
8. Add your labeled protein sample to the capture resin.
9. Incubate with rotation for 15 min at room temperature. Ensure solution is colorless before continuing.
10. Wash the resin, discarding the flow through after each wash:
  - a. 1X 200  $\mu$ L with WB1
  - b. 2X 200  $\mu$ L with WB2
  - c. 1X 200  $\mu$ L with WB3
  - d. 2X 200  $\mu$ L with water
11. Add 40  $\mu$ L EB. Incubate with rotation for 15 minutes.
12. Add 1  $\mu$ L mP-Trypsin for every 1  $\mu$ g of protein in the sample. Incubate at 37°C for 1 hour.
13. Transfer the capture tube to a collection tube and spin briefly. **This is your sample. Do not throw it away.**
14. Add 40  $\mu$ L EB and incubate with rotation for 15 minutes.
15. Transfer the capture tube back to the same collection tube and spin briefly.
16. Concentrate sample with a speed vac if desired.