Universal Protein Extraction and Cleanup Kit – Peptide (UPECK-Pep) Guide

For extraction, cleanup, and digestion of protein samples for mass spectrometry

Protocol optimized for 1-40 µg protein with concentrations 1 mg/mL or greater

Catalog number: Pep-1000

Contents:

Kit component	Amount	Catalog number
ProMTag	35 µL	T-6000
MT-Trypsin	175 µL	MT-9000
Lysis buffer (LB)	30 mL	B-3000
DTT	50 µL	Pep-1100
IAA	8 x 20 µL	Pep-1200
Wash buffer 1 (WB1)	8 mL	Pep-1300
Wash buffer 2 (WB2)	8 mL	Pep-1400
Wash buffer 3 (WB3)	8 mL	Pep-1500
Elution buffer (EB)	1 mL	Pep-1600
ProMTag capture resin	8 x 40 µL	CSB-8000
2 mL waste collection tubes	8 tubes	SB-4000
1.5 mL low protein binding tubes	16 tubes	-

Storage:

Store the entire kit at 4°C, with the exception of the Lysis Buffer which should be kept at room temperature. Make note of the expiration date on the box. Past this date, certain elements of the box may begin to lose their efficacy. If you can't find the expiration date, email us at info@impactproteomics.com with the lot number and we will find it for you.

Safety:

Always protect yourself appropriately when working with chemicals. This includes, but is not limited to, an appropriate lab coat, disposable gloves, and protective eye goggles. For more information, please read the included Safety Data Sheets about the included reagents. These are also available online at https://impactproteomics.com/product/upeck-pep/

Note: The ProMTag, WB1, WB2, and WB3 contain various amounts of acetonitrile. Please dispose of appropriately and avoid open flames.

Note: EB contains formic acid. Avoid contact with skin and eyes. If skin contact occurs, remove contaminated garments and rinse skin thoroughly with water. If eye contact occurs, remove contact lenses if applicable and rinse with plenty of water.

Equipment and reagents you will need before you start:

- Protein sample, cell lysate, or protein source
- Pipettes and pipette tips
- Benchtop centrifuge (mini or full size)
- Sample rotator (rotisserie or carousel)
- Heating block
- Boiling water bath (if using our lysis method)
- Vortex

Cell lysis and preparation of the biological sample for UPECK processing

No matter the biological source, our kits will work to separate proteins from any other undesirable biologics. For the best results, cell lysis must be as thorough as possible. We have included our favorite lysis buffer for you to use that is compatible with most tissue lysis methods, but it is certainly not the only one that is compatible.

Feel free to use your own lysis buffer **as long as it does not contain TRIS (or any other buffer with primary amines) and is ~ pH 8.0**. If your lysis technique uses TRIS, we recommend switching to 100 mM HEPES, pH 8.0 (the concentration matters so make sure it's 100 mM). If you are having trouble with your lysis step, please get in touch with us at info@impactproteomics.com so we can help you troubleshoot.

If you are not working with a strong denaturant such as high concentration SDS, we **highly** recommend using a protease inhibitor in your lysis buffer to prevent protein degradation.

Other notes to consider before you begin

1) All centrifugation steps may be performed on a benchtop centrifuge at room temperature. Our favorite mini benchtop centrifuge reaches 7,000 rpm, but if yours is a different speed just keep spinning until the liquid passes through to the collection tube. It doesn't take much centrifugal force since there aren't any filters.
2) For the best results, keep the beads suspended during all incubation steps. We do this using a 360° rotisserie, but you can also use a carousel to keep the beads suspended in solution. We do not recommend shaking the beads, but if you do make sure you use **gentle** agitation.
3) The capture tube has two holes near the top that prevent loss of liquid when closing the tubes. Make sure you don't cover these holes while closing the cap!
4) Try to avoid touching the bottom of the ProMTag capture tubes, as anything that gets on the bottom of the tube may end up in your final sample.
5) We recommend briefly vortexing the ProMTag capture tubes at multiple points throughout the protocol to aid in resuspension of the beads. **Never vortex the capture tube alone.** Always vortex using the 2 mL waste tube as an adapter to avoid touching the bottom of the capture tube.
6) We do not recommend pipetting to mix at any stage where the capture resin is present, as the resin will stick to the tip and result in suboptimal yield.
7) You selected an elution buffer compatible with mass spectrometry, and therefore you may see slightly lower yields (50-70%) than with a detergent containing buffer. That is because intact proteins have a higher tendency to stick non-specifically to resin or tubes when no detergents or salts are present. If you are not performing mass spectrometry or any other analysis where detergents will cause interference, we highly recommend using our SDS or urea elution buffers to get the highest yield possible.

Protocol for protein sample extraction, cleanup, and digestion using the UPECK-Pep kit

1) If you are starting with a prepared lysate or proteins, skip this step but be sure to read the note above regarding lysis buffers. If you are starting with un-lysed cells, start here. This is our favorite protocol to prepare a cell lysate, but if it won't work for your sample contact us and we'll help you figure out an alternative.

   a. Add LB to your pelleted cells. The volume you add is up to you, but we recommend adding about 2x the volume of your pellet (Ex: if your pellet is 100 µL, add 200 µL LB).

   b. Optional: Sonicate (we use a Brandon Probe sonicator) at 30% power, 30% duty cycle for 10-15 pulses.

   c. Incubate the cells in a boiling water bath for 15 minutes.

   d. Pellet cell debris by spinning at ~3300 x g for 10 minutes.

   e. Transfer the supernatant to a fresh tube and assay protein concentration via your favorite method. We recommend a Bradford or BCA assay.

2) Add 1 µg to 40 µg of protein in lysis buffer to a protein low bind tube provided with the kit. If the volume of your sample is less than 18 µL, bring the final volume up to 18 µL using the provided lysis buffer.

   a. If your sample is dilute, no worries! You can use a larger starting volume if necessary. However, we recommend keeping the input volume at or under 40 µL if possible.

3) Add 2 µL DTT and incubate your sample at 56°C for 30 minutes.

   a. The first time you use the kit, resuspend the provided DTT in 50 µL water. After resuspending the DTT, store it at -20°C.

   b. If your starting volume is more than 18 µL, scale up the amount of DTT and IAA you use (Ex: if you start with 30 µL of lysate, add 3.3 µL of DTT and 3.3 µL IAA in step 5).

4) While your sample is incubating, resuspend one tube of IAA in 20 µL water. Vortex or pipet thoroughly to mix.

5) Add 2 µL IAA and incubate your sample at room temperature in the dark for 30 minutes.

6) Add 3.4 µL of ProMTag to your protein sample and pipette up and down or vortex briefly to mix well. Spin briefly to collect your sample at the bottom of the tube if necessary.

7) Incubate for 30 minutes at room temperature to tag your proteins.

8) During the last 5 minutes of the incubation, prepare the capture tube for use.

   a. Take a capture tube from the bag, remove the parafilm, and place it into one of the provided 2 mL waste collection tubes.

   b. Spin briefly (~10 seconds) in a tabletop centrifuge until all the liquid has passed into the waste collection tube. Discard the flowthrough. Spin again if necessary to remove all liquid. The beads will be bright white when they are dry.

   c. Add 200 µL of WB3 buffer and vortex briefly (~2 seconds).

   d. Spin briefly in a tabletop centrifuge until all the liquid has passed into the waste collection tube. Discard the flowthrough.

9) Once step 7 and 8 are complete, bring the final volume of your tagged protein sample to 40 µL with the provided lysis buffer. If you started with 18 µL and followed the instructions above, add 14.6 µL lysis buffer.

10) Add the tagged protein sample to the capture resin. Vortex briefly to mix.

   a. Reminder: Never vortex the capture tube alone**.** Always vortex using the 2 mL waste tube as an adapter to avoid touching the bottom of the capture tube.

11) Incubate the tube at room temperature with gentle rotation for 15 minutes. We use a rotisserie, but you can use anything that works to keep the resin suspended, so long as it is gentle (no harsh shaking). As the reaction proceeds, the pink solution should turn colorless.

   a. If your sample is still pink after 15 minutes, allow it to incubate for a few more minutes.

12) Place the capture tube back into the waste collection tube. Add 200 µL WB1 to the sample. Vortex for ~2 seconds to mix. Spin briefly (~10 seconds) in a tabletop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough.

13) Add 200 µL WB2 to the sample. Vortex for ~2 seconds to mix. Spin briefly (~10 seconds) in a tabletop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough.

14) Repeat step 13 one time.

15) Add 200 µL WB3 to the sample. Vortex for ~2 seconds to mix. Spin briefly (~10 seconds) in a tabletop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough.

a. Beads may stick along the sides of the capture tube after this wash. This is normal.

16) Add 200 µL ultrapure deionized water to the sample. Vortex for ~2 seconds to mix. Spin briefly (~10 seconds) in a tabletop centrifuge until all the liquid has passed into the waste collection tube. Discard the flowthrough.

17) Repeat step 16 one time.

18) Add 40 µL EB to the capture resin. Briefly vortex the sample, then gently flick or tap the capture tube to bring most of the liquid back to the bottom of the tube. If there are beads stuck along the sides of the capture tube that are not immersed in EB, use a pipet tip to gently scrape the beads down into the EB.

a. From this stage until elution **do not** centrifuge the sample to bring the liquid back to the bottom of the tube, as doing so will result in loss of proteins or peptides.

19) Incubate the tube at room temperature with gentle rotation for 15 minutes.

20) Add 20 µL of MT-Trypsin and incubate at 37°C for 1 hour.

a. Longer digestion times will not improve yield. We recommend ensuring that digestion does not exceed 1 hour.

21) Transfer the capture tube to a provided low bind protein tube.

22) Spin briefly (~10 seconds) in a tabletop centrifuge until all the liquid has passed into the low bind protein tube. **DO NOT DISCARD THE FLOWTHROUGH.**

23) Add 40 ul EB to the capture resin. Vortex for ~2 seconds to mix.

24) Incubate the tube at room temperature with gentle rotation for 15 minutes.

25) Return the capture tube to the same low bind protein tube. Spin briefly (~10 seconds) in a tabletop centrifuge until all the liquid has passed into the low bind protein tube.

26) That tube now contains your sample of pure peptides in an acidic, volatile buffer. If desired, you can concentrate the sample by drying in a vacuum concentrator.