# ProMTag Universal Protein Extraction and Cleanup Kit – Peptide 41-150 µg (UPECK-Pep 41-150 µg) Guide

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

Protocol optimized for 41-150 µg protein

## Contents

| Kit component | Quantity | Storage |
|---|---|---|
| Lysis buffer (LB) | 30 mL | RT |
| ProMTag | 130 µL | -20°C |
| MT-Trypsin | 650 µL | -20°C |
| DTT | 200 µL | -20°C |
| IAA | 8 x 30 µL | 4°C |
| Wash buffer 1 (WB1) | 8 mL | 4°C |
| Wash buffer 2 (WB2) | 8 mL | 4°C |
| Elution buffer (EB) | 3 mL | 4°C |
| ProMTag capture resin | 1.3 mL | 4°C |
| 2 mL waste collection tube | 8 tubes | 4°C |
| 1.5 mL low protein binding tube | 16 tubes | 4°C |
| 2 mL peptide collection tube | 8 tubes | 4°C |
| 0.5 mL Resin Capture tube (RC-tube) | 8 tubes | 4°C |
| RC-tube end cap | 8 caps | 4°C |

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## Storage

Store the entire kit at 4°C, EXCEPT: Lysis Buffer, store at room temperature; and ProMTag, MT-Trypsin, and DTT, store at -20°C. We recommend using your kit within 6 months of receiving it.

## Safety Information

Always protect yourself appropriately when working with chemicals. This includes, but is not limited to, utilizing an appropriate lab coat, disposable gloves, and protective eye goggles. For more information, please consult the appropriate Safety Data Sheets. These are available online at https://www.impactproteomics.com/resources.

ProMTag, WB1, and WB2 contain various amounts of acetonitrile. Please dispose of these appropriately and avoid open flames.

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 thoroughly with 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
- Vortex
- Ultrapure deionized water

# Cell lysis and preparation of the biological sample for UPECK processing

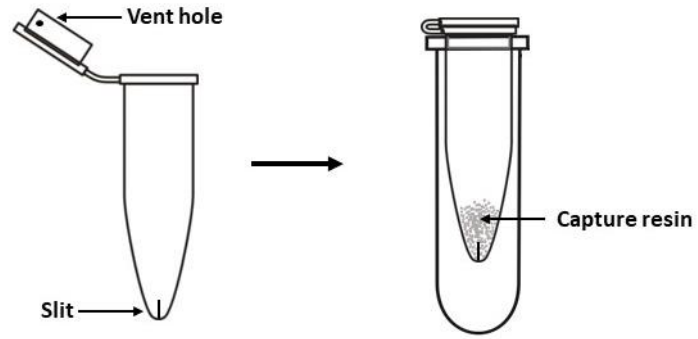
No matter the biological source, UPECK kits will work to separate proteins from any other undesirable biologics. For the best results, cell lysis must be as thorough as possible. The lysis buffer included with the kit is compatible with most lysis methods, but other lysis buffers are also compatible with UPECK kits.

You can 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. If you need advice on lysis for your particular sample, we are available to help. Simply email us at info@impactproteomics.com.

If your lysis buffer does not contain a strong denaturant such as high concentration SDS, we **highly** recommend using a protease inhibitor in your lysis buffer to prevent protein degradation. Note that certain protease inhibitors contain primary amines; we recommend using a combination of pepstatin, leupeptin, and PMSF.

# Other notes to consider before you begin

- The RC-tubes have two features that distinguish them from typical spin columns: a fine slit to retain resin instead of a frit, and a hole in the rim of the cap to prevent loss of liquid when closing the tubes.



Resin Capture (RC) Tube RC-Tube in 2 mL adapter tube

- Avoid touching the bottom of the RC-tubes. Keep the RC-tube in a waste tube or a low protein binding tube when incubating or vortexing, except when mixing on a rotisserie.
    - We recommend briefly vortexing or tapping the RC-tubes at multiple points throughout the protocol to aid in resuspension of the resin. **Never vortex the RC-tube alone.** Always vortex by placing the RC-tube in a 1.5 mL or 2 mL tube as an adapter to avoid touching the bottom of the RC-tube.

- We do not recommend pipetting to mix at any stage where the resin is present, as the resin will stick to the tip and result in suboptimal yield.
- All centrifugation steps may be performed on a benchtop centrifuge at room temperature.
- For best results, keep the resin suspended during all incubation steps. This can be done using a 360° rotisserie (recommended) or a carousel. We do not recommend shaking or vortexing to keep the resin suspended, but if you do, use **gentle** agitation.
- The entire protocol takes 5-6 hours to perform, depending on the number of samples being processed.

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

## Reduction and Alkylation

1. Add 41-150 µg of protein sample in lysis buffer (LB) to a low protein binding tube provided with the kit. If the sample volume is less than 70 µL, bring the final volume up to 70 µL using the provided LB.
    a. If using a lysis buffer other than the one included with the kit, ensure you first read our note regarding lysis buffers on Page 3.
    b. If the sample is dilute, a larger starting volume can be used. We recommend keeping the input volume at or under 150 µL.
2. Add 7.5 µL DTT and incubate at 56°C for 30 minutes.
    a. The first time using the kit, resuspend the provided DTT in 200 µL water. Store resuspended DTT at -20°C.
    b. If your input volume was more than 70 µL, scale up the amount of DTT and IAA used. The DTT should be diluted to 1X from the 10X stock and an equal volume of IAA should be used in step 4.
3. During step 2 incubation, resuspend one tube of IAA in 30 µL water. Vortex or pipet thoroughly to mix.
    a. The kit contains 8 tubes of IAA to allow use of a fresh tube with each sample. If multiple samples are being processed together, you may not use all 8 tubes of IAA.
4. Add 7.5 µL IAA and incubate at room temperature in the dark for 30 minutes.

## Protein labeling with ProMTag and binding to ProMTag capture resin

5. Add 12.8 µL ProMTag and pipette up and down or vortex briefly to mix. Spin briefly to collect the sample at the bottom of the tube if necessary.
6. Incubate for 30 minutes at room temperature.
7. During the last 5-10 minutes of step 6 incubation, prepare the ProMTag capture resin for use.
    a. Vortex the ProMTag capture resin to thoroughly resuspend.
    b. Place an RC-tube into a 2 mL waste collection tube and pipette 150 µL of ProMTag capture resin into the RC-tube.

i. We recommend using wide-bore pipette tips to pipette the resin if available.

c. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. The resin will be bright white when it is dry. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.

d. Add 300 µL of ultrapure deionized water.

e. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.

**8.** Once steps 6 and 7 are complete, add LB to bring the tagged protein sample from step 6 to 150 µL.

a. If you started with 70 µL and followed the instructions above, add 52.2 µL LB. If your sample is over 150 µL, skip this step.

**9.** Add the tagged protein sample to the capture resin. Be sure to pipette your sample directly onto the capture resin. Tap the RC-tube gently to mix.

**10.** Incubate the RC-tube at room temperature with gentle rotation for 15 minutes.

a. We recommend using a rotisserie to keep the resin suspended, but other methods can be used so long as they are gentle (no harsh shaking). Avoid positioning the RC-tube vertically to prevent spillage through the hole in the lid of the RC-tube.

b. As the reaction proceeds, the pink solution should turn colorless. If the sample is still pink after 15 minutes, allow it to continue incubating for another 5 minutes.

## ProMTag capture resin washing and protein release

**11.** Place the RC-tube back into the waste collection tube. Add 300 µL WB1 to the sample. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.

a. Note: If you started with a dilute lysate (if your lysate input was more than 150 µL) we recommend adding a brief vortex of ~1 second after each addition of WB.

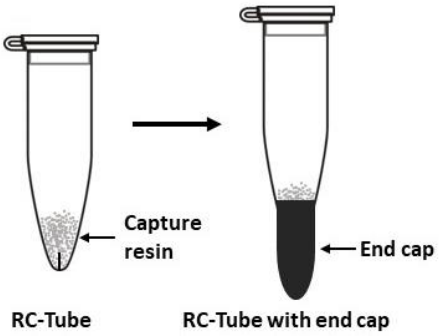
b. Reminder: Never vortex the RC-tube alone**.** Always vortex by placing the RC-tube in a 2 mL waste tube as an adapter to avoid touching the bottom of the RC-tube.

**12.** Add 300 µL WB2 to the sample. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.

**13.** Repeat step 12 one more time.

a. If your starting sample was dilute and you added more than 150 µL of lysate to the capture resin, we recommend doing a third wash with WB2.

14. Add 300 µL ultrapure deionized water to the sample. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.
    a. Resin may stick along the sides of the RC-tube after this wash. This is normal.
15. Repeat step 14 one more time.
16. Cap the RC-tube with a RC-tube end cap as shown in the diagram to the right.
    a. The cap will not fully push onto the RC-tube. Part of it will extend from the bottom of the RC-tube as shown in the diagram.




17. Add 150 µL EB directly to the capture resin. Tap the tube gently if necessary to ensure the capture resin is fully immersed in 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.
18. Incubate the RC-tube at room temperature with gentle rotation for 15 minutes.

## Protein digestion with MT-Trypsin

19. Transfer the RC-tube to a provided 2 mL peptide collection tube, keeping the end cap on, and add 75 µL MT-Trypsin directly to the capture resin. Tap the tube to ensure the resin and sample are mixed 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.
    b. Ensure the RC-tube is properly heated during this incubation. Some heating blocks position the RC-tube too high when nested in a 2 mL tube. If necessary, remove the RC-tube from the 2 mL peptide collection tube and incubate without an adapter tube.
20. Remove the RC-tube end cap and place RC-tube in a 2 mL peptide collection tube.
    a. Squeeze the bottom of the RC-tube end cap as it is removed to prevent leakage or sample loss.
21. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all the liquid has passed into the 2 mL peptide collection tube. **DO NOT DISCARD THE FLOWTHROUGH.**
22. Add 150 µL EB to the capture resin and tap to mix.
23. Return the RC-tube to the same 2 mL peptide collection tube from step 21. Spin briefly (~5-10 seconds) until all of the liquid has passed into the 2 mL peptide collection tube.

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

a. If desired, transfer the peptides to a low protein binding tube for drying and storage.

# Quick start guide for UPECK-Pep 41-150 µg

For extraction, cleanup, and digestion of protein samples for mass spectrometry.
Protocol optimized for 41-150 µg protein.
This abbreviated guide is intended for users familiar with the UPECK-Pep protocol. We highly recommend first time users follow the full-length guide.

Prior to starting: Resuspend the provided DTT in 200 µL water (first use only) and resuspend one tube of IAA per sample in 30 µL water.
Note: Never vortex the Resin Capture (RC)-tube alone. Always vortex by placing the RC-tube in a 1.5 mL or 2 mL tube as an adapter to avoid touching the bottom of the tube.

1. Add up to 150 µg of protein sample in up to 70 µL lysis buffer (LB) to a provided 1.5 mL tube.
    a. Consult the full-length guide if your input volume is larger than 70 µL.
2. Bring sample volume to 70 µL with the provided LB.
3. Add 7.5 µL DTT and incubate at 56°C for 30 minutes.
4. Add 7.5 µL IAA and incubate at room temperature in the dark for 30 minutes.
5. Add 12.8 µL ProMTag and vortex to mix. Incubate for 30 minutes at room temperature.
6. During the last 5-10 minutes of incubation, prepare the ProMTag capture resin as follows:
    a. Vortex ProMTag capture resin to resuspend.
    b. Place an RC-tube in a 2 mL waste collection tube and pipette 150 µL of ProMTag capture resin into the RC-tube.
    c. Spin briefly to remove liquid.
    d. Add 300 µL water and spin briefly to dry resin.
7. Bring tagged protein sample to a final volume of 150 µL with LB.
8. Add protein sample directly to capture resin and tap gently to mix. Incubate with gentle rotation at room temperature for 15 minutes.
9. Wash the resin, discarding the flowthrough after each wash:
    a. 1X 300 µL with WB1
    b. 2X 300 µL with WB2
    c. 2X 300 µL with water
10. Cap the RC-tube with a RC-tube end cap.
11. Add 150 µL EB directly to capture resin and tap gently to mix. Incubate with gentle rotation at room temperature for 15 minutes.
12. Transfer RC-tube to a provided 2 mL peptide collection tube, keeping the end cap on. Add 75 µL MT-Trypsin to the capture resin and tap gently to mix. Incubate at 37°C for 1 hour.
    a. Ensure the RC-tube is properly heated. Some heating blocks position the RC-tube too high when nested in a 2 mL tube. If necessary, remove the RC-

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tube from the 2 mL peptide collection tube and incubate without an adapter tube.

13. Spin briefly to collect peptide sample. **DO NOT DISCARD**.
14. Add 150 µL EB to the capture resin and tap gently to mix.
15. Transfer the RC-tube back to the same peptide collection tube from step 13 and spin briefly.
    a. Transfer peptides to a low protein binding tube if desired.
16. Concentrate sample with a vacuum concentrator if desired.