Universal Protein Extraction and Cleanup Kit – Intact Protein (UPECK-WP) Guide

For extraction and cleanup of intact protein samples compatible with mass spectrometry

Protocol optimized for 1-40 µg protein

Contents

Kit component	Quantity	Storage
Lysis buffer (LB)	30 mL	RT
ProMTag	35 µL	-20°C
DTT	50 µL	-20°C
IAA	8 x 20 µL	4°C
Wash buffer 1 (WB1)	8 mL	4°C
Wash buffer 2 (WB2)	8 mL	4°C
Elution buffer (EB)	1 mL	4°C
ProMTag capture resin	400 µL	4°C
2 mL waste collection tube	8 tubes	4°C
1.5 mL low protein binding tube	16 tubes	4°C
0.5 mL Resin Capture tube (RC-tube)	8 tubes	4°C

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Storage

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

Safety

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

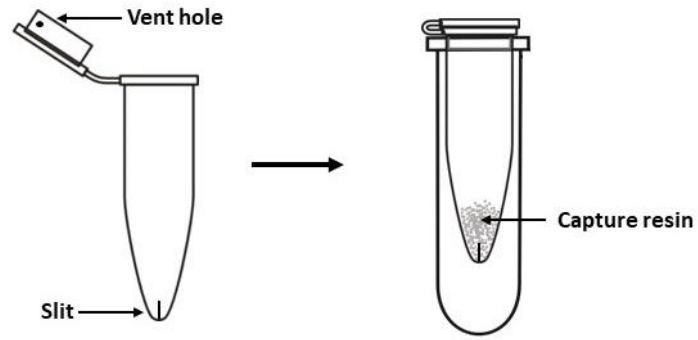
No matter the biological source, UPECK kits will work to separate proteins from any other undesirable biologics. For the best results, cell lysis must be as thorough as possible. The lysis buffer included with the kit is compatible with most lysis methods, but other lysis buffers are also compatible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



- 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.
    a. 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.
- You selected an elution buffer compatible with mass spectrometry, and therefore you may see slightly lower yields (50-70%) than with an elution buffer that contains detergent. 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-WP kit

Reduction and Alkylation

1. Add 1-40 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 18 µL, bring the final volume up to 18 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 40 µL
2. Add 2 µL DTT and incubate at 56°C for 30 minutes.
    a. The first time using the kit, resuspend the provided DTT in 50 µL water. Store resuspended DTT at -20°C.
    b. If your input volume was more than 18 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 20 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 2 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 3.4 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 a RC-tube into a 2 mL waste collection tube and pipette 40 µL of ProMTag capture resin into the RC-tube.

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

c. Add 200 µL of ultrapure deionized water.

d. 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.

8. Once steps 6 and 7 are complete, add LB to bring the tagged protein sample from step 6 to 40 µL.

a. If you started with 18 µL and followed the instructions above, add 14.6 µL LB. If your sample is over 40 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 200 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 40 µL) we recommend adding a brief vortex of ~1 second after each addition of WB. 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 200 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 40 µL of lysate to the capture resin, we recommend doing a third wash with WB2.

14. Add 200 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. Add 40 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.
17. Incubate the RC-tube at room temperature with gentle rotation for 15 minutes.
18. Transfer the RC-tube to a provided low protein binding tube.
19. Spin briefly (~2-6 seconds) in a benchtop centrifuge until all the liquid has passed into the low protein binding tube. **DO NOT DISCARD THE FLOWTHROUGH.**
20. Add 40 µL EB to the capture resin and tap to mix.
21. Incubate the RC-tube at room temperature with gentle rotation for 5 minutes.
22. Return the RC-tube to the same low protein binding tube from step 19. Spin briefly (~5-10 seconds) until all of the liquid has passed into the low protein binding tube.
23. That tube now contains your sample of pure proteins in an acidic, volatile buffer. If desired, you can concentrate the sample by drying it in a vacuum concentrator.

Quick start guide for UPECK-WP

For extraction and cleanup of intact protein samples compatible with mass spectrometry.
Protocol optimized for 1-40 µg protein.
This abbreviated guide is intended for users familiar with the UPECK-WP protocol. We highly recommend first time users follow the full-length guide.

Prior to starting: Resuspend the provided DTT in 50 µL water (first use only) and resuspend one tube of IAA per sample in 20 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 40 µg of protein sample in up to 18 µL lysis buffer (LB) to a provided 1.5 mL tube.
    a. Consult the full-length guide if you input volume is larger than 18 µL.
2. Bring sample volume to 18 µL with the provided LB.
3. Add 2 µL DTT and incubate at 56°C for 30 minutes.
4. Add 2 µL IAA and incubate at room temperature in the dark for 30 minutes.
5. Add 3.4 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 a RC-tube in a 2 mL waste collection tube and pipette 40 µL of ProMTag capture resin into the RC-tube.
    c. Add 200 µL water and spin briefly to dry resin.
7. Bring tagged protein sample to a final volume of 40 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 200 µL with WB1
    b. 2X 200 µL with WB2
    c. 2X 200 µL with water
10. Add 40 µL EB directly to capture resin and tap gently to mix. Incubate with gentle rotation at room temperature for 15 minutes.
11. Transfer RC-tube to a provided 1.5 mL low protein binding tube.
12. Spin briefly to collect protein sample. **DO NOT DISCARD**.
13. Add 40 µL EB to the capture resin and incubate with rotation for 5 minutes.
14. Transfer the RC-tube back to the same collection tube from step 12 and spin briefly.
15. Concentrate sample with a vacuum concentrator if desired.

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