Universal Protein Isolation Kit – Whole Protein (UPIK-WP) Guide

For clean up and purification of whole protein samples

Catalog number: WP-2000

Contents:

Kit component	Amount	Catalog number
ProMTag	150 µL	T-6000
Lysis buffer (LB)	30 mL	B-3000
Wash Buffer 1 (WB1)	8 mL	WP-2100
Wash Buffer 2 (WB2)	20 mL	WP-2200
Wash Buffer 3 (WB3)	8 mL	WP-2300
Elution Buffer (EB)	1 mL	WP-2400
Neutralization buffer (NB)	500 µL	WP-2500
ProMTag capture resin	8	CSB-8000
2 mL waste collection tubes	8	SB-4000
1.5 mL LoBind Eppendorf tubes	16	-

Storage:

All elements should be kept refrigerated until use (4-10 degrees C), except buffer A. Some of the buffers may crystalize when refrigerated but will go back into solution readily upon reaching room temperature before use. Make note of 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-wp/

Note: The ProMTag and WB2 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.

Applications:

The UPIK-WP kit may be used to prepare protein samples for a number of applications including:

- Two-dimensional gel electrophoresis preparation
- Protein quantification preparation
- Detergent removal
- Salt/small molecule removal
- Removal of other biological components after cell lysis
- Buffer exchange

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) or a shaker that fits 1.5/2 mL tubes
- Boiling water bath (if using our lysis method)
- Vortex

Cell lysis and preparation of the biological sample for UPIK processing

No matter what biological source our kits will work to separation proteins from any other undesirable. 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 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 or a shaker to keep the beads suspended in solution. We do not recommend shaking the beads if possible, 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. Try to avoid touching the resin with a tip at any point.

Protocol for whole protein sample clean up using the UPIK-WP 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. Incubate the cells in a boiling water bath for 15 minutes.
    c. Pellet cell debris by spinning at ~3300 x g for 10 minutes.
    d. Transfer the supernatant to a fresh tube and assay protein concentration via your favorite method. We recommend a Bradford or BCA assay.
2) Add 41 µg to 200 µg of protein in lysis buffer to a provided low protein binding tube. Dilute your sample to 1 mg/mL using the provided lysis buffer. If your protein concentration is lower than 1 mg/mL, do not dilute it any further.
3) Add 16.7 µL of ProMTag to your protein sample and pipette up and down to mix well.

4) Incubate for 30 minutes at room temperature to tag your sample.

5) 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 flow through. Spin again if necessary to remove all liquid. The beads will be bright white when they're 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 flow through.

6) Once step 4 and 5 are complete, add the tagged protein sample to the prepared ProMTag capture resin tube and pipette up and down to mix well. 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.

7) Incubate the tube at room temperature with gentle rotation for 30 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.

    b. If you are fluorescently tagging your samples for two-dimensional gel electrophoresis, do it during this incubation step. We recommend using Amersham CyDye™ DIGE Fluor minimal dyes.

8) Place the capture tube back into the waste collection tube. Add 300 µL WB1 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 flow through.

9) Repeat step 8 two times.

10) Add 300 µL WB2 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 flow through.

11) Repeat step 10 four times.

    a. This is the wash buffer that gets rid of SDS, so it is really important to actually do four washes here.

12) Add 300 µL WB3 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 flow through.

13) Repeat step 12 three times.

14) Add 50 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.

15) Incubate for 15 minutes with rotation.

16) Place capture tube into a provided low protein binding tube. Spin briefly (~10 seconds) until all the liquid has passed into the low protein binding tube. This is the first half of your sample so don't throw it away.

17) Repeat step 14 and 16 one time. Skip the incubation period.

18) That tube now contains your sample of pure proteins. It is in an acidic buffer, so if you need a neutral pH add X µL of the provided neutralization buffer.