ab65400 Plasma Membrane Protein Extraction Kit

For the rapid and sensitive extraction and purification of Plasma Membrane proteins from cultured cells and tissue samples. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab65400
(use abcam.cn/ab65400 for China, or abcam.co.jp/ab65400 for Japan)

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Materials Supplied and Storage

Item	Quantity
Homogenization Buffer I/Homogenize Buffer	100 mL
Upper Phase Solution	20 mL
Lower Phase Solution	20 mL
Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail (Lyophilized)	1 vial

Store kit at -20°C. Read the entire protocol before beginning the procedure. Be sure to keep all buffers and reagents on ice at all times during the experiment.
Upper phase solution and lower phase solutions should not be subject to freeze thaw cycles and should be aliquoted and stored at -20°C.

Protease Inhibitor Cocktail I/PROTEASE INHIBITOR COCKTAIL: Reconstitute Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail by adding 250 µl of DMSO, mix well.
HOMOGENIZE BUFFER MIX: Aliquot enough Homogenization Buffer I/Homogenize Buffer, add 1/500 volume of the reconstituted Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail (e.g., Add 10 µl to 5 ml buffer) to make the Homogenize Buffer Mix.
Note: Some precipitation may occur after adding the Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail. You may continue using the buffer or simply remove the precipitates by centrifugation.

Materials Required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Dounce homogeniser
- PBS
- Triton-X-100

Assay Procedure

The following protocol is described for extraction of ~5-10 x 10^8 cells. If more cells are used, scale up the volume proportionally.

A. Extraction of Total Cellular Membrane Proteins

1. Collect cells ~1 g wet weight (0.2-10 x 10^8) by centrifugation at 600 x *g* for 5 minutes at +4°C.
   For ***adherent cells***, scrape cells in PBS and then spin down (3000 rpm for 5 minutes) to pellet cells.
2. Wash cells once with 3 ml of ice cold PBS.
3. Re-suspend cells in 2 ml of the Homogenize Buffer Mix in an ice-cold Dounce homogenizer. Homogenize cells on ice for 30-50 times.
   For ***tissue samples***, we recommend a minimum starting amount of tissue of 50-100mg homogenize tissues in 2 volumes of the 1X Homogenization Buffer I/Homogenize Buffer, until it is completed lysed (30-50 times).
   Note: Efficient homogenization may depend on the cell type. To check the efficiency of the homogenization, pipette 2-3 µl of the homogenized suspension onto a cover slip and observe under a microscope.
   A shiny ring around the cell indicates that cells are still intact. If 70-80 percent of the cells do not have the shiny ring, proceed to the next step. Otherwise, perform 10-30 additional passes.
4. Transfer the homogenate to multiple 1.5 ml microcentrifuge tubes. Centrifuge in 700 x *g* for 10 minutes at +4°C. Collect supernatant and discard the pellet.
5. Transfer the supernatants to new vials and centrifuge at 10,000 x *g* for 30 min at +4°C.
6. Collect supernatant (This is the **Cytosol Fraction**). The pellet is the **total cellular membrane protein** (containing proteins from both plasma membrane and cellular organelle membrane).
   Note: You may stop here if you only need the total cellular membrane proteins. If you would like to further isolate the plasma membrane proteins specifically, continue to section B.

B. Purification of Plasma Membrane Proteins

1. Re-suspend the total membrane proteins pellet in 200 µl of the Upper Phase Solution. Add 200 µl of the Lower Phase Solution. Mix well and incubate on ice for 5 minutes (Mark the tube as A).
2. Prepare a fresh phase tube without samples. Adding 200 µl of Upper Phase Solution and 200 µl of Lower Phase Solution (Mark the tube as B).

   Note: Tube B is useful as a reference for the user to observe the phase separation, if not needed, in the next steps Upper Phase Solution or Lower Phase Solution can be taken directly from the stock/bottles provided

3. Centrifuge both A & B tubes in a microcentrifuge at 3500 rpm (1000 x *g*) for 5 minutes at 4°C.
4. Carefully transfer the upper phase from tube A to a new tube (tube C), keep on ice.
5. To maximize the yield, extract the tube A lower phase again by adding 100 µl of the Upper Phase Solution from tube B. Mix well and centrifuge at 3500 rpm (1000 x *g*) for 5 minutes at 4°C.
6. Carefully collect the upper phase. Combine with the upper phase from Step 4 (tube C). Extract the combined upper phase by adding 100 µl of the Lower Phase Solution from tube B, Mix well and centrifuge at 3500 rpm (1000 x *g*) for 5 minutes at 4°C.
7. Carefully collect the upper phase. Dilute the upper phase in 5 volume of water. Keep on ice for 5 minutes.
8. Spin at top speed at a microcentrifuge tube for 10 minutes at +4°C. Remove the supernatant. The pellet is the **plasma membrane protein**.
9. Store the plasma membrane proteins at -80°C for further studies. The membrane fraction can be dissolved in 0.5% Triton X-100 in PBS or other buffers before use. Generally 30-100 µg plasma membrane proteins can be obtained.

FAQs:

What is the expected yield with this kit?
The expected membrane protein recovery is 100 ug/50 million cells. The expected amount of cytosolic protein is 3-30 mg depending on cell types.
My yield is very low. Please advise.
A few suggestions regarding your questions: A few ways to get efficient yield:

1. Use ~10(E8) cells to start.
2. Make sure most of the cells are lysed.
3. Keep on ice for overnight at step B. 7. after 5 volume dilution.
4. After overnight incubation, transfer to new tubes just before the centrifugation, so that you can get a tight visible pellet.

Does the kit work on the protein complex of plasma membrane protein?
In the kit procedure, getting total cellular membrane protein procedure should not dissociate protein complex. You can use the kit to get total cellular membrane proteins, then do IP. If you further purify the plasma membrane, the yield will be low.

Does the kit work after we co-immunoprecipitate the protein?
The protocol we have with this kit gives native proteins. It may depend on how strong the association of the proteins. The phase solution we
use with the kit contains PEG and Dextran. We do not use any denaturing detergents.

In the final plasma membrane pellet, are the membranes still intact (i.e. are there lipids present)?
Most unbound lipids are removed during the process.

What type of membrane protein does this kit extracts?
The kit mainly extracts trans-membrane proteins. The efficiency should depend on the property of individual protein. It should extract multiple trans-membrane domain proteins well.

Do you know if your membrane protein isolation kit can be used to isolate membrane proteins together with membrane associated proteins?
This will depend on the association strength. If the association with the trans membrane protein is strong, it can be isolated.

Is it possible to homogenize the cells with a needle and syringe instead of a Dounce homogenizer for this protocol?
Using a Dounce homogenizer is actually the most efficient way of lysing the cells. You can try using sonication along with the needle and syringe, but ensure efficient lysis before you proceed to the next step.

Can we use frozen samples with this assay?
Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

What is the exact volume of sample required for this assay?
There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot experiment with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

How do I normalize my samples against protein concentration?
You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.
Should I dissolve the final pellet in a buffer imperatively containing 0.5% Triton X-100 or PBS alone would be enough? What is the exact role of Triton?
In general, detergents help to solubilize and stabilize membrane proteins preventing aggregation.

Technical Support

For all technical or commercial enquiries please go to:
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