Product Information

Membrane Protein Extraction Kit

I. Kit Contents:

Components	K2113-50 50 assays	Cap Color	Part Number
Homogenization Buffer	100 ml	NM	K2113-C-1
Upper Phase Solution	20 ml	NM	K2113-C-2
Lower Phase Solution	20 ml	WM	K2113-C-3
Protease Inhibitor Cocktail	1 vial	Red	K2113-C-4

II. Introduction:

The Membrane Protein Extraction Kit contains optimized buffers and reagents for fast and convenient extraction of membrane proteins from mammalian tissues and cells. This kit can extract total cellular membrane proteins and also purify plasma membrane proteins with consistent yield and high purity. The membrane protein extracted is suitable for various applications (e.g. western blotting, 2-D gels and enzyme analyses, etc.).

III. General Consideration and Reagent Preparation:

Read the entire protocol before beginning the procedure. Be sure to keep all buffers and reagents on ice at all times during the experiment.

Reconstitute Protease Inhibitor Cocktail by adding 250 µl of DMSO, mix well.

Before use, prepare sufficient Homogenization Buffer for the number of samples to be prepared, add 1/500 volume of the reconstituted Protease Inhibitor Cocktail (e.g., Add 2 µl Protease Inhibitor Cocktail per 1 ml buffer) to make the Homogenization Buffer Mix. (Note: Some precipitation may occur after adding the Protease Inhibitor Cocktail. You may continue using the buffer or simply remove the precipitates by centrifugation).

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

IV. Membrane Protein Extraction Protocol:

A. Extraction of Total Cellular Membrane Proteins:

1. Collect cells ~1 g wet weight (0.2 - 10 x 10^8) by centrifugation (700 x g, 5 minutes at 4℃).

For adherent cells, scrape cells in PBS and then spin down (700 x g, 5 minutes) to pellet cells.

2. Wash cells once with 3 ml of ice cold PBS.

3. Resuspend cells in 2 ml of the Homogenization Buffer Mix in an ice-cold Dounce Homogenizer. Homogenize cells on ice for 30-50 times.

4. For tissue samples, homogenize tissues in 2 volume of the 1X Homogenization Buffer, until completed lysed (30 - 50 times).

Note: Efficient homogenization depends 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 nuclei indicates that cells are still intact. If 70 - 80 percent of the nuclei do not have the shiny ring, proceed to the next step. Otherwise, perform 10 - 30 additional passes.

5. Transfer the homogenate to a 1.5 ml microcentrifuge tube. Centrifuge at 700 x g for 10 minutes at 4℃. Collect supernatant and discard the pellet.

6. Transfer the supernatant to a new vial and centrifuge at 10,000 x g for 30 min at 4℃.

7. 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 with the following steps.

B. Purification of Plasma Membrane Proteins:

1. Resuspend 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 sample. Adding 200 µl of Upper Phase Solution and 200 µl of Lower Phase Solution (Mark the tube as B).

3. Centrifuge both tubes A & B in a microcentrifuge at 1000 x g for 5 minutes.

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 1000 x g for 5 minutes.

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 1000 x g for 5 minutes.

7. Carefully collect the upper phase. Dilute the upper phase in 5 volumes of water. Keep on ice for 5 minutes.

8. Spin at 15,000 x g in a microcentrifuge tube for 10 minutes at 4℃. Remove the supernatant. The pellet is the plasma membrane protein. 9. Store the plasma membrane proteins at –70℃ 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.

Frequently Asked Questions:

1. What is the expected yield with this kit?

The expected membrane protein recovery is 100 µg/50 million cells. The expected amount of cytosolic protein is 3 - 30 mg depending on cell types.

2. My yield is very low. Please advise.

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 C 13 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.

3. 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.

4. Does the kit works 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.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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 expt 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.

11. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the data sheet for storage information and shelf life of each of the components.

12. Why are my standard curve values lower than those shown on the data sheet?

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.

13. 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.

14. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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