Membrane Protein Extraction Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K268
Description	The PromoKine Membrane Protein Extraction Kit provides optimized buffers and reagents for effective extraction of membrane proteins from mammalian tissues and cells. Unlike other available procedures that can only extract the total cellular membrane proteins (combinations of plasma and organelle membrane proteins), PromoKine's kit was designed to not only extract the total cellular membrane proteins, but also purify the plasma membrane proteins specifically. The procedure offers consistent yields and high purity (over 90%). Membrane proteins prepared using the kit can be utilized in a variety of applications, such as Western blotting, 2-D gels, enzyme analyses, etc. The entire procedure takes less than 1 hour.
Quantity	50 assays

Kit Components

Components	Quantity	Cap code
Homogenization Buffer	100 ml	NM
Upper Phase Solution	20 ml	NM
Lower Phase Solution	20 ml	WM
Protease Inhibitor Cocktail (lyophilized)	1 vial*	Red

*Reconstitute Protease Inhibitor Cocktail by adding 250 µl of DMSO

Applications / Assay Protocol

A. 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, aliquot enough Homogenization Buffer, add 1/500 volume of the reconstituted Protease Inhibitor Cocktail (e.g. add 2 µl Protease Inhibitor Cocktail to 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.

B. Extraction of Total Cellular Membrane Proteins:

1. Collect cells (0.2-10 x 10^8; ~1 g wet weight) by centrifugation at 700 x g for 5 minutes at 4°C. For adherent cells, scrape cells in PBS and then spin down (700x g for 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 (cat. No. PK-CA577-1998). Homogenize cells on ice 30-50 times. For tissue samples, homogenize tissues in 2 volumes of the 1X Homogenization Buffer until samples are completly 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 monitor under a microscope. A shiny ring around the nuclei indicates that cells are still intact. If 70-80% of the nuclei do not have the shiny ring, proceed with the next step. Otherwise, perform 10-30 additional passes (Step 3).

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

5. Transfer the supernatant to a new vial and centrifuge at 10,000 x g for 30 minutes at 4°C.

6. Collect supernatant (This is 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.

	C. Purification of Plasma Membrane Proteins: 7. 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**). 8. 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**). 9. Centrifuge both A & B tubes in a microcentrifuge at 3,500 rpm (1000 x g) for 5 minutes. 10. Carefully transfer the upper phase from tube A to a new tube (tube **C**), keep on ice. 11. 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. 12. Carefully collect the upper phase. Combine with the upper phase from Step 10 (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. 13. Carefully collect the upper phase. Dilute the upper phase in 5 volumes of water. Keep on ice for 5 minutes. 14. Spin at 15,000 x g in a microcentrifuge tube for 10 minutes at 4°C. Remove the supernatant. The pellet is the plasma membrane protein. 15. Store the plasma membrane proteins at –70°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.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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