ab284937 – Plasma Membrane Isolation Kit

For the isolation of plasma membrane fractions from tissues and cultured cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284937

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light, except for the Density Gradient Media II, which should be stored at 4°C upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Protease Inhibitor Cocktail (EDTA-free)	1 mL	-20°C
Gradient Dilution Buffer II	100 mL	-20°C
Homogenization Buffer	50 mL	-20°C
Density Gradient Media II	85 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Centrifuge (refrigerated) and Tubes
- Ultracentrifuge (capable of 200,000 x g) and Compatible (>17 ml) Ultracentrifuge Tubes
- Dounce Homogenizer
- Microsonicator
- Scalpel/Scissors
- 1X PBS
- Dithiothreitol (optional)

Reagent Preparation

Read the entire protocol before performing the assay.
Briefly centrifuge small vials prior to opening.

Homogenization Buffer: Thaw completely and chill bottles on ice prior to use. Store at -20°C or 4°C.

Gradient Dilution Buffer II: Store at 4°C. Mix thoroughly before use

Protease Inhibitor Cocktail (EDTA-free): Thaw at room temperature prior to use.

Density Gradient Media II: Thaw completely and store at 4°C. Mix thoroughly before use.

Working Buffer Solution (WB): Add 10 µl of Protease Inhibitor Cocktail (EDTA-free) per every 5 ml Homogenization Buffer prior to use. Keep on ice and discard unused solution after 8 hours.

Gradient Working Solution (GWS): Prepare 10 ml GWS (equivalent to one isolation) by mixing Density Gradient Media II with Gradient Dilution Buffer II at a 5:1 ratio (8.33 ml of Density Gradient Media II and 1.67 ml Gradient Dilution Buffer II). Keep GWS on ice at all times.

2.5% Gradient Solution: Prepare 3 ml of 2.5% Gradient Solution (equivalent to one isolation) by mixing GWS with Gradient Dilution Buffer II at a 1:19 ratio (150 µl GWS and 2.85 ml Gradient Dilution Buffer II). Keep 2.5% Gradient Solution on ice at all times.

25% Gradient Solution: Prepare 10 ml of 25% Gradient Solution (equivalent to one isolation) by mixing GWS with Gradient Dilution Buffer II at a 1:1 ratio (5 ml GWS and 5 ml Gradient Dilution Buffer II). Keep 25% Gradient Solution on ice at all times.

Assay Protocol

1. Sample Preparation:

Cultured cells:

1. Collect 0.2-10 x 10^8 cells by centrifugation (700 x g, for 5 min at 4°C). For adherent cells, aspirate growth medium and wash cells once with 1X PBS, remove cells using a cell scraper and pellet by centrifugation (700 x g, for 5 min at 4°C).
2. Wash both types of cells once with 5 ml of ice cold 1X PBS and centrifuge samples at 1000 x g, for 5 min at 4°C.
3. Re-suspend the cell pellet in 1 ml of ice-cold Working Buffer Solution in a pre-chilled Dounce Homogenizer and homogenize cells on ice for 20-25 strokes*****.

Mammalian Tissues:

1. Mince Tissues, ~0.2-0.5 g, into small pieces using a very clean scissors or scalpel.
2. Wash samples with 1X PBS.
3. Transfer samples to an ice-cold Dounce homogenizer.
4. Add 1 to 1.5 ml of the Working Buffer Solution and homogenize tissues using a tight fitting homogenizer until tissues have been thoroughly lysed (20-40 times*****).

***Δ Note:** *Efficient homogenization depends on the cell or tissue 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% of the nuclei do not have the shiny ring, proceed to the next step. Otherwise, perform 10-30 additional strokes.*

Plasma Membrane Isolation (Perform All Steps on Ice or at 4°C):

1. Transfer the homogenate to a clean, pre-chilled 1.5 ml microcentrifuge tube.
2. Sonicate samples using two 10-second pulses, with 30 seconds between pulses, using a microsonicator. Keep samples on ice and keep probe away from the sample-air interface to minimize foaming.
3. Centrifuge at 700 x g for 10 min at 4°C. Carefully remove and discard the fatty residue from the top of the Supernatant. Collect the remaining supernatant and transfer it to a new pre-chilled tube. Store the supernatant on ice.
4. (Optional) Using the pellet from step 3, add 0.5 ml of Working Buffer and repeat steps 2 and 3.
5. Pool the supernatant fractions. This is the post-nuclear fraction or PNS. Discard the pellet.
6. Sonicate the PNS using two 10 second pulses, 30 seconds between pulses, using a microsonicator. Keep samples on ice and keep probe away from the sample-air interface to minimize foaming.

Ultracentrifugation and Fraction Collection:

1. Mix 1 ml of the PNS with 4 ml of the GWS. Place it on the bottom of a fresh, clean, ice-cold ultracentrifuge tube.
2. Layer 10 ml of the 25% Gradient Solution carefully on top of the PNS mix. Do not mix or perturb the layers.
3. Layer 2 ml of the 2.5% Gradient Solution carefully on top of the 25% solution. Do not mix or perturb the layers.
4. Ultracentrifuge the tube(s) at 200,000 x g for 90 minutes at 4°C.
5. At the end of the ultracentrifugation, the plasma membrane fraction(s) will be in the visible band at the interface of the 2.5%/25% gradient solutions.

6. Carefully remove the clear portion of the 2.5% gradient layer and then collect the band below it in a separate, ice-cold tube. This is the isolated 'plasma membrane fraction.'

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
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