ReadiPrep™ Plasma Membrane Protein Extraction Kit

Catalog number: 60209
Unit size: 50 Tests

Component	Storage	Amount (Cat No. 60209)
Component A: ReadiPrep™ Lysis Buffer	Refrigerated (2-8 °C)	1 bottle (25 mL)
Component B: ReadiPrep™ Wash/Dilution Buffer	Refrigerated (2-8 °C)	1 bottle (50 mL)
Component C: ReadiPrep™ Protease Cocktail Mix	Freeze (< -15 °C)	1 vial (1 mL)

OVERVIEW

The ReadiPrep™ Plasma Membrane Protein Extraction Kit is designed for efficient and reproducible isolation of plasma membrane proteins from cultured mammalian cells. Leveraging a detergent-based phase separation system, the kit selectively enriches plasma membrane proteins while minimizing contamination from cytosolic and nuclear components.

The protocol involves sequential lysis, temperature-controlled incubations, and phase separations that yield a fraction containing plasma membrane proteins, thus offering lower viscosity option compared to conventional plasma membrane protein extraction methods. The included buffers are optimized for maintaining protein integrity and compatibility with downstream analytical techniques such as Western blotting, proteomics, and functional assays. This kit is ideal for researchers studying membrane-bound receptors, transporters, or surface markers and suitable for both routine and high-throughput workflows.

AT A GLANCE

1. Add ReadiPrep™ Lysis Buffer working solution to the cell pellet and incubate for 30 minutes on ice.
2. Centrifuge the cells at 13000 X g for 5 minutes, and collect the supernatant in a new tube.
3. Incubate the supernatant at 37°C for 10 minutes to separate the detergent phase.
4. Centrifuge the cells at 13000 X g for 10 minutes at RT, and remove the aqueous phase (top layer).
5. Add ReadiPrep™ Wash Buffer to the detergent phase and incubate on ice followed by 10 minutes at 37°C.
6. Repeat the steps 4 and 5 to remove the aqueous phase for 3 times.
7. Add ReadiPrep™ Dilution Buffer working solution to detergent phase and mix well.

Important: Check the buffers to see if they contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

PREPARATION OF WORKING SOLUTION

ReadiPrep™ Lysis Buffer working solution
Add 10 µL of ReadiPrep™ Protease cocktail mix (Component C) into 1 mL of ReadiPrep™ Lysis Buffer (Component A) and mix well.
Note: We recommend making the ReadiPrep™ Lysis Buffer working solution fresh as per need.

ReadiPrep™ Dilution Buffer working solution
Add 10 µL of ReadiPrep™ Protease cocktail mix (Component C) into 1 mL of ReadiPrep™ Wash/Dilution Buffer (Component B) and mix well.
Note: We recommend making the ReadiPrep™™ Dilution Buffer working solution fresh as per need.

SAMPLE EXPERIMENTAL PROTOCOL

1. Harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes.
2. Resuspend the cells in 500 µL of ReadiPrep™ Lysis Buffer working solution and lyse the cells on ice for 30 minutes.
   Note: It is recommended to use 80 to 90% confluence on a 10-cm plate. For optimal extraction, cell number must be optimized with indicated buffer volume.
3. Centrifuge the cells at 13000 X g for 5 minutes.
4. Collect the supernatant into a new tube.
5. Incubate the supernatant at 37°C for 10 minutes to separate the detergent phase.
6. Centrifuge the cells at 13000 X g for 10 minutes at room temperature.
   Note: It is necessary to perform the centrifugation step at room temperature to better separate the aqueous layer from the detergent phase.
7. Remove the aqueous phase (top layer) after the separation. The bottom layer is the detergent phase.
8. Add equal volume (Approx. 300 to 350 µL) of ReadiPrep™ Wash Buffer (Component B) to the detergent phase.
9. Incubate the mix on ice until you observe the solution getting cloudy.
10. Incubate the mix at 37°C for 10 minutes to separate the detergent phase.
11. Centrifuge at 13000 X g for 10 minutes at room temperature.
12. Repeat Steps 7 to 11 for 3 to 4 times to completely remove the aqueous phase.
13. Add equal volume of ReadiPrep™ Dilution Buffer working solution to the detergent phase. This mix contains the membrane proteins. Store at <-20 °C for downstream applications.

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EXAMPLE DATA ANALYSIS AND FIGURES

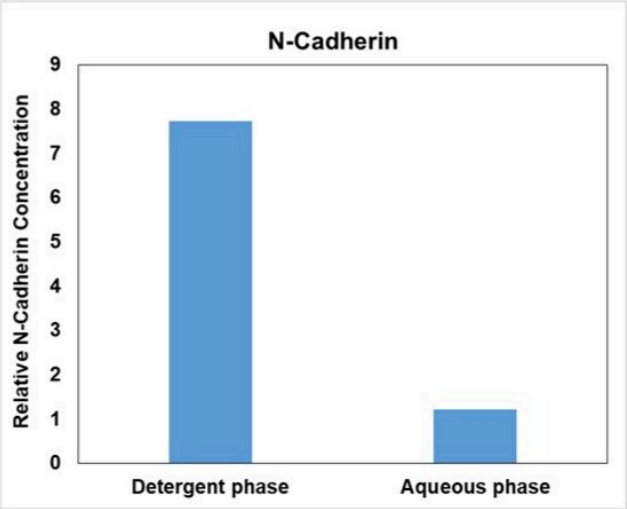

Figure 1. SDS-PAGE analysis of plasma membrane proteins collected from HeLa cells with ReadiPrep™ Plasma Membrane Protein Extraction kit (Cat# 60209). Normalized levels of N-Cadherin to tubulin levels in detergent and aqueous fractions were shown.

DISCLAIMER

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