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Qproteome® Plasma Membrane Protein Handbook

For fractionation of plasma membrane proteins from adherent cell culture samples

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Contents

Kit Contents

Qproteome Plasma Membrane Protein Kit	
Number of preps	**6**
Lysis Buffer PM (10x)	7 ml
Lysis Solution PL	100 µl
Wash Buffer PW	7 ml
Elution Buffer PME	For 15 ml
Binding Ligand PBL	120 µl
Strep-Tactin™ Magnetic Beads	2 x 1 ml
Protease Inhibitor Solution (100x)	300 µl

Storage

All kit components should be stored at 2–8°C upon arrival. Once reconstituted, Binding Ligand PBL can be stored for 4 months at 2–8°C. Elution Buffer should be stored at –30 to –15°C after reconstitution.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, Qproteome Kits are tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Product Use Limitations

The Qproteome Plasma Membrane Protein Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding Qproteome Kits or QIAGEN products in general, please do not hesitate to contact us.

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Introduction

Proteomic analysis of organelles or specific groups of proteins is a potentially powerful strategy for the discovery of proteins that are involved in specific cellular functions or disease. Targeted enrichment of specific groups of proteins or subcellular organelles reduces the complexity of samples and simplifies such approaches.

The subset of cellular proteins that is associated with plasma membranes is of high biological importance. The plasma membrane delineates the cell and provides a physical boundary between the cell and its environment. Plasma membrane proteins play important roles in cell–cell interactions, material transport, and signal transduction. Integral and peripheral membrane proteins e.g., G-protein coupled receptors (GPCRs), receptors for growth factors and cytokines, receptor-associated signaling proteins, and ion-channels are major focuses for new drug targets.

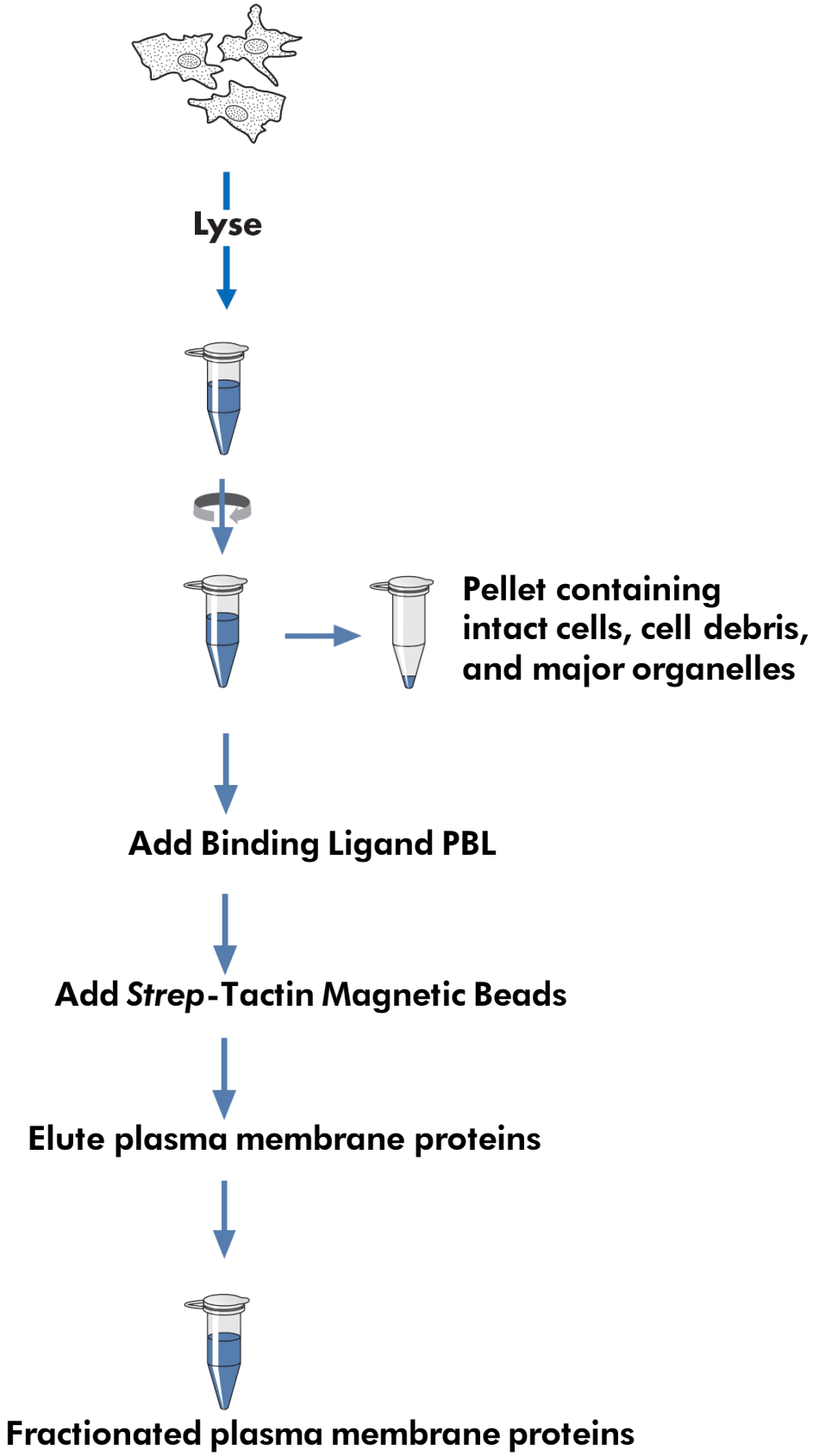
Principle and Procedure

The Qproteome Plasma Membrane Kit is designed for fast and easy fractionation of plasma membrane proteins. Cells are incubated in a hypotonic buffer, causing them to swell. After the addition of a mild detergent, the resulting cell suspension is homogenized by mechanical disruption using a needle and syringe. Intact cells, cell debris, nuclei and the major organelles are removed by centrifugation. The resulting supernatant contains cytosolic proteins and microsomes — small vesicles (20–200 nm in diameter) formed from the endoplasmatic reticulum, Golgi vesicles, and plasma membranes.

A ligand specific for molecules on the plasma membrane is added to the supernatant. The ligand binds to the plasma membrane vesicles and the ligand–vesicle complexes are precipitated using magnetic beads that bind to the ligand. After washing, plasma membrane vesicles are eluted under native conditions and the ligand remains bound to the beads.

Starting material for one fractionation procedure is 1 x 10^7 adherent cells. This corresponds to three to four 75 cm^2 cell flasks at 60–70% confluence. The procedure has been used successfully with several different adherent mammalian cell lines, including HeLa, HEK293, and NIH3T3. Depending on the cell line, the yield from a single fractionation procedure is 30–100 μg protein. For some downstream applications, concentration of the elution fractions may be necessary. A protocol for protein concentration using acetone precipitation can be found on page 14.

Plasma Membrane Protein Fractionation Procedure



Efficient Separation of Plasma Membrane Proteins

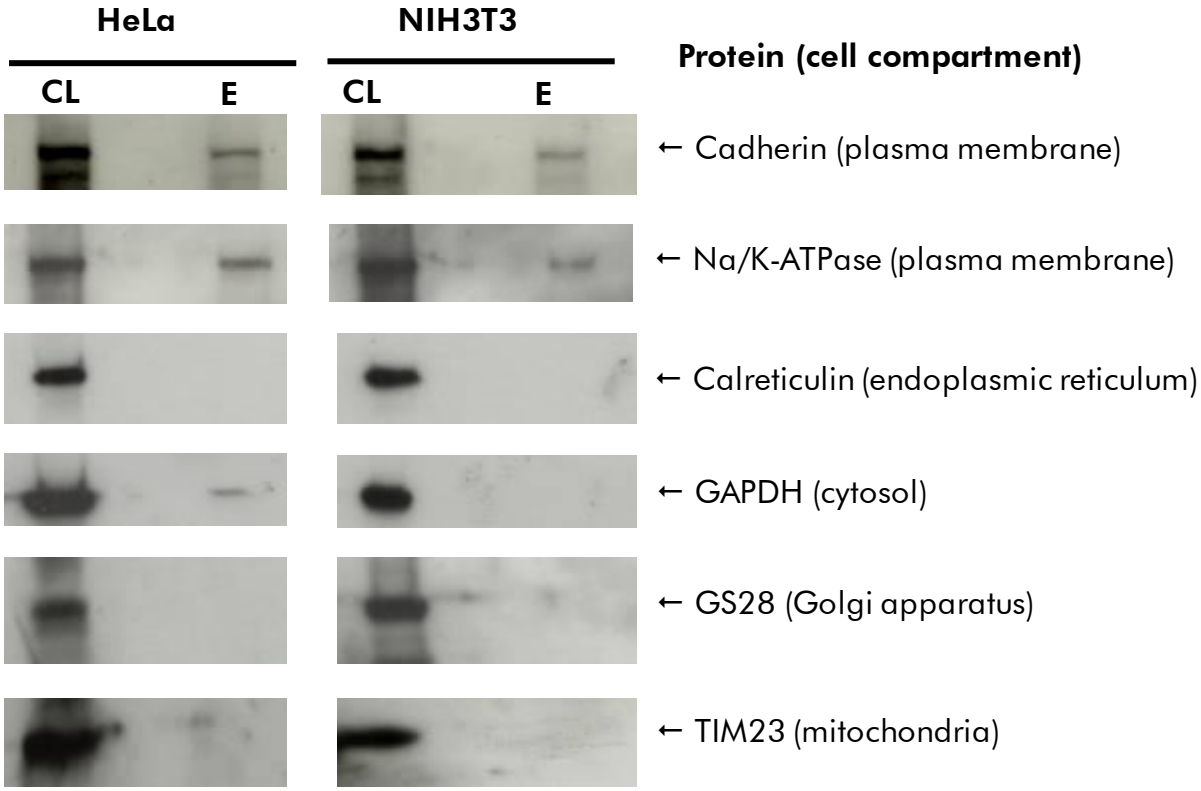


Figure 1 Plasma membrane proteins were purified from either HeLa or NIH3T3 cell cultures using the Qproteome Plasma Membrane Protein Kit. Cell lysates (**CL**) and elution fractions (**E**) were separated by SDS-PAGE and transferred to a nitrocellulose membrane by western blotting. Proteins regarded as markers for different cell compartments were detected using protein-specific antibodies and an HRP-conjugated secondary antibody with chemiluminescent detection.

Protocol: Fractionation of Plasma Membranes from Mammalian Cell Lysates

The volumes given in this protocol are suitable for processing of 1 x 10^7 adherent mammalian cells. This corresponds to three to four 75 cm^2 cell flasks at 60–70% confluence. When processing larger or smaller cultures, adjust the volume of buffer used accordingly.

Equipment and reagents be supplied by the user

- Ice-cold PBS
- 15 ml conical tube
- Microcentrifuge tubes
- Magnetic separator
- Needle (preferably 26 or 21 gauge) and syringe (1 ml or 2 ml volume) for cell disruption and homogenization
- Optional: Acetone stored at –20°C

Important notes before starting

- All steps are performed at 4°C. Use pre-cooled buffers. Separated protein fractions should be snap-frozen in liquid nitrogen and stored at –80°C.
- For downstream applications (e.g., SDS-PAGE analysis) elution fractions should be pooled and concentrated, e.g., by acetone precipitation.
- Binding Ligand PBL and Elution Buffer PME must be reconstituted in the supplied containers before lysing cells. Once reconstituted, Binding Ligand PBL should be stored at 2–8°C and Elution Buffer PME should be stored at –20°C.

Procedure

Preparation of Binding Ligand PBL

1. **Pipet 120 µl water into the tube containing Binding Ligand PBL. Place on ice for 5 min. Vortex for 10 s at maximum speed and incubate for 20 min on an end-over-end shaker at 4°C.**

Preparation of Elution Buffer PME

2. **Reconstitute Elution Buffer PME in 15 ml of Lysis Buffer PM (1x) by pipetting 13.5 ml water, 1.5 ml Lysis Buffer PM (10x), and 150 µl Protease Inhibitor Solution (100x) into the Elution Buffer PME bottle. Vortex for 10 s at maximum speed and incubate for 20 min on an end-over-end shaker at 4°C. Place buffer on ice.**

Preparation of Lysis Buffer PM (1x) with and without protease inhibitors

3. **For each preparation (1 x 10^7 cells) prepare 7 ml Lysis Buffer PM (1x). Pipet 700 µl Lysis Buffer PM (10x) into a 15 ml conical tube and add 6.3 ml water. Mix by briefly vortexing.**

   Lysis Buffer PM (1x) without protease inhibitors is used for washing cells in steps 6 and 7.

4. **Pipet 2.5 ml of Lysis Buffer PM (1x) into a separate tube and add 25 µl Protease Inhibitor Solution (100x). Mix by vortexing briefly.**

   Lysis Buffer PM (1x) with protease inhibitors is used for cell resuspension in step 8, and *Strep*-Tactin Bead equilibration and washing (steps 15–17 and 21–22).

Cell collection

5. **Collect cells by using a cell scraper. Centrifuge for 5 min at 450 x *g* and wash the cell pellet with PBS.**
6. **Resuspend the cell pellet from step 5 by adding 2 ml Lysis Buffer PM without protease inhibitors and gently pipetting up and down. Centrifuge for 5 min at 450 x *g*. Remove and discard supernatant.**
7. **Repeat step 6 using a second 2 ml aliquot of Lysis Buffer PM without protease inhibitors.**

Cell lysis

8. **Resuspend the cell pellet in 500 µl Lysis Buffer PM with protease inhibitors and transfer the cell suspension to a new microcentrifuge tube.**
9. **Incubate for 15 min at 4°C. Vortex briefly every 5 min.**
10. **Add 2.5 µl of Lysis Solution PL to the cell suspension. Mix by briefly vortexing and incubate for 5 min at 4°C.**
11. **Complete cell disruption using a needle and a syringe (not provided). Draw the lysate slowly into the syringe and eject with one stroke. Repeat 15 times.**
12. **Centrifuge the cell lysate at 12,000 x *g* and 4°C for 20 min.**

Binding plasma membrane vesicles

13. **Transfer the supernatant into a new microcentrifuge tube and add 20 µl of reconstituted Binding Ligand PBL prepared in step 1.**

    Remaining Binding Ligand PBL should be stored at 2–8°C and used within 4 months.

14. **Incubate the reaction with gentle agitation for 60 min on an end-over-end shaker at 4°C.**

Separating ligand-bound vesicles

15. **During the incubation in step 14 equilibrate an aliquot of *Strep*-Tactin Magnetic Beads. Vortex the magnetic beads vigorously to obtain a homogenous suspension. Transfer 300 µl of the bead suspension into a new microcentrifuge tube. Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.**
16. **Remove tube from the magnet, add 500 µl Lysis Buffer PM with protease inhibitors, mix the suspension, place the tube on a magnetic separator for 1 min, and remove the buffer completely.**
17. **Remove tube from the magnet and add 100 µl Lysis Buffer PM with protease inhibitors. Resuspend beads by gently vortexing. Place the tube on ice.**
18. **Add the equilibrated magnetic beads prepared in step 17 to the reaction mix from step 14.**
19. **Incubate the reaction with gentle agitation for 60 min on an end-over-end shaker at 4°C.**
20. **Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.**
21. **Remove tube from the magnet, add 500 µl of Lysis Buffer PM with protease inhibitors, resuspend beads by gently vortexing, incubate on ice for 5 min, place the tube on a magnetic separator for 1 min, and completely remove and discard supernatant.**
22. **Repeat step 21.**
23. **Remove tube from the magnet, add 500 µl of Wash Buffer PW, resuspend beads by gently vortexing, incubate on ice for 5 min, place the tube on a magnetic separator for 1 min, and completely remove and discard supernatant.**
24. **Repeat step 23.**

Eluting plasma membrane proteins

25. **Remove tube from the magnet, add 500 µl of Elution buffer PME (prepared in step 2), mix by gently vortexing, incubate on ice for 5 min, place the tube on a magnetic separator for 1 min, remove supernatant completely and transfer the eluate into a new microcentrifuge tube. Place the tube with the eluate on ice.**
    Remaining Elution Buffer PME should be stored at –20°C.
26. **Repeat step 25 three times. Combine all four eluates in a single tube.**

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications such as 2D-PAGE.

1. **Add four volumes of ice-cold acetone to the protein fraction and incubate for 15 min on ice.**
2. **Centrifuge for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Discard the supernatant and air dry the pellet.**
   Do not overdry the pellet as this may make it difficult to resuspend.
3. **Depending on the application, resuspend the pellet in the required sample buffer.**

Troubleshooting Guide

	Comments and Suggestions
Inconsistent results in protein quantification assays	
a) The elution buffer contains components that may interfere with protein quantification assays	Use a protein assay that includes a precipitation step to remove interfering substances. Alternatively precipitate a portion of the eluate using acetone and dissolve the protein pellet in a reagent for suitable your protein assay.
b) A precipitate is visible in Elution Buffer PME after thawing	This does normally not affect experimental results. To clear the buffer, gently warm to 37°C, mix well, and cool on ice before use.
Poor recovery of plasma membrane proteins	
a) Recommended buffer not used	Use only the buffers supplied with the kit, for which the protocol is optimized.
b) Inefficient cell lysis	Check cell lysis by trypan blue staining. Increase the number of syringe strokes in protocol step 11.

Ordering Information

Product	Contents	Cat. no.
Qproteome Plasma Membrane Protein Kit	Buffers and reagents for 6 high-purity plasma membrane protein preparations	37601
Related products		
Qproteome Mitochondria Isolation Kit	Buffers and reagents for 12 high-purity mitochondrial preparations	37612
Qprotome Mammalian Protein Prep Kit	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase®	37901
Qproteome Nuclear Protein Kit	For 6 nuclear protein preparations: Buffers, Reagents, Protease Inhibitor Solution, Benzonase®	37582
Qproteome Nuclear Subfractionation Kit	For 6 nuclear protein preparations: Buffers, Reagents, Nuclear protein Fractionation Columns (6), Nuclear Protein Fractionation Resin, Protease Inhibitor Solution, Benzonase®	37531
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
Qproteome Murine Albumin Depletion Kit	For albumin depletion of 6 murine serum or plasma samples: Murine Albumin Depletion Spin Columns (6)	37591
Qproteome Total Glycoprotein Kit	For 6 total glycoprotein preps: Buffers, Lectin Spin Columns (6), Detergent Solution, Protease Inhibitor Solution, Collection Tubes (6 x 2 ml)	37541
Qproteome Mannose Glycoprotein Kit	For 6 mannose glycoprotein preps: ConA, GNA, and LCH Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37551

Product	Contents	Cat. no.
Qproteome Sialic Glycoprotein Kit	For 6 sialic acid glycoprotein preps: WGA, SNA, and MAL Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37561
Qproteome O-Glycan Glycoprotein Kit	For 6 O-glycan glycoprotein preps: AIL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase®	37512
Qproteome Cell Compartment Kit	For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase®	37502
PhosphoProtein Purification Kit (6)	6 PhosphoProtein Purification Columns, 6 Nanosep® Ultrafiltration Columns, Reagents, Buffers	37101

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Notes

Notes

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