

DUALXtract

Total membrane protein extraction kit

User Manual

Article number P07115s

Product

Kit for optimal extraction of membrane proteins from cells and tissue.

Contents

- Cell Permeabilization Buffer (10 ml)
- Membrane Protein Extraction Buffer (5 ml)
- Cell Wash Solution (25 ml)

Storage

The kit should be stored at 4°C.

Background

The DUALXtract Membrane Protein Extraction kit is designed for efficient isolation of membrane proteins from mammalian cells and tissue samples. The simple and rapid procedure isolates high quality membrane and cytoplasmic protein extracts without cross-contamination between the two fractions. Both fractions contain pure, non-denatured functional proteins which can be used directly in a variety of proteomics applications. The kit is supplied with sufficient reagents for 5 extractions from 5 million cells or 20-40 mg of tissue per experiment.

Instructions

The procedure is based on stepwise differential protein solubilization. Treatment with different mild, non-denaturing detergent based buffers selectively separates membrane and membrane-associated proteins from the cytoplasmic fraction.

Cells are first permeabilized with the Cell Permeabilization Buffer to release the cytoplasmic protein fraction. The cell debris is then treated with the Membrane Protein Extraction Buffer which selectively solubilizes the majority of integral and membrane-associated proteins and isolates them as a separate fraction.

Important notes

The extraction should be performed on ice. Ice-cooled buffers should be used.

The provided protocols apply to membrane protein extraction from up to 5 million cells and 20- 40 mg of tissue sample. For larger amounts of cells or tissue, scale up the volume of buffers proportionally.

A protease inhibitor cocktail may be added to Permeabilization and Membrane Protein Extraction Buffers to minimize proteolysis.

Protocols

Suspension cells

Cell harvesting and permeabilization

- Pellet up to 5×10^6 cells by centrifugation for 5 minutes at 250 x g. Discard the supernatant.
- Resuspend cells in 3 ml of ice-cold Cell Wash Solution and repeat centrifugation. Discard the supernatant.
- Resuspend cells in 1.5 ml of ice-cold Cell Wash Solution and transfer into a 2 ml eppendorf tube. Centrifuge cells for 5-7 minutes at 250 x g. Discard the supernatant.
- Add 1.5 ml of ice-cold Cell Permeabilization Buffer and vortex briefly to eliminate cell clumps. Incubate for 10 minutes at 4°C while continuously rocking.

Extraction of cytoplasmic proteins

- Centrifuge the permeabilized cells at 16000 x g for 15 minutes at 4°C.
- Carefully remove the supernatant (cytoplasmic protein extract) and transfer it into a new tube. Use directly or store aliquots at -70°C for later analysis.
- Set the cell pellet on ice.
Note. The cell debris pellet contains membrane proteins. Make sure to remove all liquid, which contains cytoplasmic proteins.

Extraction of membrane proteins

- Add 1 ml of ice-cold Membrane Protein Extraction Buffer to the cell debris pellet. Pipet thoroughly to mix.
- Incubate mixture for 30 minutes at 4°C in the thermomixer, shaking at 1400 rpm.
Note. Alternatively, shake at 1400 rpm in a shaker placed in a 4°C coldroom.
- Clear membrane protein extract by centrifugation at 16000 x g for 15 minutes at 4°C.
- Transfer the supernatant (membrane protein fraction) into a new tube. Use directly or store aliquots at -70°C for later analysis.

Adherent cells

Plate cells in a 6 cm tissue culture plate. The cells should be 60-90% confluent on the day of analysis. Permeabilization of overgrown cells is less efficient and the membrane protein fraction may become contaminated with cytoplasmic proteins.

Cell harvesting and permeabilization

- Remove the growth medium from the cells. Rinse the cells once with 4 ml of ice-cold Cell Wash Solution.
Note. Pipette the buffer down the inside wall of the tissue culture plate to avoid disruption of the cells.
- Add 1.5 ml of ice-cold Cell Permeabilization Buffer (slowly and gently) into the plate (do not add directly to the cells).
Incubate for 10 minutes at 4°C, rocking gently.

Extraction of cytoplasmic proteins

- Collect the cytoplasmic fraction from the permeabilized cell monolayer and transfer it into the microfuge tube. Use directly or store aliquots at -70°C for later analysis.
Note. If cell debris is observed in the collected sample, centrifuge the tubes at 16000 x g for 15 minutes at 4°C and transfer the supernatant into a new microfuge tube.
- Make sure to remove all liquid from the permeabilized cell monolayer, which contains cytoplasmic proteins.
Note. If the cells have detached during this procedure, scrape the cells off and collect everything into the microfuge tube. Continue as with suspension cells from the step Extraction of cytoplasmic proteins (p.3).

Extraction of membrane proteins

- Add 1 ml of ice-cold Membrane Protein Extraction Buffer on to the monolayer of permeabilized cells and incubate mixture for 30 minutes at 4°C shaking constantly at 450 rpm.
Note. Alternatively, shake at 450 rpm in a shaker placed in a 4°C coldroom.
- Collect the membrane protein extract into a microfuge tube.
- Clear membrane protein extract by centrifugation at 16000 x g for 15 minutes at 4°C.
- Transfer the supernatant (membrane protein fraction) into a new tube. Use directly or store aliquots at -70°C for later analysis.

Tissue

Tissue preparation and permeabilization

Fresh tissue

- Dissect and clean the tissue (remove connective tissue, fat, etc.). Rinse 20-40 mg of fresh tissue in 4 ml of ice-cold Cell Wash Solution and place tissue into a microfuge tube.
- Add 1 ml of ice-cold Cell Permeabilization Buffer. Cut the tissue into small ($\sim 2 \text{ mm}^3$) pieces using scissors.
- Homogenize the tissue with a homogenizer to obtain a uniform cell suspension. Add an additional 1 ml of ice-cold Cell Permeabilization Buffer.
- Incubate mixture for 10 minutes at 4°C while rocking continuously.

Frozen tissue

- Transfer the appropriate amount of frozen tissue into a precooled container.
- Grind the tissue in liquid nitrogen using a mortar and pestle. Collect into 2 ml eppendorf tube and resuspend in 2 ml of ice-cold Cell Permeabilization Buffer.
- Incubate mixture for 10 minutes at 4°C while rocking continuously.

Extraction of cytoplasmic proteins

- Centrifuge the permeabilized cells at 16000 x g for 15 minutes at 4°C.
- Carefully remove the supernatant (cytoplasmic protein extract) and transfer it into a new tube. Use directly or store aliquots at -70°C for later analysis.
- Set the cell pellet on ice.
Note. The cell debris pellet contains membrane proteins. Make sure to remove all liquid, which contains cytoplasmic proteins.

Extraction of membrane proteins

- Add 1 ml of ice-cold Membrane Protein Extraction Buffer to the cell debris pellet. Pipet thoroughly to mix.
- Incubate mixture for 30 minutes at 4°C in the thermomixer, shaking at 1400 rpm.
Note. Alternatively, shake at 1400 rpm in a shaker placed in a 4°C coldroom.

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- Clear membrane protein extract by centrifugation at 16000 x g for 15 minutes at 4°C.
 - Transfer the supernatant (membrane protein fraction) into a new tube. Use directly or store aliquots at -70°C for later analysis.

Troubleshooting

Membrane protein fraction contaminated with cytoplasmic proteins

Cytoplasmic protein fraction (supernatant) incompletely removed from cellular debris pellet.
Ensure that following the permeabilization step all supernatant (cytoplasmic fraction) is removed after the centrifugation.

Cytoplasmic protein is bound to membrane proteins and is extracted with membrane protein fraction.
Check the properties of the contaminating cytoplasmic protein.

Cytoplasmic protein fraction contaminated with membrane proteins

Part of cellular debris pellet was transferred along with cytoplasmic fraction (supernatant).
Be careful not to disturb the cell debris pellet while transferring the cytoplasmic fraction during the "Extraction of Cytoplasmic Proteins" step.

Membrane protein is partially degraded and cut off from its transmembrane domain.
Use fresh (>90% viable) cells.
Add protease inhibitors to the buffers.

Low protein yield

Incomplete cell permeabilization / lysis.
Increase the permeabilization or membrane protein solubilization time.

Old buffers.
Check the expiration date on the bottles of the kit.

Related products

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| P07001 | DUALXtract buffer set (trial size) |
| P07002 | DUALXtract buffer set (standard size) |
| P07113 | DUALXtract Mammalian Cell Lysis Reagent |
| P07114 | DUALXtract Cytoplasmic and Nuclear Protein Extraction Kit |
| P07501 | DUALrefold membrane protein refolding kit |
| P07701 | QuickSpin protein desalting kit |
| P07702 | QuickSpin detergent removal kit |

Support

Please see www.dualsystems.com for support and protocols. Please direct support inquiries to support@dualsystems.com or call +41 44 738 50 00.

Research use

This product is intended for research use only, not for diagnostic or therapeutic uses.

MSDS

Please see the accompanying MSDS for safety and handling instructions. Observe good laboratory practice guidelines and wear gloves, laboratory coat and glasses when handling the product.