Membrane and Cytosol Protein Extraction Kit

Catalog# BWR1007

Size: 100 tests

Lot # Check on the product label

Introduction

1. Membrane and Cytosol Protein Extraction Kit provides a simple and convenient way for extracting membrane and cytosol protein from cultured cells and tissues. This kit can extract not only the membrane protein on the plasma membrane, also the membrane protein on the Mitochondrial, Endoplasmic reticulum and Golgiapparatus.
2. This kit breaks cells properly by homogenizing, and through low speed centrifugation, discards nucleus and precipitate produced by the unbroken cells. Then, collect the supernatant and centrifuge at high speed to obtain the cell membrane pellets and the cytosol protein included supernatant. At last, extract the membrane protein from the pellets through Membrane Protein Extraction Reagent.
3. It takes about 90 min to complete separating and extracting the membrane and cytosol protein from cultured cells and tissues. Extracted proteins can be used for SDS-PAGE, Western, enzyme activity and other subsequent experiment.
4. The Membrane Protein Extraction Reagent contains protease and phosphatases inhibitor, EDTA, etc. Thus, it is not suitable for determining the activities of enzymes which affected by these inhibitors (like protease, phosphatases), but the extracted membrane or cytosol proteins can be used for detection of protein phosphorylation levels.
5. The kit can be used for 100 samples. User can adjust the volume of reagent according to different sample volumes.

Kit Components

Components	Size	Storage Instruction
Membrane Protein Extraction Reagent A (MER A)	100 ml	Store at -20℃ for one year.
Membrane Protein Extraction Reagent B (MER B)	30 ml	Store at -20℃ for one year.

Protocol

1. Prepare solutions: thaw MER A and MER B at room temperature and mix thoroughly, then put them on ice once completely dissolved. Take the proper volume of MER A & B for use, 2-3 min before use, add PMSF and make its final concentration to 1 mM.
2. Prepare cells and tissues:

   a. **For cells**

   1) For **adherent cells**: culture about 2×10^7-5×10^7 cells, wash with PBS and scrape

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cells, or treat cells by EDTA, and stroke cells with pipette. Then centrifuge for several minutes to collect cells, discard supernatant, and keep cell pellets for use. Do not digest with pancreatin to avoid degradation of proteins.

For **suspending cells**: culture about 2×10^7-5×10^7 cells, centrifuge for several minutes to collect cells directly, discard supernatant, and keep cell pellets for use.

2) Wash cells: re-suspense cell pellets slightly with ice-cold PBS, take a small number of cells for counting, then centrifuge the rest cells at 600 g at 4℃ for 5 min to precipitate cells. Discard the supernatant, then centrifuge at 600 g at 4℃ for 1 min to precipitate the residual liquid of the centrifugal tube wall and precipitate the cells further. Discard the supernatant thoroughly.

3) Pretreat cells: add 1ml of MER A (containing PMSF) into 2×10^7-5×10^7 cells, suspend cells slightly and thoroughly, then, incubate cells on ice for 10-15 min.

b. **For tissues**

Take 100 mg tissues, cut into small slices. Add 1 ml of MER A (containing PMSF) to the tissue slices and suspend slightly, then, incubate on ice for 10-15 min.

Note: This kit can also work for less tissue samples, for example 30-50 mg, the reagent dosage and the following protocols are the same, but less tissue samples will get less membrane proteins.

3. Cells & tissues break and the break efficiency evaluation: transfer the cell suspension or tissue samples to a proper precooling glass homogenizer, homogenize about 30-50 passes. However, efficient homogenization may depend on the cell and tissue type, so the optimal condition should be determined by the end user.

To check the efficiency of homogenization, pipette 2-3 μl of the homogenized suspension or tissue homogenate onto a coverslip 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 and intact cellular morphology, proceed to next step. Otherwise, perform 10-30 additional passes using the homogenizer until 70% cells were broken. Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components.

Note: If no proper homogenizer, freeze-thaw method can be used to break cultured cells. Repeated freeze-thaw the samples of Step 2 in liquid nitrogen and at room temperature in turn twice, pipette small volume of sample to a coverslip and observe under a microscope to detect the break efficiency. If 70% of nuclei do not have the shiny ring, proceed to next step, otherwise, increase the times of freeze-thaw.

4. Remove nuclei and unbroken cells: centrifuge at 700 g at 4℃ for 10 min, pipette the supernatant (cytosol protein) into a new tube. **Do not touch the precipitate, leave 30-50 μl of supernatant to ensure the purity of supernatant**.

5. Precipitate cell membrane fragment: centrifuge at 14,000 g at 4℃ for 30 min.

6. Collect cytosol protein: the supernatant of Step 4 is the cytosol protein, assay it immediately or store at -70℃ for use. Do not touch the precipitate, leave 30-50 μl of supernatant to avoid touching.

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7. Extract membrane protein: centrifuge at 14,000 g at 4℃ for 10 seconds, discard supernatant (Can touch the precipitate slightly, even pipette very small volume of precipitate to discard supernatant thoroughly). Add 200 μl of MER B (can add 300 μl if necessary), vortex at maximum speed for 5 seconds to suspend pallets, incubate on ice for 5-10 min. Repeat this vortex and incubation operation for 1-2 times to extract the membrane protein thoroughly. Then, centrifuge at 14,000 g at 4℃ for 5 min, collect the supernatant (membrane protein). Assay the membrane protein immediately or store at -70℃ for use.

Note: For the membrane protein for special purpose, end user can prepare the membrane protein extraction reagent to extract.

Notes

1. PMSF (phenylmethylsulfonyl fluoride) buffer is not included in this product, we provide it separately (Catalog # BWR1014). 2-3 min advanced before use, add PMSF buffer and make its final concentration to1mM.
2. Please wear the lab coat and disposable gloves to operate.
3. The extracted protein of this kit can be assayed by our BCA Protein Assay Kits (Catalog # BWR1023, BWR1024, BWR1025, BWR1026). And the Bradford Protein Assay Kit is not recommended.

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