Plasma Membrane Protein Extraction Kit (mammalian cells or tissues)

Cat. #: P503S (5 rxn); P503 (20 rxn); P503L (50 rxn)

Storage: Store Buffer A and Buffer B at -20°C, and the rest at room temperature

Shelf Life: 12 months

Product Description:

This kit is for rapid extraction of native total membrane proteins (organelle membrane proteins) and native plasma membrane proteins from **cultured mammalian cells or tissues**.

- ✓ Simple and user friendly
- ✓ Wide range of starting cells (1 - 50 millions / sample)
- ✓ Detergent and EDTA free
- ✓ No need for Dounce homogenizer or tissue blender
- ✓ **45 minutes or less protocol.**
- ✓ High yield

This product is for research use only.

Product Components

Component	Amount			Storage temperature
	Cat.#: **P503S**	Cat.#: **P503**	Cat.#: **P503L**	
Buffer A	2.5 mL	10 mL	25 mL	–20°C
Buffer B	1 mL	4 mL	10 mL	–20°C
Protein extraction filter cartridges	5	20	50	Room temperature
Collection tubes with cap	5	20	50	Room temperature
Tissue dissociation beads	0.5g	2g	5g	Room temperature
Plastic rod	1	1	4	Room temperature

Additional Materials Required: 1 X PBS, Vortexer, Table-Top Microcentrifuge, etc.

Important Notes:

1. All centrifugation steps should be performed at 4°C (either in a cold room or in a refrigerated microcentrifuge).
2. To study protein phosphorylation, phosphatase inhibitors (such as PhosStop from Roche) should be added to Buffer A prior to use. The use of protease inhibitor cocktails is optional.
3. It is recommended to use BCA Protein Assay Kit for determination of protein concentration.
4. The rpm in this protocol is based on A Eppendoff 5415C table top microcentrifuge.

Protocol

Before experiment, thaw Buffer A or/and Buffer B completely, invert the bottles a few times then keep on ice. Place the filter cartridges in collection tubs, and pre-chill on ice. Pre-chill PBS on ice.

A. Extraction of Total Membrane Proteins

1. **For cultured cells** (For tissue samples, start from step 2.)

   collect 1 to 50 x 10^6 cells by low speed centrifugation (**500 ~ 600x g, 5 minutes**).

   Note: If plasma membrane proteins are to be extracted from cultured cells, we recommended to start with 20 ~ 50 X 10^6 cells.

   Wash cells once with cold PBS. Aspirate supernatant completely and resuspend the pellet in cold Buffer A (**200 µL for a starting cell number less than 5 million and 500 µL for a starting cell number greater than 5 million**). Incubate the cell suspension **on ice** for **5-10 minutes**. Vortex the tube vigorously for 10-30 seconds. Immediately transfer the cell suspension to the filter cartridge, and go to step 3.

2. **For tissue samples,** place a piece of fresh tissue (10-30 mg) or frozen tissue (20-30 mg) in a filter cartridge. Add 200 µL Buffer A to the filter and grind the tissue with a plastic rod for **1 minute** by pushing the tissue against the surface of the filter repeatedly with twisting force.

   Note: if you are working with **skeletal or cardiac muscles,** it is recommended to **add 100-120 mg tissue dissociation beads** to the filter prior to grinding.

   Add additional 300 µL Buffer A to the same filter cartridge, mix by pipette up and down a few times and incubate the tube **on ice with cap open for 5 min**. Go to step 3.

   Note: The presence of a small amount of un-homogenized tissue will not affect the sample quality. The plastic rod is reusable: after use, wipe it with 75% alcohol or rinse it with distilled water.

3. Cap the filter cartridge and centrifuge at **14,000 rpm (16,000x g)** for **30 seconds**.

   Optional: For cultured cells we recommend to resuspend the pellet in collection tube from step 3, transfer the cell suspension back to the same filter and spin at 14,000 rpm for another 30 seconds. Re-passing the cells through the filter can increase the yield by 20-30%.

4. Discard the filter and resuspend the pellet by **vigorously vortex** for 10 seconds.

The following procedure separates total cellular components into 4 fractions: nuclei, cytosol, organelles and plasma membrane.

5. Centrifuge at **3,000 rpm** (700x g) for **1 minute**, the pellet contains intact nuclei.

Transfer the supernatant to a fresh 1.5 mL microcentrifuge tube and centrifuge at **4ºC** for **10-30 minutes at 14,000 rpm (16,000x g)** (longer centrifugation time will increase yield), the supernatant is cytosol fraction.

Remove the supernatant and **save the pellet.** This pellet is the total membrane protein fraction including organelles and plasma membranes.

- The typical yield is 10-500 µg / sample.
- Continue to **step 6** if plasma membrane protein is to be extracted. Do not freeze total membrane protein fraction if further isolation of plasma membrane proteins is desired.
- You may stop here if isolation of plasma membrane proteins is not necessary. Store the pellet at −70ºC or dissolve it in detergent-containing buffers of your choice.

B. Extraction of Plasma Membrane Proteins

6. Resuspend the total membrane protein fraction from step 5 in 200 µL Buffer B by repeatedly pipetting up and down or vortex. Centrifuge at **10,000 rpm** (7,800x g) for **5 minutes** at **4ºC**. The pellet contains organelle membrane proteins.

   Note: if final plasma membrane prep is contaminated by organelle membranes, increasing centrifugation time to 20 minutes can improve the purity.

7. Carefully transfer the supernatant to a fresh 2.0 mL microcentrifuge tube and add **1.6 mL cold PBS**. Mix by inverting the tube a few times. Centrifuge at **14,000 rpm** (16,000x g) for **15-30 minutes.** (longer centrifugation will improve yield). Discard the supernatant and save the pellet. This is the extracted plasma membrane proteins.

   Typically **10 ~ 300 µg** plasma membrane proteins can be obtained. Pellet of plasma membrane proteins can be dissolved in 20-200 µL detergent containing buffers of your choice such as 0.5% Triton X-100 in PBS.

About Evaluation of Isolated PM Proteins

Many researchers use Western blotting to access the purity of isolated membrane proteins. Some commonly used "cytosolic markers" are not exclusively cytosolic. For example, actin (1), GAPDH (2) and tubulin (3) are mainly cytosolic but they are also associated with plasma membranes. It's not surprising to detect week signals of these marker proteins in PM preps in certain cell and tissue types. For more information please refer to following publications:

(1). Gruenstein E., et al. (1975). Actin associated with membranes from 3T3 mouse fibroblast and Hela cells. Journal of cell Biology. 64:223-234.

(2). Terrasse R., et al. (2012). Human and pneumococcal cell surface glyceraldehydes-3-phosphate dehydrogenase (GAPDH) proteins are both ligands of human C1q protein. J. Biol. Chem. 287:42620-42633.

(3). Wolff J. (2009). Plasma membrane tubulin. Biochemica et Biophysica Acta.

Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting cell numbers Increase incubation time to 10 minutes (**step 1 or 2**)
Low protein activity	Keep lysate cold/add protease inhibitors
Retention of cell lysate in protein filter cartridge after 30 seconds of centrifugation	Reduce amount of starting material or increase centrifugation time to 2 minutes.
Contamination of PM by cytosolic proteins	Wash PM pellet with 0.5 ml cold PBS containing 0.3 M NaCl, PH 9.5

Related products

Cat.#	Kit Name	Application		Protein Status	Minute
P501	Total protein kit	cells	→ total protein	denatured / native	1 ~ 8
P502	Total protein kit	tissues	→ total protein	denatured / native	1 ~ 8
P503	Membrane protein kit	cells / tissues	→ membrane protein	native , detergent-free	20 ~ 45
P504	Nuclear protein kit	cells / tissues	→ nuclear & cytosol protein	native	6 ~ 8
P505	Detergent-free kit	cells	→ total protein	denatured / native	5 ~ 8
P506	Detergent-free kit	Tissues	→ total protein	denatured / native	5 ~ 8
P507	Mitochondria kit	cells / tissues	→ mitochondria	native, detergent-free	25 ~ 30
P508	Plant total protein	plant tissues	→ total protein	denatured/native	5 ~ 8
P510	Plant detergent-free	plant tissues	→ total protein	native	6 ~ 8
P511	Plant chloroplast kit	plant tissues	→ intact chloroplast		5
P518	Plant Microsomal Membrane Extraction	plant tissues	→ microsomal membrane	native	1 hr
P512	Bacteria total protein	bacteria	→ total protein	denatured	2 ~ 3
P513	Nuclear envelope kit	cells	→ nuclear envelope	native	< 45
P514	Histone/DNA binding protein extraction kit	cells	→ histone & dna binding protein	denatured	< 10
P515	Thick cell wall microbes protein kit	microbes	→ total protein	denatured / native	< 10
P519	Gel slice recovery kit	PAGE gel	→ protein	denatured / native	10 ~ 20
P521	Hair & nail protein kit	hair, nail	→ protein	denatured	5 min. hands on
P522	Adipose protein kit	adipose	→ total protein	denatured / native	20
P523	Adipose fractionation	adipose	→ water soluble/insoluble protein	native	40
P524	Nuclei isolation kit	cells / tissues	→ intact nuclei	native, detergent-free	20