VISUAL PROTEIN

Manual

FracPRO™ Protein Fractionation Kit

FP01-5/FP01-50

V2.0

Store at 2-8 °C
For Research Use Only

Introduction

FracPRO™ Protein Fractionation Kit is a simple, reproducible and ultracentrifuge-independent method to separate four subcellular protein fractions, including nuclear, cytosol, membrane/organelle, and cytoskeletal fractions, in a detergent-based procedure. The **FracPRO™ Protein Fractionation Kit** takes advantage of the differential solubility of proteins in various subcellular compartments and utilizes highly specialized fractionation buffers to target specific subcellular compartments and simultaneously preserve the structural integrity of the proteins before and during each sequential fractionation. The **FracPRO™ Protein Fractionation Kit** effectively separates four fractions from cultured cells using a simple microcentrifuge procedure. All of the separated fractions from **FracPRO™ Protein Fractionation Kit** can directly be used in SDS-PAGE, enzyme analyses, Western blotting, gel mobility shift assays, and other procedures.

Product Components

FracPRO™ Protein Fractionation Kit (FP01-5)

Solution 1 (Cytosol Buffer)	10 mL	1 bottle
Solution 2 (Membrane/Organelle Buffer)	10 mL	1 bottle
Solution 3 (Nuclear Buffer)	7.5 mL	1 bottle
Solution 4 (Cytoskeletal Buffer)	1.5 mL	1 bottle
User's manual		

FracPRO™ Protein Fractionation Kit (FP01-50)

Solution 1 (Cytosol Buffer)	100 mL	1 bottle
Solution 2 (Membrane/Organelle Buffer)	100 mL	1 bottle
Solution 3 (Nuclear Buffer)	75 mL	1 bottle
Solution 4 (Cytoskeletal Buffer)	15 mL	1 bottle
User's manual		

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Safety Information

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

Storage

FracPRO™ Protein Fractionation Kit should be stored at 2-8 °C. Expiration date is labeled on the bottle or box.

Materials needed but not provided

1. 1.5 mL microcentrifuge tubes
2. PBS buffer: 10 mM KH_2PO_4, 150 mM NaCl, pH 7.4
3. Protease inhibitor cocktails

Instruction

A. Sample preparation

Suspension cell

1. Collect cells (~5×10^6) by centrifugation for 5 minutes at 250 x g. Discard the supernatant.
2. Wash cells in 5 mL of ice-cold PBS and discard the supernatant after centrifugation. Repeat this step three times.
3. Add 1 mL Solution 1 (Cytosol buffer) and proper amount of protease inhibitor cocktails.
4. Incubate for 15 minutes at 4 °C with gentle agitation.

Adherent cell

1. Remove the medium from the cells.
2. Add 2 mL of ice-cold PBS into cells and scrape cells carefully.
3. Centrifuge cells for 5 minutes at 250 × g and discard the supernatant.
4. Wash cells in 5 mL of ice-cold PBS and discard the supernatant after centrifugation. Repeat this step three times.
5. Add 1 mL Solution 1 (Cytosol buffer) and proper amount of protease inhibitor cocktails.
6. Incubate for 15 minutes at 4 °C with gentle agitation.

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A. Sample preparation (~continued)

- **Fresh tissue**

1. Dissect and clean the tissue (remove connective tissue, fat, etc.). Rinse 20-40 mg of fresh tissue in 4 mL of ice-cold PBS and place tissue into a centrifuge tube.
2. Add 1 mL Solution 1 (Cytosol buffer). Cut the tissue into small (~2 mm^3) pieces using scissors.
3. Homogenize the tissue with a homogenizer to obtain a uniform cell suspension.
4. Incubate mixture for 15 minutes at 4 °C with gentle agitation.

- **Frozen tissue**

1. Transfer the appropriate amount of frozen tissue into a pre-cooled container.
2. Grind the tissue in liquid nitrogen using a mortar and pestle.
3. Re-suspend in 1 mL Solution 1 (Cytosol buffer) and collect into 1.5 mL centrifuge tube.
4. Incubate mixture for 15 minutes at 4 °C with gentle agitation.

B. Isolation of cytosol proteins

1. Centrifuge the tube at 16,000 x g for 10 minutes at 4 °C.
2. Carefully transfer the supernatant (cytosol protein fraction) into a new tube (CY tube). Sample can be stored at -80 °C.
3. Wash the pellet with 1 mL Solution 1 (Cytosol buffer).
4. Wash the pellet with 1 mL ice-cold PBS twice.
5. Keep the washed pellet on ice before isolating the membrane/organelle proteins (see below).

C. Isolation of membrane/organelle proteins

1. Add 1 mL Solution 2 (Membrane/Organelle Buffer) to the pellet.
2. Thoroughly pipet to mix and incubate mixture for 30 minutes at 4 °C with gentle agitation.
3. Isolate membrane/organelle protein fraction by centrifugation at 16,000 x g for 10 minutes at 4 °C.
4. Immediately transfer the supernatant (membrane/organelle protein fraction) into a new tube (MEM tube). Sample can be stored at -80 °C.
5. Wash the pellet with 1 mL Solution 2 (Membrane/Organelle Buffer).
6. Wash the pellet with 1 mL ice-cold PBS twice.

D. Isolation of nuclear proteins

1. Re-suspend the pellet with 0.5 mL Solution 3 (Nuclear Buffer) and thoroughly mix by pipetting.
2. Incubate for 20 minutes at 4 °C with gentle agitation.
3. Centrifuge at 16,000 x g for 10 minutes at 4°C.
4. Transfer the supernatant (nuclear protein fraction) into a new tube (NU tube). Sample can be stored at -80 °C.
5. Wash the pellet with 1 mL Solution 3 (Nuclear Buffer) for one times.
6. Wash the pellet with 1 mL ice-cold PBS twice.
7. Keep the washed cell pellet on ice before isolating the cytoskeletal proteins (see below).

E. Isolation of cytoskeletal proteins

1. Re-suspend the pellet with 0.3 mL Solution 4 (Cytoskeletal Buffer) and thoroughly mix by pipetting.
2. Incubate for 20 minutes at 4 °C with gentle agitation.
3. Centrifuge at 16,000 x g for 10 minutes at 4 °C.
4. Transfer the supernatant (cytoskeletal protein fraction) into a new tube (SK tube). Sample can be stored at -80 °C.

Troubleshooting

Problem	Possible cause	Remedy
Low cytosol protein yield	Cells not lysed	Increase the incubation time with Cytosol buffer
	Cell pellet not dispersed	Disperse cells in Cytosol buffer thoroughly
Low membrane protein yield	Membranes solubilized with Cytosol buffer	Decrease the incubation time with Cytosol buffer
	Incomplete membrane protein isolation	Increase the incubation time with Membrane/Organelle buffer
Low soluble nuclear protein yield	Nuclei not extracted	Vortex the sample more vigorously
	Incomplete nuclei isolation	Increase the centrifugation time after adding Membrane/Organelle buffer