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PURExtract™ Protein Fractionation Kit

Description

PURExtract™ Protein Fractionation Kit is especially designed for extracting cytoplasmic, membrane, nuclear and cytoskeletal proteins from mammalian tissues (fresh or frozen) and cultured cells. Intact proteins can be easily extracted by sequential addition of different extraction buffers to a cell pellet, proteins in the different cellular compartments can be selectively isolated.

PURExtract™ Protein Fractionation Kit includes four bottles of ready-to-use buffers. Cytoplasmic, membrane, nuclear and cytoskeletal proteins can be easily extracted within 2 hours with minimal cross-contamination. The extracted protein can be directly used in many downstream applications, including DNA-protein interaction, SDS-PAGE, gel mobility shift, immunoassays (Western blot, ELISA, RIA), protein assays (PKA, PKC, tyrosine kinase), reporter assays (luciferase, β-galactosidase, chloramphenicol acetyltransferase), enzyme analysis or other affinity purification procedures.

Contents

Cat. No.	Product Name	Specifications
PPF050	PURExtract™ Protein Fractionation Kit	PURExtract™ Cytoplasmic Buffer : 100 ml PURExtract™ Membrane Buffer : 100 ml PURExtract™ Nuclear Buffer : 75 ml PURExtract™ Cytoskeletal Buffer : 15 ml

-Sufficient reagent to extract protein from 2.5 x 10^7 of cells or from 2 g of tissues.

Features

Ready-to-use, optimal buffer sets.

Intact proteins can be easily extracted minimizing protein loss.

Extract cytoplasmic, membrane, nuclear and cytoskeletal proteins within 2 hours.

Extracted protein is ready for direct use in many downstream applications.

Compatible with enzyme analysis, SDS-PAGE, gel mobility shift, immunoassays...etc.

Applications

The extracted protein can be directly used in many downstream applications, including enzyme analysis, DNA-protein interaction, SDS-PAGE, gel mobility shift, immunoassays (Western blot, ELISA, RIA), protein assays (PKA, PKC, tyrosine kinase), reporter assays (luciferase, β-galactosidase, chloramphenicol acetyltransferase) or other affinity purification procedures.

Storage

PURExtract™ Protein Fractionation Kit should be stored at 4℃ upon receipt.

Protocol

Caution:

During operation, always wear a lab coat, disposable gloves and protective goggles. Prevent contact product directly. In case of contacting, wash with large amount of water.

Reagents to Be Supplied by User:

1. 1.5 mL microcentrifuge tubes.
2. PBS buffer: 10 mM KH2PO4, 150 mM NaCl, pH 7.4.
3. Protease inhibitor cocktails (Please refer to Cat. No. PRIC02 PURExtract™ Protease Inhibitor Cocktail).

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 of PURExtract™ Cytoplasmic Buffer and proper amount of protease inhibitor cocktails. 4. Incubate for 15 minutes at 4 ℃ with gentle agit ation.
	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 of PURExtract™ Cytoplasmic Buffer and proper amount of protease inhibitor cocktails. 6. Incubate for 15 minutes at 4 ℃ with gentle agit ation.
	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 of PURExtract™ Cytoplasmic 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 ℃ with gen tle 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 of PURExtract™ Cytoplasmic Buffer and collect into 1.5 mL centrifuge tube. 4. Incubate mixture for 15 minutes at 4 ℃ with gen tle agitation.
Isolation of Cytosol Proteins	1. Centrifuge the tube at 16,000 x g for 10 minutes at 4 ℃. 2. Carefully transfer the supernatant (cytosol protein fraction) into a new tube (CY tube). Sample can be stored at -80 ℃. 3. Wash the pellet with 1 mL PURExtract™ Cytoplasmic 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.
Isolation of Membrane /Organelle Proteins	1. Add 1 mL PURExtract™ Membrane Buffer to the pellet. 2. Thoroughly pipet to mix and incubate mixture for 30 minutes at 4 ℃ with gentle agitation. 3. Isolate membrane/organelle protein fraction by centrifugation at 16,000 x g for 10 minutes at 4 ℃. 4. Immediately transfer the supernatant (membrane/organelle protein fraction) into a new tube (MEM tube). Sample can be stored at -80 ℃. 5. Wash the pellet with 1 mL PURExtract™ Membrane Buffer. 6. Wash the pellet with 1 mL ice-cold PBS twice.
Isolation of Nuclear Proteins	1. Re-suspend the pellet with 0.5 mL PURExtract™ Nuclear Buffer and thoroughly mix by pipetting. 2. Incubate for 20 minutes at 4 ℃ with gentle agit ation. 3. Centrifuge at 16,000 x g for 10 minutes at 4℃. 4. Transfer the supernatant (nuclear protein fraction) into a new tube (NU tube). Sample can be stored at -80 ℃. 5. Wash the pellet with 1 mL PURExtract™ Nuclear Buffer. 6. Wash the pellet with 1 mL ice-cold PBS twice. 7. Keep the washed cell pellet on ice before isolating the cytoskeletal proteins.
Isolation of Cytoskeletal Proteins	1. Re-suspend the pellet with 0.3 mL PURExtract™ Cytoskeletal Buffer and thoroughly mix by pipetting. 2. Incubate for 20 minutes at 4 ℃ with gentle agit ation. 3. Centrifuge at 16,000 x g for 10 minutes at 4 ℃. 4. Transfer the supernatant (cytoskeletal protein fraction) into a new tube (SK tube). Sample can be stored at -80 ℃.

Troubleshooting

Problem	Possible Reasons / Solution
Low cytosol protein yield	**Cells not lysed** Increase the incubation time with PURExtract™ Cytoplasmic Buffer. **Cell pellet not dispersed** Disperse cells in PURExtract™ Cytoplasmic Buffer thoroughly.
Low membrane protein yield	**Membranes solubilized with PURExtract™ Cytoplasmic Buffer** Decrease the incubation time with PURExtract™ Cytoplasmic Buffer. **Incomplete membrane protein isolation** Increase the incubation time with PURExtract™ Membrane Buffer.
Low soluble nuclear protein yield	**Nuclei not extracted** Vortex the sample more vigorously **Incomplete nuclei isolation** Increase the centrifugation time after adding PURExtract™ Membrane Buffer.