PURExtract™ Protein Extraction Reagent

Extracting proteins from animal / plant tissues, cultured cells or fungus

Store at 4°C

Cat. No. PPE030

Sufficient reagent to extract protein from 3 x 10^6 of cells
Sufficient reagent to extract protein from 3 g of tissues
PURExtract™ Protein Extraction Reagent : 30 ml

Description

PURExtract™ Protein Extraction Reagent is especially designed for extracting proteins from a wide range of biological samples, such as any types of animal tissues or cells, plant tissues, bacteria or fungus. Without freeze-thaw cycles and without using protease inhibitors, proteins can be efficiently extracted within 30 minutes. The yield is much higher than the one from freeze-thaw cycles.

PURExtract™ Protein Extraction Reagent is a ready-to-use detergent. It can be used as extraction buffer and it can also be used as storage buffer. During batch sample collection, collected protein samples treated with PURExtract™ Protein Extraction Reagent can be storaged at room temperature for 6 months without showing degradation without using protease inhibitor.

The extracted total protein can be well preserved for long term with PURExtract™ Protein Extraction Reagent, or it can be directly used in many downstream applications, including DNA-protein interaction, SDS-PAGE, gel mobility shift, immunoassays, protein assays, reporter assays or affinity purification procedures.

Features

Ready-to-use detergent can be used as extraction buffer or storage buffer.
Without freeze-thaw cycles and without using protease inhibitors.
Proteins can be easily extracted from animal tissues or cells, plant tissues, bacteria or fungus.
The yield is higher than the one from freeze-thaw cycles.
Extracted protein is ready for direct use in many downstream applications.
Compatible with coomassie (Bradford), BCA protein assays or SDS-PAGE.

Applications

The extracted total 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) or other affinity purification procedures.

Storage Conditions

PURExtract™ Protein Extraction Reagent should be stored at 4°C upon receipt.

Safety Information:

Please always work in a chemical fume hood. Always wear gloves, lab coat and goggles while operating. Prevent contact with product directly because contact to skin, eyes, or respiratory tract may cause chemical burns to the exposed area. In case of contacting, wash with large amount of water for 15 minutes.

Additional materials required:

1. 1.5 mL microcentrifuge tubes
2. PBS buffer: 10 mM NaH_2PO_4, 130 mM NaCl, pH 7.0
3. Chemical fume hood
4. Vortex
5. Chloroform
6. 100% Ethanol
7. Isopropyl alcohol
8. Acetone

Things to do / to know before starting:

Please extract protein in chemical fume hood due to PURExtract™ Protein Extraction Reagent contain organic solvent.

Protocol:

Sample Preparation

Cultured Cells

1. Harvest cells (10^6~10^7) by centrifugation, scraping or trypsin treatment.
2. Discard the supernatant carefully.
3. Wash cells in 5 ml of ice-cold PBS and discard the supernatant after centrifugation for 5 minutes at 250 ×g. Repeat this step three times.
4. Add 1 ml of PURExtract™ Protein Extraction Reagent.
5. Transfer the cells to a 1.5 ml microcentrifuge tube.

Note: Disruption of some yeast and bacterial cells may require the use of a power homogenizer.

Tissues

1. Add 1 ml of PURExtract™ Protein Extraction Reagent in 50~100 mg of tissue sample.
2. Homogenize tissue sample using a Tissue Grinder or power homogenizer.
3. Transfer the tissue sample to a 1.5 ml microcentrifuge tube.

Note: The sample volume should not exceed 10% of the volume of PURExtract™ Protein Extraction Reagent used for homogenization.

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Protein Extraction

1. Vortex vigorously for 15 minutes.
2. Centrifuge the tube at 12,000 × g for 10 minutes at 4°C.
3. Transfer the supernatant to a new tube
4. Add 200 µl of chloroform. Shake for 15 seconds.
5. Incubate for 3 minutes at room temperature.
6. Centrifuge at 12,000 × g for 15 minutes at 4°C.

Note: PURExtract™ Protein Extraction Reagent should be separated into three phases (aqueous phase, interphase, organic phase from top to bottom respectively)

7. Discard aqueous phase carefully.
8. Add 300 µl of 100% ethanol. Invert for 15 seconds.
9. Centrifuge at 2,000 × g for 5 minutes at 4°C.
10. Divide equally the supernatant into two new tubes.

Protein Precipitation

1. Add 750 µl of Isopropyl alcohol per tube. Shake for 15 seconds.
2. Incubate for 10 minutes at room temperature.
3. Centrifuge at 16,000 × g for 10 minutes at 4°C.
4. Discard supernatant carefully.
5. Add 1 ml of ice-cold acetone. Vortex the protein pellets for 1 minute.
6. Centrifuge at 16,000 × g for 10 minutes at 4°C.
7. Discard acetone carefully.
8. Repeat Step 5~7 twice.
9. Evaporate residual acetone by open tube cover for few minute.
   Do not allow the pellet to dry out.
10. Pour the protein pellets to a 1.5 ml microcentrifuge tube using tip.
11. Resuspend protein pellets using suitable buffer depending on downstream experiment.

Troubleshooting:

PURExtract™ Protein Extraction Reagent was not separated into three phases.
Incomplete mixing in protein extraction steps
→Shake vigorously for 15 seconds.
Centrifugation speed is incorrect in protein extraction steps
→Make sure to centrifuge at 12,000 × g.

Protein Yield is Low.
Incomplete homogonization in sample preparation steps
→Decrease sample amount. Mince tissues into smaller pieces.
Remove exceed organic phase.
→Discard aqueous phase carefully.
Incubation time or method of protein precipitation is incorrect.
→Mix thoroughly and incubate at room temperature for 10 minutes.

Related Products:

PRIC02	PURExtract™ Protease Inhibitor Cocktail (1 ml x 2)
PHIC02	PURExtract™ Phosphatase Inhibitor Cocktail (1 ml x 2)
PTP500	PURExtract™ Tissue Protein Extraction Reagent (500 ml)
PPF050	PURExtract™ Protein Fractionation Kit (50 preps)
PEC006	PURExtract™ Phosphoprotein Enrichment Kit (6 preps)
PER003	PURExtract™ Phosphoprotein Enrichment Kit (3 ml resin)
PPC006	PURExtract™ Phosphoprotein Purification Kit (6 preps)
PPR003	PURExtract™ Phosphoprotein Purification Kit (3 ml resin)
PHM020	PURExtract™ His-tagged Protein Purification Mini Kit (20 preps)
PHP005	PURExtract™ His-tagged Protein Purification Midi Kit (5 preps)

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