ExtractPRO™ Protein Extraction Reagent

EP05-30

V2.0

Store at 2-8 °C
For Research Use Only

Introduction

ExtractPRO™ Protein Extraction Reagent is a new formula reagent for extracting most of bio-samples and reduce the most contaminations including DNA, RNA and cell debris. The **ExtractPRO™ Protein Extraction Reagent** not only effectively extract proteins from cultured cells and mammalia tissues without using protease inhibitor, but also easily precipitate proteins after protein extraction. **ExtractPRO™ Protein Extraction Reagent** omit the tedious and time-consuming traditional methods, and can maintain protein profile after long-term sample preservation in reagent.

Product Components

ExtractPRO™ Protein Extraction Reagent (EP05-30)

Protein Extraction Reagent	30 mL	1 bottle
User's manual		

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.

Storage

ExtractPRO™ Protein Extraction Reagent should be stored at 2-8 °C. Expiration date is labeled on the bottle or box.

VISUAL PROTEIN

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. Chemical fume hood
4. Vortex
5. Chloroform or 1-Bromo-3-chloropropane
6. 100% Ethanol
7. Isopropyl alcohol
8. Acetone

Instruction

> **NOTE:** Please extract protein in chemical fume hood due to Protein Extraction Reagent contain organic solvent.

A. 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 x g. Repeat this step three times.
4. Add 1 mL of 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 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 Protein Extraction Reagent used for homogenization.

B. 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 or 1-Bromo-3-chloropropane and shake for 15 seconds.
5. Incubate for 3 minutes at room temperature.
6. Centrifuge at 12,000 x g for 15 minutes at 4 °C.

> **NOTE:** The solution should be separated three phase (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 x g for 5 minutes at 4 °C.
10. Divide equally the supernatant into two new tubes.

C. Protein precipitation

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

Troubleshooting

Problem	Possible cause	Remedy
Not any phase separated	Mix procedure is not well	Shake vigorously for 15 seconds
	Centrifuge rotor speed is incorrect	Check the rotor speed of centrifugal machine
Protein yield low	Incompletely homogenize or lyse bio-samples	Decrease the amount of bio-sample material or mince tissues into smaller pieces
	Remove exceed organic phase	Discard aqueous phase carefully
	Incubation time or method of protein precipitation is incorrect	Mix well and incubate at room temperature for 10 minutes

Appendix

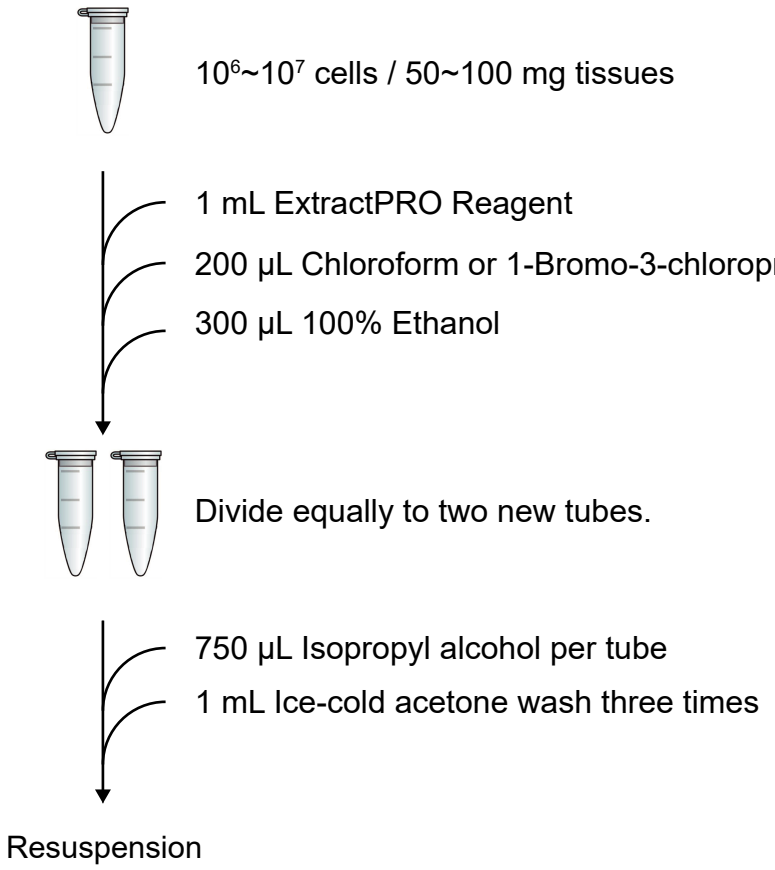

Figure 1. **ExtractPRO™ Protein Extraction Reagent Procedure**

Related Visual Protein Products

Bovine Serum Albumin Standard (2 mg/mL)	AS02-1ML	1 set
Dual-Range™ BCA Protein Assay Kit	BC03-500	1 kit
Dual-Range™ Bradford Reagent	BR01-500	500 mL
Dual-Range™ Bradford Reagent (5X)	BR05-500	500 mL
Dual-Range™ Bradford Protein Assay Kit	BR05-500-K	1 kit

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