PRODUCT INFORMATION & MANUAL

Total Protein Extraction Kit

NBP3-24539

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Total Protein Extraction Kit

Catalog No: NBP3-24539

Specification: 100 assays

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to extract total protein from animal tissues and cells, and the obtained protein can be used for subsequent studies such as Western Blot and co-immunoprecipitation.

▲Detection principle

Cell and tissue samples are treated with lysates containing protease inhibitors and phosphatase inhibitors to prevent the enzymes in the sample from being released to hydrolyze or dephpsphprylate protein due to the disruption of the membrane system.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Lysis Buffer	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Phosphatase Inhibitor	1.2 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Protease Inhibitor	1.2 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Phenylmethylsulfonyl Fluoride	1.2 mL × 1 vial	-20℃ , 12 months, shading light
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

High-speed freezing centrifuge, 5mL Glass homogenizer

Reagents:

Double distilled water, PBS(0.01 M, pH=7.4)

⚠Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. It is recommended to prepare the lysis working solution after weighting the sample.

2.The whole process of extraction should be kept in ice bath or low temperature.

▲ Reagent preparation

1. Place all reagents in ice water for pre-cooling for at least 15 min until it has returned to the solution state before use.
2. Preparation of lysis working solution:
   Mix reagent 1, reagent 2 and reagent 3 with a ratio of 1000:10:10 and preserved on ice with shading light. Prepare the fresh needed amount before use and the prepared solution should be used within 20 min.

▲Operation steps

1.Total Protein Extraction of Tissue

(1) Take 0.1g of fresh tissue, wash with PBS(0.01 M, pH 7.4) at 2-8°C to remove blood. Blot the water with absorbent paper.

(2) Cut the tissue into pieces with scissors and place them into a pre-cooled 5mL glass homogenizer.

(3) Add 1 mL of pre-cooled lysis working solution and 10 μL of precooled reagent 4.

(4) Grind the tissue up and down in the ice bath for about 30 times.

(5) Transfere tissue homogenate to a 2 mL pre-cooled EP tube, place in the ice bath for 15 min.

(6) Centrifuge at 12000 g at 4°C for 15 min. The supernatant was the total protein extract. Place it on ice for detection.

(7) The prepared total protein solution should be stored at -70°C with avoiding of repeated freeze-thaw.

2.Total Protein Extraction of Cell

a. Cell collection

Suspension cell: Transferre cell suspension to pre-cooled EP tubes, centrifuge at 4°C at 1000 g for 10 min to remove supernatant, wash with PBS(0.01 M, pH 7.4) at 2-8°C once, centrifuge at 4°C at 1000 g for 10 min to remove supernatant, leaving precipitation for use.

Adherent cell: Discard the culture solution and wash the cells twice with PBS (0.01 M, pH 7.4) at 2-8°C. Scrape down cells with cell scraping, or treat with EDTA solution, blown the cells off with a pipettor and transferre the cell suspension to a pre-cooled EP tube. Centrifuge at 4°C at 1000 g for 10 min to remove supernatant, wash with PBS (0.01 M, pH 7.4) at 2-8°C once, centrifuge at 4°C at 1000 g for 10 min to remove supernatant, leaving precipitation for use.

b. Cell extraction

(1) Take 5×10^6 cells and add 0.5 mL of pre-cooled lysis working solution and 10 μL of pre-cooled reagent 4.

(2) Place on ice box for 15 min, vortex and mix every 5 min for 10 s each time.

(3) Centrifuge at 12000 g at 4°C for 15 min. The supernatant was the total protein extract, which was placed on ice for detection.

(4) The prepared total protein supernatant should be stored at -70°C to avoid repeated freeze-thaw.