Cat.:BR4C131

Cell Total Protein Extraction Kit

V01

Product Description

Cell Total Protein Extraction Kit is designed for the extraction of total proteins from various animal cells, fungi, or bacterial samples. The provided lysis buffer enables efficient extraction of total proteins, including those from cytoplasm, cell membranes, nuclei, nuclear matrix, mitochondria, endoplasmic reticulum, and Golgi apparatus. The resulting total protein samples can be utilized for protein immunoblotting, immunoprecipitation, co-immunoprecipitation (Co-IP), ELISA assays, protein analysis, and small-scale protein chromatographic purification, among other applications. The kit includes protein loading buffer and a protease inhibitor mixture. For phosphorylation sample preparation, the dedicated phosphatase inhibitor (Product Code: BR4C122) is recommended.

Components

Components	BR4C131-01 30 T
Lysis Buffer	30 mL
5×SDS-PAGE Protein Loading Buffer	7.5 mL
Protease Inhibitor Cocktail，EDTA-Free（100×）	0.3 mL

Storage

Store at -20±5°C, Mix thoroughly before use. The Lysis Buffer may be temporarily stored at 2–8°C after thawing.

Notes

1.This product is only for scientific research purposes, not for clinical diagnosis.
2.All steps of sample lyysis must be performed on ice or at 4°C.
3.During cell sample lysis, the collection of cells should prioritize intrabottle lysis method, which effectively prevents cell disruption during centrifugation, significantly improves total protein yield, and preserves extracellular matrix (ECM). For drug-treated adherent cells, centrifugation is recommended for collection as these cells often exhibit poor adhesion and are prone to loss during PBS washing. Pancreatin digestion is not recommended, as it not only causes complete loss of ECM but also cleaves proteins sensitive to pancreatic enzymes. Cells with high viscosity (e.g., HEK-293) or high-density suspension cells (e.g., Jurkat, K562) may form flocs upon addition of cell lysis buffer, necessitating immediate use of an ultrasonic disruptor for dispersion.
4.For your safety and health, wear lab coats and disposable gloves during operation.

Protocol

Cell sample lysis procedure:

1. Vial lysis method (most effective): Suitable for all adherent cells.
Aspirate the culture medium and gently wash the cell surface twice with pre-cooled PBS (using gentle motions to prevent cell detachment), aspirating residual PBS as much as possible. Add 1 mL of pre-cooled lysis buffer (with fresh protease inhibitor mixture added before use) per 10 million cells on ice to cell bottles, culture plates, or dishes. Disperse adherent cells using a pipette, or collect tightly adhered cells with a cell scraper. Collect the lysis buffer and incubate at ice for 30 minutes, stirring gently every 10 minutes.
2. Centrifugation method: Suitable for suspended cells and drug-treated cells.
Collect cell suspension (along with culture medium) using a cell scraper, centrifuge at 500 g for 5 minutes at 4°C, collect the precipitate, wash the precipitate twice with pre-cooled PBS, collect the cell pellet, and aspirate residual PBS as much as possible. Add 1 mL of pre-cooled lysis buffer per 10 million cells, incubate on ice for 30 minutes, and invert the mixture to mix thoroughly every 10 minutes during this period.

Usage and Storage of Samples

1.For non-denaturing protein analysis experiments such as immunoprecipitation and ELISA, centrifuge at 4°C and 10,000 g for 5 minutes to separate the supernatant. Take a portion of the sample for concentration determination, while the remaining protein samples can be directly divided into multiple tubes and stored at-20°C or-80°C.
2.For denatured protein analysis experiments used in immunoblotting, select a portion of samples for concentration determination. The remaining protein samples are mixed with SDS-PAGE loading buffer at a ratio of 4:1. Heat the mixture in a boiling water bath at 100°C for 5 minutes to fully denature the proteins. After cooling to room temperature, centrifuge at 10,000 g for 5 minutes to separate the supernatant. The supernatant can be directly loaded into SDS-PAGE gel wells or aliquoted into multiple tubes for storage at-20°C or-80°C.
Note: For denatured protein analysis used in immunoblotting, boiling samples with SDS-PAGE loading buffer followed by centrifugation can improve total protein yield.

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