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RIPA Lysis Buffer, Strong

Product Number: BF33

Shipping and Storage

2-8°C

Components

Component	BF33(100mL)
RIPA Lysis Buffer, Strong	100mL

Description

RIPA lysis buffer is a traditional tissue and cell rapid lysis buffer, mainly used to extract soluble proteins from animal tissues and mammalian cells, and can be used to lyse adherent cells and suspended cells. According to the strength of its cracking solution, it can be divided into three categories: strong, medium, and weak. Please refer to Appendix 2 for specific characteristics and differences. Corresponding products can be selected according to experimental needs. RIPA lysis buffer (strong) can effectively extract nuclear, membrane, and cytoplasmic proteins, and the extracted proteins can be used for protein quantification, Western Blot, IP, and other detection and analysis.

The main component of RIPA Lysis Buffer, Strong is 50 mM Tris(pH 7.4)，150mM NaCl，1%Triton X-100，1% sodium deoxycholate，0.1% SDS And sodium orthovanadate, sodium fluoride, EDTA, leupeptin, etc.

Note

1. To prevent protein degradation, all operations should be carried out on ice as much as possible.
2. The protein sample obtained using RIPA lysis buffer can be quantified using BCA method to determine protein concentration. The product can be ordered separately from our company, such as the BCA protein quantification kit. This product contains a high concentration of detergent and cannot be quantified for protein using the Bradford method.
3. It is recommended to add protease inhibitors or phosphatase inhibitors to RIPA lysis buffer before use to prevent protein degradation or maintain protein phosphorylation. Products can be ordered separately from our company, such as protease inhibitor mixtures and phosphatase inhibitor mixtures. If higher concentrations of protein are required, the use of mammalian protein extraction reagents should be reduced.
4. For adherent cells in culture bottles, it is recommended to first digest the cells using conventional methods, and then follow the steps for extracting suspended cell proteins.
5. For cells obtained by centrifugation, if the cell volume is uncertain, the amount of RIPA lysis buffer used can be calculated based on the number of cells. Approximately 500μL of RIPA lysis buffer needs to be added to 5 × 106 Hela cells, and so on.

Protocol

1. Cell sample: Protein extraction from adherent cells

1.1. Be careful to pour out the culture medium of adherent cells.

1.2. Optional steps: If the culture medium contains phenol red or other substances that may affect the experimental results, rinse the cells with pre cooled PBS first.

1.3. Add an appropriate amount of RIPA Lysis Buffer, Strong (protease inhibitor should be added within 2-3 minutes before use). Please refer to the table below for the amount of reagent used. Blow the adherent cells on ice with a gun tip.

Cell culture plate type or culture area	RIPA cracking solution usage
100mm	500-1000μL
60mm	250-500μL
6-well culture plate	200-400μL/hole

24-well culture plate	100-200μL/hole
96-well culture plate	50-100μL/hole

1.4. Transfer the lysate to a new centrifuge tube and incubate on ice for 20 minutes to fully lyse the cells.

1.5. Centrifuge at 14000×g for 10 minutes. Transfer the supernatant to a new tube for further analysis.

2. Cell sample: Extraction of suspended cell protein

2.1. Suspend the cells, centrifuge at 2500×g for 5 minutes, and discard the supernatant.

2.2. Optional steps: If the culture medium contains phenol red or other substances that may affect the experimental results, rinse the cells with PBS. After rinsing, centrifuge the cell suspension at 2500×g for 5 minutes and discard the supernatant.

2.3. Add an appropriate amount of RIPA Lysis Buffer (Strong) (protease inhibitor should be added within 2-3 minutes before use), and add about 200-500μL of RIPA Lysis Buffer (Strong) every 5 × 106 cells. Blow evenly.

2.4. Leave it on ice for 20 minutes to allow the cells to fully lyse.

2.5. Centrifuge at 14000 × g for 10 minutes. Transfer the supernatant to a new tube for further analysis.

3. Tissue sample

3.1. Take an appropriate RIPA Lysis Buffer (Strong) and add protease inhibitors 2-3 minutes before use.

3.2. Weigh the experimental tissue and add RIPA Lysis Buffer in a ratio of 1:10 (g/ml), After Strong, the tissue was cut into small fragments and homogenized using an electric homogenizer. If concentrated protein extract is required, the amount of tissue protein extraction reagents used can be appropriately reduced. Incubate on ice for 20 minutes to fully lyse the cells.

3.3. Centrifuge at 14000 × g for 10 minutes. Transfer the supernatant to a new tube for further analysis.

Note: A small transparent gel like substance may appear in the cracking products of RIPA cracking solution, which is a normal phenomenon. The transparent gelatinous substance is the genome.

Product name	RIPA Lysis Buffer, Strong	RIPA Lysis Buffer, medium	RIPA Lysis Buffer, weak	SDS cracking solution
Effective cracking components	1%Triton X-100, 1% sodium deoxycholate, 0.1% SDS	1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS	1% NP-40, 0.25% sodium deoxycholate	1%SDS
Cracking strength	Strong	Centre	Mild	Strong
Membrane protein extraction	Very good	Better	General	Very good
Cytoplasmic protein extraction	Very good	Very good	Very good	Very good
Nuclear protein extraction	Very good	Better	Better	Very good
Main purpose	WB, IP	WB, IP	WB, IP, CO-IP	WB, CHIP

Table: Performance and parameter comparison of protein lysate