Plus RIPA Lysis Buffer

Catalog number: AR0102-100

Boster's Plus RIPA Lysis Buffer is a enhanced complete cell lysis solution reagent used for rapid and efficient total cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells, effectively extracting cytoplasmic, nuclear and membrane proteins.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566

Phone: 888-466-3604 Fax: 925-215-2184 Email:support@bosterbio.com Web: www.bosterbio.com

Plus RIPA Lysis Buffer

Catalog Number: AR0102-100

Overview

Form Supplied	Ready-to-use 1X solution
Physical State	Liquid
Pack Size	100 mL
Content	50mM Tris•HCl pH 7.6, 150mM NaCl, 1% TritonX-100, 1% sodium deoxycholate, 0.1% SDS
Recommended working concentration	10 mL Plus RIPA Lysis Buffer per gram of tissue 0.5 mL Plus RIPA Lysis Buffer per $5.0x10^6$ cells in suspension 0.5 mL Plus RIPA Lysis Buffer per $5.0x10^6$ adherent mammalian cells
Storage & Expiration	Upon receipt store at 4°C. Plus RIPA Lysis Buffer is stable for one year. Product is shipped on ice.
Assays per kit	200 assays for $5.0x10^6$ cells 100 assays for 0.1g tissue
Compatibility with reagents	Fully compatible with Broad Spectrum Protease Inhibitor Cocktail and Broad Spectrum Phosphatase Inhibitor Cocktail
Equivalent	Thermofisher (Product No. 89900, 89901), Millipore Sigma (Product No. R0278)
Reagent Type	Western Blotting related reagent; Cell lysis buffer; Universal tissue extraction buffer; Detergent solution
Usage	Extraction of cytoplasmic, membrane and nuclear proteins
Cite This Product	Plus RIPA Lysis Buffer (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR0102-100)

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Description

Plus RIPA Lysis Buffer is a enhanced complete cell lysis solution reagent used for rapid and efficient total cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells, effectively extracting cytoplasmic, nuclear and membrane proteins. It is more suitable for extracting membrane proteins compared to RIPA Lysis Buffer (Product No. AR0105-100). Protein lysis can be finished in 60 minutes.

Background

RIPA lysis extraction buffer contains non-ionic and ionic detergents which are able to extract protein from wide variety of cell types and membrane structures. RIPA buffer ensures efficient cell lysis and protein solubilization preventing protein degradation and interference with protein immunoreactivity and biological activity. Since most antibodies and protein antigens are not adversely affected by the components of this solution, RIPA buffer-conducted protein extraction is compatible with various downstream immunoprecipitation and molecular pull-down assays, including reporter assays, protein assays, immunoassays and protein purification. RIPA buffer reagent minimizes non-specific protein-binding interactions to keep background low, while allowing most specific interactions to occur, enabling studies of relevant protein-protein interactions.

Important Product Information

- If desired, add protease inhibitor (Product No. AR1182) and phosphatase inhibitor (Product No. AR1183) to the lysis buffer to prevent proteolysis and maintain phosphorylation status of proteins.
- Some protein kinases and other enzymes may be sensitive to the components of the Plus RIPA Lysis Buffer, resulting in their decreased activity. In such cases, prepare a Plus RIPA Lysis Buffer that does not contain sodium deoxycholate and SDS.

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Additional Materials Required

- Protease inhibitor (Product No. AR1182) and phosphatase inhibitor (Product No. AR1183)
- 2 ml microcentrifuge tubes
- Tissue homogenizer
- Microcentrifuge capable of spinning at 10,000 x g
- Cell scraper

Procedure for Lysis of Monolayer-cultured Adherent Mammalian Cells

Note: Pre-chill an appropriate volume of Plus RIPA Lysis Buffer at 4°C. If desired, add protease inhibitor and phosphatase inhibitor to the lysis buffer immediately before use.

1. In a microcentrifuge tube, resuspend 5×10^6 cells in the growth media by scraping the cells off the surface of the plate with a cell scraper. Centrifuge harvested cell suspension at 600xg for 5min, then carefully remove and discard the supernatant.
2. Resuspend the cells in chilled PBS. Centrifuge at 600xg for 5min, then carefully remove and discard the supernatant.
3. Add 0.5 mL of chilled plus RIPA lysis buffer to the cell pellet. Vortex briefly. Incubate on ice for 30 minutes.
4. Centrifuge samples at 14000xg for 10 minutes.
5. Transfer supernatant to a new tube for further analysis.

Note: Plus RIPA lysis buffer can be added directly to the flask containing cells. Please see the following procedures.

1. Carefully remove culture medium from adherent cells.
2. Wash cells with chilled PBS. Carefully remove PBS.
3. Add chilled plus RIPA lysis buffer to the cells. Vortex briefly. Incubate on ice for 30 minutes. (For the volume of the lysis buffer, follow the instructions listed below)

SIZE of the plate/surface area	Volume of the lysis buffer
100mm	500-1000μL
60mm	250-500μL
6-well plate	200-400μL per well
24-well plate	100-200μL per well
96-well plate	50-100μL per well

4. Centrifuge samples at 14000xg for 10 minutes.
5. Transfer supernatant to a new tube for further analysis.

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Procedure for Lysis of Suspension-cultured Mammalian Cells

Note: Pre-chill an appropriate volume of Plus RIPA Lysis Buffer at 4°C. If desired, add protease inhibitor and phosphatase inhibitor to the lysis buffer immediately before use.

1. In a microcentrifuge tube, harvest 5×10^6 cells by centrifugation at 600xg for 5min. Carefully remove and discard the supernatant.
2. Resuspend the cells in chilled PBS. Centrifuge at 600xg for 5min, then carefully remove and discard the supernatant.
3. Add 0.5 mL of chilled plus RIPA lysis buffer to the cell pellet. Vortex briefly. Incubate on ice for 30 minutes.
4. Centrifuge samples at 14000xg for 10 minutes.
5. Transfer supernatant to a new tube for further analysis.

Procedure for Lysis of Tissues

Note: Pre-chill an appropriate volume of Plus RIPA Lysis Buffer at 4°C. If desired, add protease inhibitor and phosphatase inhibitor to the lysis buffer immediately before use.

1. Place the fresh tissue into chilled PBS and rinse several times. Mince the tissue into small pieces.
2. Add Plus RIPA Lysis Buffer to the tissue at 10:1. (i.e., Add 10 mL chilled lysis buffer per gram of tissue.) Use a smaller volume of reagent if a more concentrated protein extract is required.
3. Homogenize for several minutes at high speed until no tissue chunks remain.
4. Incubate on ice for 30 minutes.
5. Centrifuge at ~10000 x g for 10 minutes.
6. Transfer supernatant to a new tube for further analysis.

Precautions

- All steps of protein lysis should be operated on ice or at 4°C.
- Use BCA Protein Assay kit (Product No. AR0146) to quantify lysed proteins. Bradford Protein Assay kit is not recommended.
- There might be some transparent gel complex containing genomic DNA in lysed proteins. The protein fractions can be used for further analysis after centrifugation if target proteins have little connection with genomic DNA. When detecting target proteins related closely to genomic DNA, sonicate gel complex and then centrifuge to collect supernatant for further analysis. Common transcription factors such as NFKB, p53 can be detected without sonication.

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Troubleshooting

Problem	Possible Cause	Solution
Low total protein yield	Some cells are more resistant to lysis than others	Make sure the cell pellet is thoroughly suspended in RIPA Buffer and incubate for longer with occasional swirling − sonicate the pellet to increase yield
Low concentration of proteins	Excess buffer used	Use less buffer
Proteolysis	No protease inhibitors added	Add protease inhibitor to the buffer before use
Low phosphorylation of proteins	Phosphatase activity	Add phosphatase inhibitor to the buffer before use

Related Boster Products

AR1182 Broad Spectrum Protease Inhibitor Cocktail
AR1183 Broad Spectrum Phosphatase Inhibitor Cocktail
AR0146 BCA Protein Assay Kit
AR0138 SDS-PAGE Gel Preparation Kit