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Technical Datasheet

RIPA Buffer

Sterile filtered

Product Code: TCL131

Product Description:

Radio-immunoprecipitation assay (RIPA) buffer is a ready to use cell lysis buffer designed to extract membrane, nuclear and cytoplasmic proteins. RIPA buffer's ability for rapid and efficient cell lysis is further augmented by its compatibility with protease and phosphatase inhibitors, ability to minimize non-specific protein binding leading to a lower background in immunoprecipitation, and its suitability for protein quantitation. It can be used with both adherent and suspension cells.

RIPA buffer effectively solubilizes a wide range of proteins, making it a standard for Western blotting. Besides western blotting, it is compatible with a myriad of applications including reporter assays, protein assays, immunoassays and protein purification. Lysis buffers fractionate proteins on the basis of their molecular weight. Extracellular matrix (ECM) proteins are not efficiently extracted by RIPA buffer. It is recommended to use a two step extraction process for applications involving study of ECM proteins as in cancer research.

Directions:

Precautions

1. Maintain the cell lysate at 2 - 8ºC throughout thelysis process. An increase in temperature may cause inactivation and/or denaturation of heat sensitive proteins in the lysate.
2. TCL131 does not contain protease or phosphatase inhibitor. If desired, add directly to the buffer before use.

For adherent cells

1. Aseptically aspirate entire medium from the culture flask/dish without disturbing the monolayer.
2. Wash the cells twice with appropriate volume of wash solution like Dulbecco's Phosphate Buffered Saline (DPBS, Product Code TL1006) to remove residual medium.
3. Add appropriate quantity of chilled RIPA buffer and incubate on ice or in a refrigerator at 2–8°C for 5 minutes.
   (Ensure that the quantity is sufficient to cover the entire monolayer)
4. Scrape the plate with a cell scraper to remove the lysed cells.
5. Immediately transfer the cell lysate to a sterile tube.
6. Centrifuge the lysate at 8000x*g* for 10 minutes at 4°C to pellet the cell debris.
7. Transfer the supernatant carefully to a separate tube and use it for further analysis.

For suspension cells

1. Aseptically transfer the cell suspension to a sterile centrifuge tube.
2. Pellet the cells by centrifuging at 450x*g* for 5 minutes.
3. Discard the supernatant.
4. Add appropriate volume of DPBS to the pellet and wash the cells by pipetting up and down gently.
5. Pellet the cells by centrifuging at 450 x *g* for 5 minutes.
6. Repeat steps 4 and 5 to remove all the traces of medium.
7. Add appropriate quantity of chilled RIPA buffer. Resuspend the cells by pipetting up and down or vortex briefly.
   (We recommend to add 1ml RIPA buffer for 0.5 to 5X 10^7 cells. However, quantity of the RIPA buffer can be determined empirically.)
8. Incubate on ice or in a refrigerator at 2–8°C for 5 minutes.
9. Cold centrifuge the lysate at 8000x*g* for 10 minutes at 4°C to pellet the cell debris.
10. Transfer the supernatant carefully to a separate tube and use it for further analysis.

Quality Control:

Appearance
Clear colorless solution.

pH
7.80 - 8.20

Sterility
No bacterial or fungal growth was observed after 14 days of incubation as per USP specification.

Protease detection
Not detected

Storage and Shelf Life:

Store at 2 - 8ºC.
Shelf life is 12 months.
Use before expiry date given on the product label.

Troubleshooting:

Sr.no.	Problem	Probable causes	Suggestions
1	Low protein content	a. Inadequate exposure time of cells to lysis buffer. b. Some cells are more resistant to lysis.	a. Incubate the cells in lysis buffer for a longer period. b. Ensure complete cell disruption by vigorously pipetting the cell lysate in tube for 6-7 times.
2	Low concentration of protein	Dilution of cell lysate	Use lesser quantity of RIPA buffer
3	Proteolysis	Liberation of endogenous proteases from the lysed cells.	Add protease inhibitor to the buffer.
4	Inadequate protein phosphorylation	Phosphatases released into the cell lysate during protein extraction bring about dephosphorylation of proteins.	Add phosphatase inhibitor to the buffer.