RIPA Solution

Catalog No. TAAR-ZBZ5
Size: 100 mL
Storage: at 4°C for 1 year.
Components: SDS, NP-40, and sodium deoxycholate.

Introduction

The extraction of cellular proteins requires efficient cell lysis and protein solubilization while avoiding protein degradation and/or interference from protein immunoreactivity and biological activity. Radioimmunoprecipitation assay (RIPA) solution enables rapid, efficient cell lysis and the solubilization of proteins from both adherent and suspension-cultured mammalian cells. It has long been widely used as a lysis and wash solution for small-scale affinity pull-down applications, such as immunoprecipitation, because most antibodies and protein antigens are not adversely affected by its components. In addition, RIPA solution minimizes nonspecific protein-binding interactions to maintain a low background while allowing most specific interactions to occur, enabling studies of relevant protein–protein interactions. RIPA solution is supplied as a ready-to-use solution that requires no preparation. Protease and phosphatase require no preparation, and protease and phosphatase inhibitors may be added to the lysis solution as required.

Protocol

Cell sample

1. Pipette the appropriate volume of RIPA solution and mix until distributed well. A few minutes before use, add phenylmethylsulfonyl fluoride (PMSF) buffer to bring its final concentration to 1 mM.
2. Anchorage-dependent cells: Wash the sample with phosphate-buffered saline (PBS), NS, or serum-free medium to remove the culture solution. Add RIPA solution and then stroke with a pipette until the cells are completely immersed in the solution. Shake slightly for 5–10 minutes. Subsequently, centrifuge at 10,000–14,000 × *g* for 10 minutes, collect the supernatant, and move on to the next step.

Instruction for RIPA USAGE

SIZE of well/surface area	Kit volume
100mm	500-1000μl
60mm	250-500μl
6-well plate	200-400μlper well
24-well plate	100-200μlper well
96-well plate	50-100μlper well

3. Suspending cells: After centrifugation, wash the sample with PBS, NS, or serum-free medium. Add RIPA solution, then stroke with a pipette until the cells separate. Vortex for 5–10 minutes to lyse completely. Subsequently, centrifuge at 10,000–14,000 × *g* for 10 minutes, collect the supernatant, and move on to the next step.

Tissue Sample:

1. Put the tissue sample into precooled NS quickly and remove blood by washing it several times. Weigh the sample, cut it into small slices, and then place them in a tissue homogenizer.
2. Pipette the appropriate volume of RIPA solution and mix until distributed well. A few minutes before use, add PMSF buffer to bring its final concentration to 1 mM.
3. Add RIPA solution to tissues at a 10:1 ratio (RIPA lysate solution: tissue net weight = 10:1; specifically, add 10 mL of RIPA lysate solution to 1 g of tissue) and homogenize. (If the lysis is incomplete, add more RIPA lysate solution; if high-concentration protein samples are required, reduce the volume of lysate solution.)
4. Homogenize with a glass homogenizer until samples are lysed completely.
5. Centrifuge at 10,000–14,000 × *g* for 3–5 minutes, collect the supernatant, and move on to the next step.

Notes:

1. All protein extraction steps should be operated on ice or at 4°C. We suggest aliquoting the sample into subpackages at an appropriate volume and then freeze-drying or storing at −20°C in liquid form. Avoid freeze-thawing repeatedly.

2. PMSF buffer is not included. A few minutes before use, add PMSF buffer to brings its final concentration to 1 mM.

3. After lysing, the protein sample will contain a high concentration of detergent. If the Bradford detective method does not work, then attempt to use a BCA Protein Assay Kit (TAAR-ZBE6) to detect the concentration.

FAQ:

Question	Reason	Solution
Low output	Protein expression is low	Optimize the step of transfection, or condense lysed protein sample.
	The volume of lysate solution is not enough	Add more volume of solution
	The dispersion of cell mass is not completely	Stroke (vortex) strongly and completely, prolong the time of ice bath during lysing
Low activity or degradation	Protease activity is too high	Add protease inhibitor;
	When operate, the temperature is not low enough or sample was freeze and thawing.	Operate on ice; Aliquot the sample into sub-packages at proper volume, then freeze-drying or store at -20℃ in liquid form. Avoid freeze thawing repeatedly.

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