Product Information Sheet

RIPA Lysate Solution

Catalog No. AR0105

Size 50ml

Storage

At 4℃for one year.

Introduction

Extraction of cellular proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and/or interference with protein immunoreactivity and biological activity. RIPA (Radio-Immunoprecipitation Assay) Solution enables rapid, efficient cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells. It has long been a widely used lysis and wash solution for small-scale affinity pull-down applications, such as immunoprecipitation, since most antibodies and protein antigens are not adversely affected by the components of this solution. In addition, RIPA Solution minimizes non-specific protein-binding interactions to keep background low, 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 requires no preparation. Protease and phosphatase inhibitors may be added to the lysis solution as needed.

Protocol

1. Cell sample

1) Pipette proper volume of RIPA solution and mix to well-distributed. Few minutes advanced before use, add PMSF buffer to make its final concentration to1mM.
2) **Anchorage-dependent cell:** wash sample with PBS, NS or serum-free medium to remove culture solution. Add RIPA solution, then stroke with pipette until solution immerse cells completely. Shake slightly for 5-10 min. Then centrifuge at 10000-14000g for 10 min, collect the supernatants 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 cell：** After centrifuge, wash sample with PBS, NS or serum-free medium. Add RIPA solution, then stroke with pipette until cells separate. Vertex for 5-10 min to lyses completely. Centrifuge at 10000-14000g for 10 min, collect the supernatants and move on to the next step.

2. Tissue Sample:

1) Put the tissue sample into precooling NS quickly, remove blood by washing it several times. Weight sample and cut it into small slices, then put them into tissue homogenizer.
2) Pipette proper volume of RIPA Solution and mix to well-distributed. Few minutes advanced before use, add PMSF buffer to make its final concentration to 1mM.
3) Add RIPA Solution to tissues in 10:1 (RIPA lysate solution: tissue net weight = 10:1, i.e. add 10ml of RIPA lysate solution to 1g tissues) and homogenate. (If lyses incompletely, add more RIPA lysate solution; if high concentration protein samples are required, reduce the volume of lysate solution.)
4）Homogenate with glass homogenizer until samples were lysed completely.
5) Centrifuge at 10000-14000g for 3-5 min, collect the supernatant and move on to next step.

Notes:

1. All steps of protein extraction should be operated on ice or at 4℃. We suggest aliquot the sample into sub-packages at proper volume, then freeze-drying or store at -20℃ in liquid form. Avoid freeze thawing repeatedly.
2. PMSF buffer is not included. Few minutes advanced before use, add PMSF buffer to make its final concentration to1mM.
3. After lysing, the protein sample contains detergent with high concentration. If Bradford detective method cannot work, try to use BCA Protein Assay Kit (AR0146) 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.