RIPA Buffer

Cell lysis buffer for protein extraction from mammalian cells

RIPA buffer is one of the most reliable cell lysis buffers for fast and efficient lysis of mammalian cells from adhesion or suspension cultures.

Composition

Ingredients	Conc.
TRIS hydrochloride	50 mM
Sodium chloride	150 mM
Tergitol™15-S-9	1 %
Deoxycholic acid sodium salt	0,5 %
SDS	0,1 %
Water	

General information

The name radioimmunoprecipitation buffer, short RIPA buffer, is based on the original application, the radioimmunoprecipitation assay. Today this method is often not applied anymore, but the RIPA buffer is still considered one of the most common reagents for cell lysis of mammalian cells. It has proven to be a very reliable method for fast and efficient cell lysis of numerous cell types, enabling effective protein extraction and solubilization. The unique composition of the RIPA buffer has several advantages:

- Extraction of proteins from numerous cell types
- Efficient cell lysis and solubilization of proteins
- Protein degradation is prevented
- No interference with most antibodies and protein antigens
- Minimizes non-specific protein binding interactions, ensuring low background in immunoprecipitation and molecular pull-down assays
- Compatible with many applications, including reporter assays, protein assays, immunoassays and protein purifications

Despite its many advantages, RIPA buffer is not suitable for all downstream analyses. For example, it generates a particularly strong absorption signal at a wavelength of 280 nm, which should be taken into account in absorption analyses. It should also be checked beforehand whether the detergent formulation is compatible with your downstream application.

Below you will find a protocol for cell lysis of mammalian cells from adherent cultures and suspension cultures. The protocol serves as a guide and should be adapted according to your specific needs.

Attention! The use of this product is intended for qualified personnel only. Suitability for use must be determined by the end user.
Read the centrifuge manufacturer's instructions and tare the samples properly before using the centrifuge.

Cell lysis of adherent cells

Note: Buffer does not contain protease and phosphatase inhibitors. These should be added as required immediately before use.

1. Carefully remove the medium in the cell culture flask.
2. Wash the cell layer twice as usual with cold DPBS or PBS. Remove the wash buffer.
3. Add cold RIPA buffer to the cells. Dosage guide: 1 ml buffer for 75 cm2 cell layer (0.5 to 5 x 107 cells).
4. Allow the buffer to incubate for 5 min on ice or in the refrigerator and make sure that the buffer is evenly distributed on the cell layer throughout.
5. Immediately afterwards scrape the still adherent cells from the bottom with a cell scraper. Transfer the entire cell lysate to a centrifuge tube on ice. The lysate can either be used immediately or frozen in liquid nitrogen. Freeze the cells before the centrifugation step.
6. Centrifuge the cell lysate for 15 min at 14000 x g at 4°C.
7. The soluble protein is now in the supernatant. Carefully transfer it to a new tube for further analysis. Leave the protein solution on ice.

Cell lysis of suspension cells

Note: Buffer does not contain protease and phosphatase inhibitors. These should be added as required immediately before use.

1. Transfer your cells including medium into a suitable centrifuge tube and centrifuge the solution for 5 minutes at 450 x g.
2. Decant the medium so that the cell pellet remains in the tube.
3. Wash your cells twice as usual with cold DPBS or PBS. Then decant wash buffer.
4. Add cold RIPA buffer to the cell pellet and resuspend the pellet completely by mixing or vortexing. Dosage guide: 1 ml buffer for 0.5 to 5 x 107 cells.
5. Allow the buffer to incubate for 5 min on ice or in the refrigerator and then vortex the cell buffer solution to lyse all cells.
6. The lysate can either be used immediately or frozen in liquid nitrogen. Freeze the cells before the centrifugation step.
7. Centrifuge the cell lysate for 15 min at 14000 x g at 4°C.
8. The soluble protein is now in the supernatant. Carefully transfer it to a new tube for further analysis. Leave the protein solution on ice.

Warning H319

Full text of hazard- and precautionary statements see material safety data sheet section 2.2

RIPA Buffer at Carl Roth		
RIPA Buffer	**100 ml**	**23T1.1**
RIPA Buffer	**250 ml**	**23T1.2**
RIPA Buffer	**500 ml**	**23T1.3**

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