

HiPurA[®] RIPA Lysis Buffer

<u>Product Name</u>	<u>Product Code</u>	<u>Kit Packing</u>
HiPurA [®] RIPA Lysis Buffer	ML242-50ML	50ml
	ML242-100ML	100ml

Intended Use:

Recommended for extraction of Protein from cultured Mammalian cells

Introduction:

The HiMedia's RIPA (Radio-Immunoprecipitation Assay) Lysis Buffer is designed to provide highly efficient total soluble protein extraction from cultured mammalian cells. The protocol is rapid and buffer enables efficient lysis of cells which permit easy extraction of cytoplasmic and nuclear protein. The buffer is formulated for minimal interference with downstream biological applications and has been tested for use with several cell lines, including primary, suspension and adherent cell types.

Description:

The HiPurA[®] RIPA Lysis Buffer effectively breaks the protein bonds enabling efficient extraction of the soluble proteins from mammalian cells while preventing protein degradation making it useful for a variety of purposes, such as: protein purification, electrophoresis and many other downstream applications.

Application:

HiPurA[®] RIPA Lysis Buffer is compatible with most applications, including enzyme assays, various chromatography procedures and electrophoresis. After preparation the resulting cell lysate is compatible with many downstream assays including SDS-PAGE, Immunoassays (Immunoprecipitation, Western, ELISA, RIA etc.), enzyme assays, a variety of common reporter assays, protein assays such as Coomassie Blue Bradford and the BCA Protein Assays and Protein Purifications.

Composition:

HiPurA[®] RIPA Lysis Buffer contains a proprietary non-ionic detergent in 50mM Tis-CL (pH 8.0) and 150mM NaCL buffer system along with 0.5% sodium deoxycholate and 0.1% SDS.

Properties:

Appearance/ Color/ Clarity : Colorless clear solution and free of particles
pH : 7.9-8.1
DNase & RNase : None detected
Suitability Test : This product is suitable for use in extraction of proteins from mammalian cells.

Storage Conditions:

HiPurA® RIPA Lysis Buffer can be stored at 2-8°C. The reagent is filter sterilized and should be open under aseptic conditions. Under recommended conditions, the buffer is stable of 6 months.

Material needed but not provided:

- Protease Inhibitor Cocktail (Product Code: ML051)
- Monolayer Cultured Mammalian Cells / Suspension Cultured Mammalian Cells.
- 1X PBS Solution (Product Code: ML116) / 10X PBS Solution (Product Code: ML023)
- Centrifuge Tubes
- Refrigerated Centrifuge

Protocol:**For Lysis of Monolayer Cultured Mammalian cells**

1. Carefully remove the culture medium from adherent cells.
2. Wash the cells twice with cold 1X PBS to wash off any phenol red or other reagents that may interfere with subsequent protein analysis.
3. Add the appropriate amount of cold HiMedia's RIPA Lysis buffer to the plate (Use 1 ml of buffer per 75 cm² flask containing 5 x 10⁶ cells).

NOTE: HiMedia's RIPA Lysis Buffer does not contain protease inhibitors. If desired, add Protease Inhibitor Cocktail (Product Code: ML051).

Optional: To obtain concentrated protein extracts, use less buffer.

4. Incubate for 5 minutes in cold conditions or on ice with gentle agitation or swirling.
5. Collect the lysate and transfer to a micro centrifuge tube. Centrifuge samples at ~ 14,000 x g for 15 minutes at 4°C to pellet the cell debris.
6. Transfer the supernatant to a fresh tube for further analysis.

For Lysis of Suspension - Cultured Mammalian cells

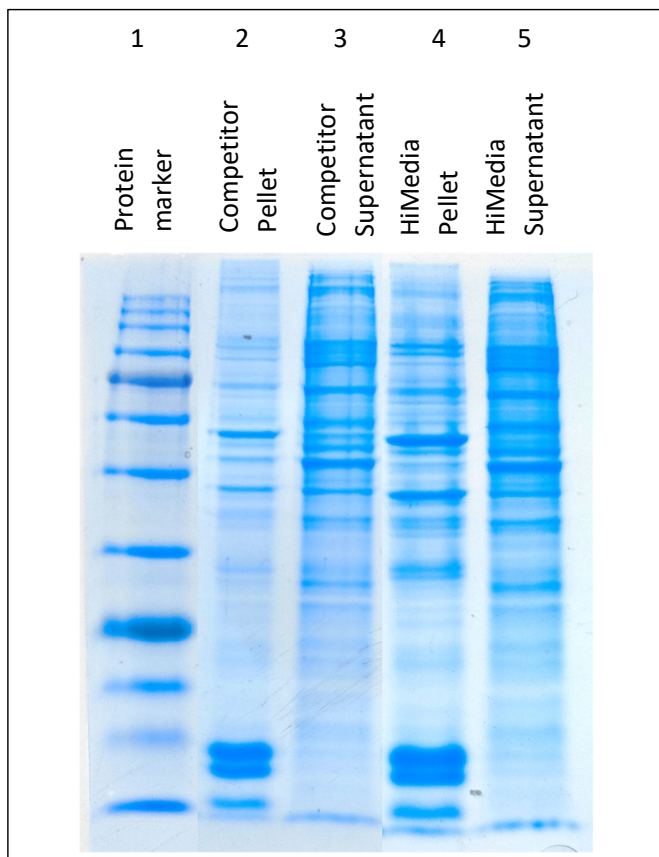
1. Pellet the suspension of cells by centrifugation at 2500 x g for 5 minutes. Discard the culture medium.
2. Wash the cells twice with cold 1X PBS to wash off any phenol red or other reagents that may interfere with subsequent protein analysis. Pellet the washed cells by centrifugation at 2500 x g for 5 minutes. Discard the supernatant.
3. Add cold HiMedia's RIPA Lysis Buffer to the cell pellet. Use 1 ml of reagent for each 40mg (~5 x 10⁶ of cells) of wet cell pellet. If larger amounts of cells are used, first add 1/10 the final recommended volume of reagent and resuspend the cell pellet by pipetting. Add the remaining amount of reagent to the cell suspension.

NOTE: HiMedia's RIPA Lysis Buffer does not contain protease inhibitors. If desired, add Protease Inhibitor Cocktail (Product Code: ML051).

Optional: The cell pellet can be sonicated for 30 seconds with 50% pulse to increase the total yield.

4. Shake mixture gently for 15 minutes on ice. Remove cell debris by centrifugation at ~ 14,000 x g for 15 minutes at 4°C.
5. Transfer the supernatant to a fresh tube for further analysis.

Data:



Well No	Samples
1	Prestained Protein Ladder
2	Mammalian proteins from the pellet using competitor reagent
3	Mammalian proteins from the supernatant using competitor reagent
4	Mammalian proteins from the pellet using HiMedia's RIPA Lysis Buffer
5	Mammalian proteins from the Supernatant using HiMedia's RIPA Lysis Buffer

Fig: SDS-PAGE Gel image representing Mammalian cell protein extraction using suspension cultured mammalian cells.

Troubleshooting:

Problem	Cause	Solution
Low Protein Yield	Low protein expression	Optimize transfection procedure
	Insufficient amount of reagent or less incubation time for cell lysis	Add more reagent and increase incubation time and shake more vigorously during incubation
Degraded Protein	Protease contamination	Add Protease Inhibitor cocktail to the reagent prior to use

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Guanidine hydrochloride causes severe irritation to eyes and skin. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

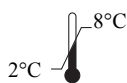
Performance of the solution is expected when the solution is stored at recommended temperature and within the expiry period.

Safety Information

The RIPA Lysis Buffer is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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10/2026

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Disclaimer :

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