

RIPA Buffer (10X)

15 ml



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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: RIPA buffer is used to lyse cells and tissues.

1X RIPA Buffer:

20 mM Tris-HCl (pH 7.5)
150 mM NaCl, 1 mM Na₂EDTA
1 mM EGTA
1% NP-40
1% sodium deoxycholate
2.5 mM sodium pyrophosphate
1 mM β-glycerophosphate
1 mM Na₃VO₄
1 μg/ml leupeptin.

Directions for Use

1. If buffer will be continually used, it is recommended that the 10x buffer be kept at 4°C for 1-2 weeks. For longer periods of time, buffer should be stored at -20°C. Aliquoting of 10x buffer is recommended if many small experiments are to be performed.
2. Thaw 10x buffer at 24-30°C, mixing end-over-end.
3. Dilute 10X RIPA Buffer to a 1X solution using ddH₂O. This product supplies enough 10X material to make 150 mls of whole cell extract.
4. Chill 1x buffer on ice and add PMSF just prior to use.

Note: CST recommends adding 1 mM PMSF immediately before use.

Lysis:

For lysis of adherent cells, we recommend the following: (all reagents and lysates must be kept cold).

1. Treat cells as desired.
2. Wash plate with PBS to remove residual media.
3. Add 400 μl of 1x RIPA buffer/10 cm dish.
4. Incubate plate on ice for 5 minutes.
5. Scrape cells.
6. Sonicate briefly.
7. Centrifuge extract for 10 minutes at 14,000 x g in a cold microfuge.
8. Remove supernatant for use.

Additional notes:

1. For non-adherent cells, add 400 μl of buffer per 10⁷ cells once they have been washed in 1X PBS and pelleted.
2. 1X RIPA Buffer can be used for lysis of tissue samples, although a homogenization step is recommended after adding lysis buffer. Extract the tissue at a ratio of 100 mg of tissue to 1 ml of buffer. Sonication of the tissue lysate is also required.
3. Additional protease inhibitors can be added to the 1x lysis buffer without any difficulties.
4. Aggregation may be present in this buffer upon arrival due to the high concentrations of reagents included at the supplied 10X formulation. At times, the aggregation persists despite warming to room temperature. We recommend warming the buffer to 37°C for 15 minutes and mixing to help eliminate the precipitate. Alternatively, diluting the 10X RIPA with ddH₂O to at least 5X will yield a precipitate-free solution. Once diluted, it can be aliquoted and stored at -20°C for future use.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

RIPA Lysis and Extraction Buffer

Catalog Numbers 89900 and 89901

Doc. Part No. 2161782 Pub. No. MAN0011565 Rev. B.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Contents and storage

Cat. No.	Description	Storage
89900	RIPA Buffer, 100 mL	Upon receipt, store at 4°C. Product shipped at ambient temperature. Note: Product shipped at ambient temperature.
89901	RIPA Buffer, 250 mL Contents: 25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS	

Product description

The Thermo Scientific™ RIPA buffer is one of the most reliable buffers used to lyse cultured mammalian cells from both plated cells and cells pelleted from suspension cultures. This buffer enables protein extraction from cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification.

Guidelines

- RIPA Buffer does not contain protease or phosphatase inhibitors. If desired, add protease inhibitors, such as Thermo Scientific™ Halt™ Protease Inhibitor Cocktail (Cat. No. 78410) and Halt™ Phosphatase Inhibitor Cocktail (Cat. No. 78420) to the reagent to prevent proteolysis and maintain phosphorylation status of proteins. Add protease and phosphatase inhibitors immediately before use.
- Use 1mL of cold RIPA Buffer for every 5×10^6 of HeLa or A431 cells (~20 μ L of packed cells, which is equivalent to ~40 mg of cells). To obtain concentrated protein extracts, directly lyse cells on plate and use less buffer.
- Some protein kinases and other enzymes may be sensitive to the components of the RIPA Buffer, resulting in their decreased activity. In such cases, prepare a RIPA buffer that does not contain sodium deoxycholate and SDS.
- RIPA Buffer is compatible with the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Cat. No. 23225).

Lyse Monolayer-cultured mammalian cells

Note: If desired, add protease and phosphatase inhibitors to the RIPA Buffer immediately before use.

1. Carefully remove (decant) culture medium from adherent cells.
2. Wash cells twice with cold PBS.
3. Add cold RIPA Buffer to the cells. Use 1 mL of buffer per 75 cm² flask containing 5×10^6 HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading.
4. Gather the lysate to one side using a cell scraper, collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 $\times$ g for 15 minutes to collect the cell debris.

Note: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.

5. Transfer supernatant to a new tube for further analysis.

Lyse Suspension-cultured mammalian cells

Note: If desired, add protease and phosphatase inhibitors to the RIPA Buffer immediately before use.

1. Collect cells by centrifugation at $2500 \times g$ for 5 minutes. Discard the supernatant.
2. Wash cells twice in cold PBS. Collect cells by centrifugation at $2500 \times g$ for 5 minutes.
3. Add RIPA Buffer to the cell pellet. Use 1 mL of RIPA buffer for 40 mg ($\sim 5 \times 10^6$ of HeLa cells) of wet cell pellet. Pipette the mixture up and down to suspend the pellet.

Note: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.

4. Shake mixture gently for 15 minutes on ice. Centrifuge mixture at $\sim 14,000 \times g$ for 15 minutes to pellet the cell debris.
5. Transfer supernatant to a new tube for further analysis.

Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For troubleshooting information and FAQs for this product: <https://www.thermofisher.com/ripabufferfaqs>
- To browse the database and search using keywords: [thermofisher.com/faqs](https://www.thermofisher.com/faqs)

Related Thermo Scientific™ products

Cat. No.	Product
78410	Halt™ Protease Inhibitor Cocktail Kit
78420	Halt™ Phosphatase Inhibitor Cocktail, 1mL
78248	B-PER™ Bacterial Protein Extraction Reagent, 500mL
78990	Y-PER™ Yeast Protein Extraction Reagent, 500mL
89826	Mem-PER™ Membrane Protein Extraction Reagent Kit
78833	NE-PER™ Nuclear and Cytoplasmic Extraction Kit
23227	Pierce™ BCA Protein Assay Kit
26148	Pierce™ Direct IP Kit
34080	SuperSignal™ West Pico Chemiluminescent Substrate, 500mL
34076	SuperSignal™ West Dura Extended Duration Substrate, 200mL

General references

Cao, F., *et al.* (2005). Identification of an essential molecular contact point on the duck hepatitis B virus reverse transcriptase. *J Virol* **79**(16): 10164-70.

Pfrepper, K.I. and Flugel R.M. (2005). Molecular characterization of proteolytic processing of the gap proteins of human spumaretrovirus. *Methods in Mol Biol* **304**:435-44.

Sefton, B.M. (2005). Labeling cultured cells with ^{32}P i and preparing cell lysates for immunoprecipitation. Unit 18.2. F. M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (eds.) *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

Documentation and support

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 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Revision	Date	Description
B.0	28 January 2020	Moved troubleshooting content to thermofisher.com .

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