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 µL 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 cm^2 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 × *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 × *g* for 5 minutes. Discard the supernatant.
2. Wash cells twice in cold PBS. Collect cells by centrifugation at 2500 × *g* for 5 minutes.
3. Add RIPA Buffer to the cell pellet. Use 1 mL of RIPA buffer for 40 mg (~5×10^6 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 ~14,000 × *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**

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 32Pi 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

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates

  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0011565

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

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.