

5X RIPA Buffer, with Protease Inhibitor Cocktail

CATALOG NUMBER: AKR-190

QUANTITY: 20 mL

COMPONENTS:

1. RIPA Buffer (5X) (Part No. AKR-191): One bottle – 20 mL containing 125 mM Tris pH 7.6, 750 mM NaCl, 5% Igepal CA-630, 5% sodium deoxycholate, 0.5% SDS.
2. Protease Inhibitor Cocktail (100X) (Part No. 217205): One vial – 1 mL containing AEBSF, Aprotinin, Bestatin, E64, Leupeptin, and Pepstatin A in DMSO.

Storage

Upon receipt, aliquot and store Protease Inhibitor Cocktail at -20°C and avoid multiple freeze/thaw cycles. Store RIPA Buffer concentrate at room temperature.

Shelf Life

1 year from date of receipt under proper storage conditions; aliquot to avoid multiple freeze thaw cycles

Background

RIPA Buffer continues to be a popular buffer to lyse plated and suspension cultured mammalian cells. The buffer extracts cytoplasmic, membrane, and nuclear proteins and is suitable for downstream assays such as reporter assays, protein assays, immunoassays and protein purification.

Preparation of Reagents

- 1X RIPA Buffer: Mix the 5X RIPA Buffer by inverting/vortexing the bottle a few minutes. Dilute the 5X RIPA Buffer to 1X with deionized water. Stir to homogeneity. Store at 4°C for up to 6 months.
- Protease Inhibitor Cocktail: Immediately before use dilute the Protease Inhibitor Cocktail 1:100 with 1X RIPA Buffer. Stir to homogeneity. Do not store diluted solutions containing protease inhibitors.

Preparation of Samples

I. Adherent Cells

1. Culture cells to approximately 80-90% confluence.
2. Aspirate the culture media and wash twice with cold PBS.
3. Add cold 1X RIPA Buffer to the cells, spreading evenly. Use 1 mL of buffer per 10-cm dish (56.7 cm²), maintaining on ice for 5 minutes. Cells may be scraped with a cell scraper.
4. Collect the lysate into a microcentrifuge tube.
5. Centrifuge for 10 minutes (14000 x g).
6. Transfer the supernatant to a clean tube and maintain at 4°C. Samples may be frozen at -80°C.

II. Suspension Cells

1. Collect the cells into an appropriate conical centrifuge tube.
2. Centrifuge for 5 minutes (600 x g).
3. Remove and discard the supernatant.
4. Wash the cells twice with cold PBS, centrifuging for 5 minutes between (600 x g).
5. Add cold 1X RIPA Buffer to the cell pellet. Use 1 mL of buffer per 40 mg of wet cell pellet. Gently resuspend the pellet by pipetting up and down.
6. Shake the suspension for 15 minutes at 4°C.
7. Centrifuge for 10 minutes (14000 x g).
8. Transfer the supernatant to a clean tube and maintain at 4°C. Samples may be frozen at -80°C.

Recent Product Citations

1. Wang, M. et al. (2021). Exendin-4 improves long-term potentiation and neuronal dendritic growth in vivo and in vitro obesity condition. *Sci Rep.* **11**(1):8326. doi: 10.1038/s41598-021-87809-4.
2. Shao, M. et al. (2020). Curcumin and wiktstroflavone B, a new biflavonoid isolated from Wikstroemia indica, synergistically suppress the proliferation and metastasis of nasopharyngeal carcinoma cells via blocking FAK/STAT3 signaling pathway. *Phytomedicine.* doi: 10.1016/j.phymed.2020.153341.
3. Liu, Z. et al. (2020). Detecting Tumor Antigen-Specific T Cells via Interaction-Dependent Fucosyl-Biotinylation. *Cell.* doi: 10.1016/j.cell.2020.09.048.
4. Wang, M. et al. (2020). Oleuropein promotes hippocampal LTP via intracellular calcium mobilization and Ca²⁺-permeable AMPA receptor surface recruitment. *Neuropharmacology.* doi: 10.1016/j.neuropharm.2020.108196.
5. Thakore, P. et al. (2020). TRPML1 channels initiate Ca²⁺ sparks in vascular smooth muscle cells. *Sci Signal.* **13**(637):eaba1015. doi: 10.1126/scisignal.aba1015.
6. Huang, M.L. et al. (2020). Plac8-mediated autophagy regulates nasopharyngeal carcinoma cell function via AKT/mTOR pathway. *J Cell Mol Med.* doi: 10.1111/jcmm.15409.
7. Yun, J. et al. (2019). Up-regulation of miR-297 mediates aluminum oxide nanoparticle-induced lung inflammation through activation of Notch pathway. *Environ Pollut.* **259**:113839. doi: 10.1016/j.envpol.2019.113839.
8. Wang, M. et al. (2019). Acute restraint stress reverses impaired LTP in the hippocampal CA1 region in mouse models of Alzheimer's disease. *Sci Rep.* **9**(1):10955. doi: 10.1038/s41598-019-47452-6.
9. Ramasamy, V.S. et al. (2019). Avenanthramide-C Restores Impaired Plasticity and Cognition in Alzheimer's Disease Model Mice. *Mol Neurobiol.* doi: 10.1007/s12035-019-01707-5.
10. Wang, M. et al. (2019). Adiponectin improves long-term potentiation in the 5XFAD mouse brain. *Sci Rep.* **9**(1):8918. doi: 10.1038/s41598-019-45509-0.
11. Yang, R. et al. (2018). Knockout of the placenta specific 8 gene radiosensitizes nasopharyngeal carcinoma cells by activating the PI3K/AKT/GSK3 β pathway. *Am J Transl Res.* **10**(2):455-464.
12. Woo, H.K. et al. (2018). Urine-based liquid biopsy: non-invasive and sensitive AR-V7 detection in urinary EVs from patients with prostate cancer. *Lab Chip.* **19**(1):87-97. doi: 10.1039/c8lc01185k.

13. Eshwar, A.K. et al. (2018). Interaction of matrix metalloproteinase-9 and Zpx in Cronobacter turicensis LMG 23827T mediated infections in the zebrafish model. *Cell Microbiol.* **20**(11):e12888. doi: 10.1111/cmi.12888.
14. Tanaka K, et al. (2017). Decreased Expression of Thrombomodulin in Endothelial Cells by Fibroblast Growth Factor-23/ α -Klotho. *Ther Apher Dial.* **21**(4):395-404. doi: 10.1111/1744-9987.12524
15. Matsumoto, Y. et al. (2014). Ezrin mediates neuritogenesis via down-regulation of RhoA activity in cultured cortical neurons. *PLoS One.* **9**: e105435.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2012-2021: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.