# 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 -20ºC 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 4ºC 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 $cm^2$), 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 4ºC. Samples may be frozen at -80ºC.

### 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 4ºC.
7. Centrifuge for 10 minutes (14000 x *g*).
8. Transfer the supernatant to a clean tube and maintain at 4ºC. Samples may be frozen at -80ºC.

## Recent Product Citation


1. 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
2. Matsumoto, Y. et al. (2014). Ezrin mediates neuritogenesis via down-regulation of RhoA activity in cultured cortical neurons. *PLoS One*. **9**: e105435.


## Warranty



***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