

## 5X RIPA Buffer

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<b>CATALOG NUMBER:</b>	AKR-191
<b>QUANTITY:</b>	20 mL
<b>COMPOSITION:</b>	125 mM Tris pH 7.6, 750 mM NaCl, 5% Igepal CA-630, 5% sodium deoxycholate, 0.5% SDS.

### Storage

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

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.

### 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<sup>2</sup>), 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 Citations**

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5. Dong, L. et al. (2020). Comprehensive evaluation of methods for small extracellular vesicles separation from human plasma, urine and cell culture medium. *J Extracell Vesicles*. **10**(2):e12044. doi: 10.1002/jev2.12044.
6. Sharma, R. et al. (2020). Chronic alcohol exposure reduces acetylated histones in the sleep-wake regulatory brain regions to cause insomnia during withdrawal. *Neuropharmacology*. doi: 10.1016/j.neuropharm.2020.108332.
7. Hortobagyi, D. et al. (2020). In vitro mechanical vibration down-regulates pro-inflammatory and pro-fibrotic signaling in human vocal fold fibroblasts. *PLoS One*. **15**(11):e0241901. doi: 10.1371/journal.pone.0241901.
8. Sharma, R. et al. (2020). Short-term sleep deprivation immediately after contextual conditioning inhibits BDNF signaling and disrupts memory consolidation in predator odor trauma mice model of PTSD. *Brain Res*. doi: 10.1016/j.brainres.2020.147155.
9. Teraoka, S. et al. (2020). Association of BRCA Mutantations and BRCAness Status With Anticancer Drug Sensitivities in Triple-Negative Breast Cancer Cell Lines. *J Surg Res*. **250**:200-208. doi: 10.1016/j.jss.2019.12.040.
10. Kirsch, A. et al. (2019). Development and validation of a novel phonomimetic bioreactor. *PLoS One*. **14**(3):e0213788. doi: 10.1371/journal.pone.0213788.
11. Li, G. et al. (2018). 4-1BB enhancement of CAR T function requires NF-κB and TRAFs. *JCI Insight*. **3**(18). pii: 121322. doi: 10.1172/jci.insight.121322.

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