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Datasheet for ABIN5067541

5X RIPA Buffer with Protease Inhibitor Cocktail

1 Publication

Overview

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| Quantity: | 20 mL |
| Application: | Biochemical Assay (BCA) |

Product Details

Application Details

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| Comment: | <ul style="list-style-type: none">• Buffer is able to extract cytoplasmic, membrane, and nuclear proteins• Suitable for downstream assays such as reporter assays, protein assays, immunoassays and protein purification• Protease Inhibitor Cocktail included |
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| Reagent Preparation: | 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. |
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| Sample Preparation: | <p>I. Adherent Cells</p> <ol style="list-style-type: none">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 2 (56.7 cm), 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. <p>II. Suspension Cells</p> |
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Application Details

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.

Restrictions: For Research Use only

Handling

Handling Advice: Avoid multiple freeze/thaw cycles.

Storage: RT/-20 °C

Storage Comment: 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.

Publications

Product cited in: Matsumoto, Inden, Tamura, Hatano, Tsukita, Asano: "Ezrin mediates neuritogenesis via down-regulation of RhoA activity in cultured cortical neurons." in: **PLoS ONE**, Vol. 9, Issue 8, pp. e105435, (2014) ([PubMed](#)).