RIPA Lysis Buffer (Strong)

Catalogue No.:abx090624

Radioimmunoprecipitation Assay (RIPA) buffer is a lysis buffer used to lyse cells and tissue, for Western Blot and Immunoprecipitation. This buffer is more denaturing than NP-40 or Triton X-100 lysis buffer because it contains the ionic detergents SDS and sodium deoxycholate as active constituents, and is particularly useful for disruption of nuclear membranes in the preparation of nuclear extracts.

The concentration of the protein in the lysed pellet can be determined by our BCA protein assay kits (abx090640 and abx090642). It is not recommended to use a Bradford Protein Assay Kit with this buffer.

Tested Applications:	WB
Storage:	Store at 4 °C for up to one year.
Buffer:	Contains 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and inhibitors (sodium orthovanadate, sodium fluoride, EDTA and leupeptin).
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Directions for use: **Cultured Cell Samples**

1. Prepare an appropriate volume of RIPA Lysis Buffer. Add PMSF Buffer (protease inhibitor) approximately 2-3 min before use to make a final PMSF concentration of 1 mM, and mix thoroughly.

Plate Size / Surface Area	Recommended Buffer Volume
60 mm^2	250-500 µl
100 mm^2	500-1000 µl
6-well cell culture plate	200-400 µl per well
24-well cell culture plate	100-200 µl per well
96-well cell culture plate	50-100 µl per well

2. For **adherent** cells, wash the sample with PBS, normal saline or serum-free culture medium to remove the culture solution. Add an appropriate volume of RIPA Lysis Buffer, then mix until the cells immerse completely in the buffer. Shake gently for 5-10 min. After lysis, centrifuge at 10,000-14,000 × g for 10 min, then collect the supernatant.

For **suspension** cells, centrifuge the sample to collect the cells, then wash the sample with PBS, normal saline or serum-free culture medium. Add an appropriate volume of RIPA Lysis Buffer, then mix until the cells are dispersed. Vortex for 5-10 min to fully lyse the cells. There should be no visible precipitate after lysis; for large volumes of cells, aliquot then lyse. After lysis, centrifuge at 10,000-14,000 × g for 10 min, then collect the supernatant.

Tissue Samples

1. Rinse the tissue sample in cold saline to remove any excess blood. Weigh the sample, then cut it into smaller pieces and place in a tissue homogeniser.
2. Add an appropriate volume of RIPA Lysis Buffer and mix thoroughly. Add PMSF Buffer (protease inhibitor) approximately 2-3 min before use to make a final PMSF concentration of 1 mM.

Note: It is recommended to use a Net Tissue Weight (g) to RIPA Lysis Buffer (ml) ratio of 1:10 (e.g. add 10 ml RIPA Lysis Buffer to 1 g of tissue). For incompletely lysed tissues, the volume of RIPA Lysis Buffer can be increased. For samples with high protein concentration, the volume of RIPA Lysis Buffer can be reduced.

3. Homogenise with a glass homogeniser until samples are completely lysed.
4. Centrifuge at 10,000-14,000 × g for 10 min, then collect the supernatant.

Notes

• It is normal to see a small transparent jelly substance as an end product after adding RIPA Lysis Buffer. This transparent jelly is a complex containing genomic DNA. If genomic-DNA binding proteins are not of interest, the end user can centrifuge and collect the supernatant as normal. Otherwise, the jelly can be dispersed by ultrasound (e.g. sonication) before centrifugation. Ultrasound is not necessary for detecting transcription factors such as NF-kappa B and p53.

• All steps of the protein extraction should be performed at 4 °C or on ice. It is recommended to aliquot large samples into smaller volumes, and then lyophilizing or storing at -20 °C in liquid form. Avoid repeated freeze/thaw cycles.

• Personal protective equipment (PPE), such as lab coats, lab glasses and disposable gloves, are recommended when using this product.

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