Store at –20°C

#9806

RIPA Buffer (10X)

☑ 15 ml

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: RIPA buffer is used to lyse cells and tissues.

1X RIPA Buffer:
20 mM Tris-HCl (pH 7.5)
150 mM NaCl, 1 mM Na_2EDTA
1 mM EGTA
1% NP-40
1% sodium deoxycholate
2.5 mM sodium pyrophosphate
1 mM β-glycerophosphate
1 mM Na_3VO_4
1 µg/ml leupeptin.

Directions for Use

1. If buffer will be continually used, it is recommended that the 10x buffer be kept at 4°C for 1-2 weeks. For longer periods of time, buffer should be stored at -20°C. Aliquotting of 10x buffer is recommended if many small experiments are to be performed.
2. Thaw 10x buffer at 24-30°C, mixing end-over-end.
3. Dilute 10X RIPA Buffer to a 1X solution using ddH_2O. This product supplies enough 10X material to make 150 mls of whole cell extract.
4. Chill 1x buffer on ice and add PMSF just prior to use.

Note: CST recommends adding 1 mM PMSF immediately before use.

Lysis:
For lysis of adherent cells, we recommend the following: (all reagents and lysates must be kept cold).

1. Treat cells as desired.
2. Wash plate with PBS to remove residual media.
3. Add 400 µl of 1x RIPA buffer/10 cm dish.
4. Incubate plate on ice for 5 minutes.
5. Scrape cells.
6. Sonicate briefly.
7. Centrifuge extract for 10 minutes at 14,000 x g in a cold microfuge.
8. Remove supernatant for use.

Additional notes:

1. For non-adherent cells, add 400 µl of buffer per 10^7 cells once they have been washed in 1X PBS and pelleted.
2. 1X RIPA Buffer can be used for lysis of tissue samples, although a homogenization step is recommended after adding lysis buffer. Extract the tissue at a ratio of 100 mg of tissue to 1 ml of buffer. Sonication of the tissue lysate is also required.
3. Additional protease inhibitors can be added to the 1x lysis buffer without any difficulties.
4. Aggregation may be present in this buffer upon arrival due to the high concentrations of reagents included at the supplied 10X formulation. At times, the aggregation persists despite warming to room temperature. We recommend warming the buffer to 37°C for 15 minutes and mixing to help eliminate the precipitate. Alternatively, diluting the 10X RIPA with ddH_2O to at least 5X will yield a precipitate-free solution. Once diluted, it can be aliquoted and stored at -20°C for future use.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.