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Product information

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Animal Cells Protein Lysis Buffer

Catalog #: BSP022
Size: 20 preps
Storage: Mixed components storage*

*: Product will be shipped with ice pack. Check storage conditions. Products have one year expiration from time of purchase.

Product Description:

The Animal Cells Protein Lysis Buffer extracts cytoplasmic and nuclear protein from cultured mammalian cells. The buffer utilizes mild non-ionic detergent with our proprietary combination of various salts and reagents to enhance protein extraction and stability. The buffer is compatible with many applications, including reporter assays (e.g., luciferase, β-galactosidase, acetyltransferase), protein assays (e.g., PKA, PKC, tyrosine kinase), immunoassays (e.g., Western blot, ELISA, RIA) and protein purification. This dialyzable reagent enables simple, rapid, mild and efficient lysis from a wide variety of Mammalian cell.

Features:

- This buffer can be used for Lysis of adherent cells directly in plate or for cells in suspension after scraping and washing.
- This buffer provides mild detergent lysis, yielding extracts that are immediately compatible with the Better BCA Protein Assay Kit (SK3051), BCA Protein Assay Kit (SK3021), or SDS-PAGE electrophoresis.
- Extracts soluble proteins in their non-denatured state, enabling direct use in immunoprecipitation and other affinity purification procedures.
- Maintains luciferase, β-galactosidase, and other reporter gene activities often better than competitor products and traditional freeze/thaw methods.

Composition:

Lysis Buffer	20 ml
Protease Inhibitor	25 µl
DTT	25 µl
PMSF	250 µl

Storage:

Upon receipt store the Lysis Buffer at room temperature, and store the Protease Inhibitor, DTT (1M), and PMSF at -20°C. These products have one year expiration from time of purchase.

Procedure:

A. Procedure for Lysis of Monolayer-Cultured Mammalian Cells

1. Carefully remove (decant) culture medium from adherent cells.

> **NOTE:** If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash cells once in wash buffer (e.g., PBS).

2. Add the appropriate amount of the Lysis buffer (before use, add 1 µl Protease inhibitor, 1 µl DTT, and 10 µl PMSF per 1 ml Lysis Buffer) to the plate or to each plate well (see Table 1). Shake gently for 5 minutes.

> **NOTE:** Cells grown in 100 mm plates typically contain 10^7 cells (50 mg) and yield ~3 mg total protein depending on cell type.

Table 1. Suggested volume of Lysis buffer to use for different sizes of standard culture plates.

Plate Size/Surface Area	Lysis Buffer Volume
100 mm*	500-1,000 µl
60 mm	250-500 µl
6-well plate	200-400 µl per well
24-well plate	100-200 µl per well
96-well plate	50-100 µl per well

3. Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 × g for 5-10 minutes to pellet the cell debris.
4. Transfer the supernatant to a new tube for analysis.

B. Procedure for Lysis of Suspension-cultured Mammalian Cells

1. Pellet the suspension of cells by centrifugation at 2,500 × g for 10 minutes. Discard the supernatant.
2. Optional Wash: If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash the cells once by resuspending the cell pellet in wash buffer (e.g., PBS). Pellet cells by centrifugation at 2,500 × g for 10 minutes.
3. Add the Lysis Buffer to the cell pellet (before use, add 1 µl Protease inhibitor, 1 µl DTT, 10 µl PMSF per 1 ml Lysis Buffer). Use at least 1 ml of the Lysis Buffer for each 100 mg (~100 µl) of wet cell pellet.

   NOTE: If a large amount of cells is used, first add 1/10 the final recommended volume of the Lysis Buffer to the cell pellet. Pipette the mixture up and down to resuspend pellet. Add the rest of the Lysis Buffer to the cell suspension.

   NOTE: Total protein yield for 100 mg of wet cell pellet is approximately 6 mg depending on cell type.
4. Shake mixture gently for 10 minutes. Remove cell debris by centrifugation at ~14,000 × g for 15 minutes.
5. Transfer the supernatant to a new tube for analysis.

Additional Notes:

1. Adherent Cells vs. Cell Pellets: The Lysis Buffer effectively lyses both plated cells and cells pelleted from suspension cultures or scraped cells. For direct, in-the-plate lysis of adherent cells, protein extraction efficiency using this Lysis Buffer is similar to freeze/thaw methods. For lysis of pelleted cells, either from cell suspension or scraped adherent cells, protein extraction efficiency is typically 25% higher than that achieved with freeze-thaw (three cycles) and 20% higher than sonication (2 minutes with 50% pulse) methods.
2. Cell Lines: The Lysis Buffer has been tested on cell lines representing several different cell types. Complete lysis of adherent cells is observed with, but is not limited to, the following cell lines: COS-7, NIH 3T3, Hepa 1-6, 293, CHO, MDA, MB 231 and FM2 cells.
3. For immunoassays, such as ELISA or RIA, extracts prepared in the Lysis Buffer alone generate satisfactory results; however, adding NaCl to a final concentration of 150 mM to the cell lysate often improves results. Depending on applications, EDTA may also be added. Prepare an appropriate volume of the Lysis Buffer for use by adding EDTA to a final concentration of 5 mM. Avoid adding EDTA when extracting 6xHis-tagged proteins, as EDTA will inactivate most nickel-chelated chromatography resins used for purification.
4. Volume for Cell Lysis: Volumes indicated in Table 1 are optimal for maximum cell lysis without scraping cells. If more concentrated extracts are preferred, use a smaller volume; however, scraping the cells is necessary for maximal recovery. If cell volume is unknown, it may be estimated. For example, 2×10^6 of HeLa cells equals ~10 µl of a packed cell volume, which is equivalent to 20 mg of cells and requires 200 µl of the Lysis Buffer .
5. Compatibility with Protein Assays: The Lysis Buffer is compatible with the Better BCA Protein Assay Kit (SK3051) and the BCA Protein Assay Kit (SK3021).