YamayBio

Lysis Buffer for IP/Co-IP

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

Lysis Buffer for IP/Co-IP

BF7474 100mL

Storage: Store at 4°C for 12 months. Product shipped at room temperature.

Introduction

This lysis buffer is primarily designed for the extraction of soluble proteins from cells and tissues under non-denaturing conditions. The resulting protein samples are particularly well-suited for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) assays. It can also be used for PAGE, Western blotting, and ELISA. This product is suitable for protein extraction from animal, plant, fungal, and bacterial samples. Protein samples lysed with this buffer can be quantified using the Ready-to-use BCA Protein Assay Kit (Cat. No.: BH5484). However, due to the presence of high concentrations of interfering substances such as Triton X-100, protein quantification using the Bradford assay is not recommended.

Quick Start Protocol

1. For cell lysis, use 50-100 µL of lysis buffer for IP/Co-IP per 1 × 10^6 cells. For tissue lysis, use 150-250 µL per 20 mg of tissue. Add Phosphatase Inhibitor Cocktail (100×) (Cat. No.: BH5482) at a 1:100 (v/v) ratio within a few minutes before use.

   Note: If phosphorylated proteins are to be extracted, supplement the lysis buffer with Protease Inhibitor Cocktail (Cat. No.: BH5481) at a 1:100 (v/v) ratio, Protease and Phosphatase Inhibitor Cocktail (Cat. No.: BH5483) at a 1:100 (v/v) ratio.

2. Sample Lysis (Perform on Ice):

A. For Adherent Cells:

2.a.1 Discard the culture medium and wash the cells once with 1× PBS or physiological saline or serum-free culture medium.

2.a.2 Remove the PBS as completely as possible.

2.a.3 Add lysis buffer for IP/Co-IP at a ratio of 50-100 μL per 1 × 10^6 cells (e.g., 150-250 μL per well in a 6-well plate). Pipette up and down several times to ensure thorough contact between the lysis buffer and the cells. Cell lysis typically occurs within 1-2 seconds of contact with the lysis buffer.

2.a.4 Transfer all liquid to a new microcentrifuge tube.

B. For Suspension Cells:

2.b.1 Transfer the cells to a centrifuge tube, centrifuge to collect the cells,and discard the culture medium.

2.b.2 Wash the cells once with 1× PBS (if serum proteins do not interfere with the experiment).

2.b.3 Remove the PBS as completely as possible.

2.b.4 Add lysis buffer for IP/Co-IP at a ratio of 50-100 μL per 1 × 10^6 cells (e.g., if processing a large pellet equivalent to a 6-well plate, use 150-250 μL). Pipette up and down several times to ensure thorough contact between the lysis buffer and the cells. There should be no visible cell pellet after sufficient lysis. If the cell number is high, it is essential to aliquot the cells into tubes containing 5 × 10^5 to 1 × 10^6 cells per tube before lysis.

C. For Bacteria or Yeast:

2.C.1 Transfer the bacterial or yeast culture to a centrifuge tube and centrifuge to discard the culture medium.

2.C.2 Wash once with 1× PBS.

2.C.3 Remove the PBS as completely as possible.

2.C.4 Add lysis buffer for IP/Co-IP at a ratio of 100-200 μL per 1 mL of bacterial or yeast culture. Pipette up and down several times to ensure thorough contact between the lysis buffer and the cells. Lyse on ice for 5 minutes. For enhanced lysis, pre-treat bacteria with lysozyme and yeast with Lyticase, followed by lysis with this buffer.

D. For Tissue Samples:

2.d.1 Cut the tissue sample into small pieces.

2.d.2 Add lysis buffer at a ratio of 150-250 µL per 20 mg of tissue sample.
Note: If sample lysis is insufficient, the volume of lysis buffer can be increased appropriately. If a high concentration of protein sample is required, the volume of lysis buffer can also be reduced appropriately.

2.d.C Homogenize the sample using a glass homogenizer until the sample is thoroughly lysed.

3. After sufficient lysis, centrifuge the lysate at 10,000-14,000 × *g* for 3-5 minutes. Carefully transfer the supernatant (protein sample) to a new microcentrifuge tube. This protein sample is now ready for downstream applications such as Immunoprecipitation (Co-IP), PAGE, Western Blotting and ELISA. The resulting protein samples can be aliquoted and stored long-term at -80°C.