YamayBio

Lysis Buffer for IP/Co-IP

QUICK START GUIDE

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

Lysis Buffer for IP/Co-IP

BF7474 100 mL

Storage: Store at 4 °C for 12 months. The 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 lysates are particularly suitable for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) assays. The buffer is also compatible with PAGE, Western blotting, and ELISA.
This product can be used to extract protein from animal, plant, fungal, and bacterial samples. Protein concentrations can be determined using the Ready-to-use BCA Protein Assay Kit (Cat. No.: BH5484). However, due to the presence of detergents 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 of lysis buffer per 20 mg of tissue.
   Immediately before use, add Phosphatase Inhibitor Cocktail (100×) (Cat. No.: BH5482) at a 1:100 (v/v) ratio.

   Note: If preservation of phosphorylated proteins is required, supplement with Protease Inhibitor Cocktail (Cat. No.: BH5481) or Protease and Phosphatase Inhibitor Cocktail (Cat. No.: BH5483) at a 1:100 (v/v) ratio.

2.Sample Lysis (Perform All Steps on Ice):

A. For Adherent Cells:

2.a.1 Discard the culture medium and wash 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).
2.a.4. Pipette up and down several times to ensure thorough contact between the lysis buffer and cells. Cell lysis typically occurs within 1-2 seconds.
2.a.5 Transfer the lysate to a new microcentrifuge tube.

B. For Suspension Cells:

2.b.1 Transfer cells to a centrifuge tube, centrifuge to pellet cells, and discard the culture medium.
2.b.2 Wash once with 1× PBS if serum proteins do not interfere with downstream applications.
2.b.3 Remove PBS completely.
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 gently until no visible cell pellet remains.
2.b.5 If cell numbers are high, aliquot cells into tubes containing 5 × 10^5 to 1 × 10^6 cells prior to lysis.

C. For Bacteria or Yeast:

2.c.1 Transfer the bacterial or yeast culture to a centrifuge tube and pellet cells.
2.c.2 Wash once with 1× PBS and remove the wash completely.
2.c.3 Add lysis buffer for IP/Co-IP at a ratio of 100-200 μL per 1 mL of culture.
2.c.4 Pipette gently to ensure thorough contact between the lysis buffer and cells.
2.c.5 Incubate on ice for 5 minutes.
2.c.6 For improved lysis, pre-treat bacteria with lysozyme and yeast with lyticase before adding the lysis buffer.

D. For Tissue Samples:

2.d.1 Cut tissue into small pieces.
2.d.2 Add lysis buffer at a ratio of 150-250 µL per 20 mg of tissue.
If lysis is insufficient, increase the volume of lysis buffer. If a higher protein concentration is required, the buffer volume may be reduced accordingly.
2.d.3 Homogenize thoroughly using a glass homogenizer until the tissue is completely lysed.

3. After complete lysis, centrifuge samples at 10,000-14,000 × *g* for 3-5 minutes. Carefully transfer the supernatant containing soluble proteins to a new microcentrifuge tube.
The resulting protein lysates are suitable for downstream applications such as immunoprecipitation (IP or Co-IP), PAGE, Western Blotting, and ELISA.
Lysates may be aliquoted and stored at -80 °C for long-term storage.