NP-40 Lysis Buffer

Introduction

NP-40 Lysis Buffer is a relatively mild cell and tissue lysis buffer used to prepare protein samples by lysing cells or tissue samples under non-denaturing conditions. The main components of this product include 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, as well as various inhibitors such as sodium pyrophosphate, β-glycerophosphate, sodium orthovanadate, sodium fluoride, EDTA, and leupeptin. These effectively prevent protein degradation and maintain native protein-protein interactions. Protein samples obtained using NP-40 Lysis Buffer can be used for routine PAGE, Western Blotting (WB), Immunoprecipitation (IP), Co-Immunoprecipitation (Co-IP), and Enzyme-Linked Immunosorbent Assay (ELISA). This product can be used for animal and plant cell or tissue samples, as well as for fungal or bacterial samples.

Protocol

1. Reagents to be prepared by the user

   - Protease inhibitors: PMSF (A2587) or Protease Inhibitor Cocktail (K1007)
   - Phosphatase inhibitors: Phosphatase Inhibitor Cocktail (K1015)
   - Deacetylase inhibitors: Deacetylase Inhibitor Cocktail (K1017)

2. Preparation of lysis buffer

   After thawing NP-40 Lysis Buffer, mix it evenly. Take an appropriate amount of the buffer and add the protease inhibitor PMSF to make the final concentration of PMSF reach 1 mM. Appropriate Cocktails of the above-mentioned protease and phosphatase inhibitors can be added according to the experimental requirements.

***Note:** 1. When more comprehensive protection is required, you can choose to replace it with protease inhibitor Cocktail (K1007); 2. If the protected protein is in phosphorylated or acetylated form, the phosphatase inhibitor Cocktail (K1015) or the deacetylase inhibitor Cocktail (K1017) can be used, respectively.

3. Cell or tissue lysis

   3.1 Adherent cells

   a. Discard culture medium. Wash once with PBS, saline, or serum-free medium (washing may be skipped if serum proteins do not interfere).

   b. Add lysis buffer at 150–250 μL per well of a 6-well plate. Pipette gently to ensure full contact.

c. Most animal cells lyse within 1–2 seconds. Plant cells are recommended to lyse on ice for 2–10 min.

d. After complete lysis, centrifuge at 10,000–14,000×g for 3–5 min. Collect the supernatant for downstream PAGE, WB, IP, Co-IP, ELISA, etc.

3.2 Suspension cells

a. Collect cells by centrifugation. Disperse cells by gentle vortexing or flicking the tube bottom.

b. Add lysis buffer at 150–250 μL per well-equivalent of a 6-well plate. Flick the tube to aid complete lysis.

c. No obvious cell pellet should remain after lysis. For large cell numbers, split into 0.5–1×10^6 cells per tube before lysis.

d. After complete lysis, centrifuge at 10,000–14,000×g for 3–5 min. Collect the supernatant for downstream PAGE, WB, IP, Co-IP, ELISA, etc.

3.3 Bacteria or yeast

a. Take 1 mL culture, centrifuge, and discard supernatant (may wash once with PBS to remove residual liquid). Disperse by gentle vortexing or flicking.

b. Add 100–200 μL lysis buffer, mix gently, and lyse on ice for 2–10 min. For improved lysis: treat bacteria with lysozyme and yeast with lyticase before using this buffer.

c. After complete lysis, centrifuge at 10,000–14,000×g for 3–5 min. Collect the supernatant for downstream PAGE, WB, IP, Co-IP, ELISA, etc.

3.4 Tissue Samples

a. Cut tissue into small pieces.

b. Add lysis buffer at 150–250 μL per 20 mg tissue. Increase volume if lysis is incomplete; decrease volume to obtain higher protein concentration.

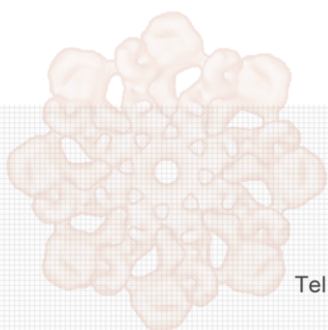
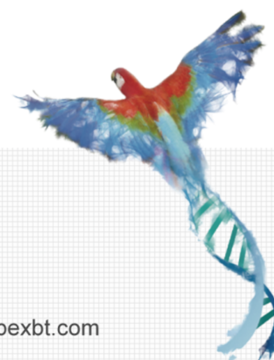
c. Homogenize with a glass homogenizer until fully lysed. Alternatively, freeze tissue, grind in liquid nitrogen, then add lysis buffer.

d. After complete lysis, centrifuge at 10,000–14,000×g for 3–5 min. Collect the supernatant for downstream assays.

Note

1. Lysis buffer volume guide: 150 μL is usually sufficient per well of a 6-well plate or per 1 mL bacterial/yeast culture. For very high cell density, increase to 200 or 250 μL. Lysate from 1×10^6 animal cells in 100 μL buffer typically yields 2–4 mg/mL protein (varies by cell type).

2. Lysate from 20 mg frozen mouse liver in 200 μL buffer typically yields 15–25 mg/mL protein (varies by tissue and condition).
3. Very fine tissue samples may be directly lysed after minimal chopping, using vigorous vortexing. This method is convenient but less thorough than homogenization or grinding.
4. For optimal performance, aliquot before use to avoid repeated freeze–thaw cycles.
5. All lysis steps must be performed on ice or at 4°C.
6. This product is for scientific research use only.

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