NP-40 Lysis Buffer (Contains inhibitors)

N748603

Storage: NP-40 Lysis Buffer store at 2-8℃,
Phosphatase Inhibitor Cocktail and PMSF Store at -20°C

Introduction:

NP-40 Lysis Buffer is a relatively mild cell and tissue lysis solution. The protein samples obtained by lysis with it can be used for routine Western blotting, immunoprecipitation (IP), and co-immunoprecipitation (co-IP), etc. For the protein samples obtained by lysis with NP-40 Lysis Buffer, the protein concentration can be determined using a BCA Protein Assay Kit. Due to the presence of a relatively high concentration of detergent, the Bradford method cannot be used to determine the protein concentration of the samples lysed by this lysis buffer.

Usage method:

1. Reagent Preparation

Take an appropriate amount of lysis buffer. Within a few minutes before use, add the protease inhibitor cocktail and/or phosphatase inhibitor cocktail to the lysis buffer at a ratio of 1:50. Alternatively, use 100 mM PMSF and adjust its final concentration to 1 mM.

2. Cell/Tissue Lysis

For adherent cells

a) Remove the culture medium and wash the cells once with PBS, normal saline, or serum - free culture medium.
b) Add the lysis buffer at a ratio of 150 - 250 μL per well of a 6 - well plate. Pipette the lysis buffer up and down several times to ensure full contact between the lysis buffer and the cells. Usually, the cells will be lysed within 1 - 2 seconds after contact with the lysis buffer.
c) If it is for ChIP (Chromatin Immunoprecipitation), continue the lysis on ice for another 10 minutes after the initial lysis.

For suspension cells

a) Collect the cells by centrifugation and flick the tube forcefully with your finger to disperse the cells.
b) Add the lysis buffer at a ratio of 150 - 250 μL per well of a 6 - well plate equivalent of cells. Then gently flick the tube to fully lyse the cells.
c) If the cell quantity is large, aliquot the cells into tubes at a density of 500,000 - 1,000,000 cells per tube before lysis. There should be no obvious cell pellet after full lysis.
d) If it is for ChIP, continue the lysis on ice for another 10 minutes after the initial lysis.

For tissue samples

a) Cut the tissue into small pieces with tissue scissors.

b) Add the lysis buffer at a ratio of 150 - 250 μL per 20 mg of tissue and homogenize the tissue using a glass homogenizer until fully lysed.

c) If it is for ChIP, continue the lysis on ice for another 10 minutes after the initial lysis.

3. Protein Sample Collection

After full lysis, centrifuge the lysate at 10,000 - 14,000 g for 3 - 5 minutes. Collect the supernatant for subsequent operations such as PAGE (Polyacrylamide Gel Electrophoresis), Western blotting, and ChIP.

Precautions:

1. For the best usage effect, it is advisable to aliquot the reagent and store it at -20°C. Avoid excessive repeated freeze - thaw cycles.
2. All steps of lysing the samples should be carried out on ice or at 2 - 8°C.
3. For your own safety, please take protective measures such as wearing a lab coat and gloves before using the reagent.