NP-40 Lysis Buffer

Catalog# BWR1002

Size: 100 ml

Lot # Check on the product label

Introduction

1. NP-40 Lysis Buffer is a kind of gentle cell and tissue lysis buffer. The end protein / sample lysed by this buffer can be used for Western Blot, Immunoprecipitation and Co-Immunoprecipitation.
2. NP-40 Lysis Buffer mainly contains: 50mM Tris (pH 7.4), 150mM NaCl, 1% NP-40 and different kinds of inhibitors (sodium pyrophosphate, β-glycerophosphate, sodium orthovanadatesodium fluoride, EDTA and leupeptin), which can effectively inhibit the protein degradation.
3. The concentration of the protein which lysed by this buffer can be determined by our BCA Protein Assay Kits (Catalog # BWR1023, BWR1024, BWR1025, BWR1026). For the high concentration of detergent, it is not recommended to use Bradford Protein Assay Kit.

Kit Components

Components	Size	Storage Instruction
NP-40 Lysis Buffer	100 ml	Store at 4°C for one year

Protocol

- **Cultured Cells Sample**

1. Pipette proper volume of NP-40 Lysis Buffer and mix thoroughly. 2-3 min advanced before use, add PMSF buffer to make its final concentration to 1mM.

2. For **adherent cells**: wash sample with PBS, normal saline or serum-free culture medium to remove culture solution. Add proper volume of NP-40 Lysis Buffer, then stroke with pipette until the buffer immerse cells completely. Shake slightly for 5-10 min. After lysis, centrifuge at 10,000-14,000 g for 10 min, then collect the supernatants and move on to the next step.

>> Instruction for NP-40 usage: for different sizes of cell culture plates <<

Sizes of plate / surface area	Volume of Buffer
100 mm	500-1,000 µl
60 mm	250-500 µl

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6-well plate	200-400 μl per well
24-well plate	100-200 μl per well
96-well plate	50-100μl per well

3. For **suspending cells**： Centrifuge to collect cells, then, wash sample with PBS, normal saline or serum-free culture medium. Add proper volume of NP-40 Lysis Buffer, then stroke with pipette until cells dispersed. Vertex for 5-10 min to lyse cells completely (There should be no obvious cell precipitates if cells lysed completely. For large volume of cells, aliquot and lyse then). After lysis, centrifuge at 10,000-14,000 g for 10 min, collect the supernatants and move on to the next step.

- **Tissues Sample**

1. Put the tissue sample into precooling normal saline quickly, remove blood by rinsing it several times. Weight sample and cut it into small slices, then put them into the tissue homogenizer.
2. Pipette proper volume of NP-40 Lysis Buffer and mix thoroughly. 2-3 min advanced before use, add PMSF buffer to make its final concentration to1mM.
3. Tissue net weight (g) to NP-40 Lysis Buffer (ml) ratio = 1:10 (i.e. add 10ml of NP-40 Lysis Buffer to 1g tissues) and homogenate (If lyses incompletely, can add more volume of NP-40 Lysis Buffer; if high concentration protein samples are required, can reduce the volume of lysis buffer).
4. Homogenize with a glass homogenizer until samples were lysed completely.
5. Centrifuge at 10,000-14,000 g for 3-5 min, collect the supernatant and move on to next step.

Note: It is normal to see a small group of transparent jelly in the end product of NP-40 Lysis Buffer. This transparent jelly is a complex containing genomic DNA. If not detect the genomic DNA binding proteins, users can centrifuge directly and collect the supernatant to do next steps of the experiment; Or, scatter the jelly by ultrasonic wave, then, centrifuge and collect the supernatant to do next steps of the experiment. For detecting the normal transcription factors (e.g. NF-kappa B, P53, etc.), ultrasonic wave processing is not necessary.

Notes

1. All steps of protein extraction should be operated on ice or at 4 ℃. It is recommended to aliquot the sample into sub-packages at proper volume, then freeze-drying or store at -20℃ in liquid form. Avoid freeze thawing repeatedly.
2. PMSF (phenylmethylsulfonyl fluoride) buffer is not included in this product, we provide it separately (Catalog # BWR1014). Few minutes (2-3 min) advanced before use, add PMSF buffer to make its final concentration to1mM.
3. Please wear the lab coat and disposable gloves to operate.

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