NP40 Lysis Buffer (with Inhibitors)

（LY004）

Product Description

NP40 Lysis Buffer is a mild lysis buffer for cells and tissues, with the main components being 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, and various inhibitors including sodium pyrophosphate, β-glycerophosphate, sodium orthovanadate, sodium fluoride, EDTA and leupeptin. It exhibits strong lytic activity on both cytoplasmic subfractions and nuclear components, facilitating the extraction of cytoplasmic proteins, nuclear proteins, cytoplasmic phosphorylated proteins and nuclear transcription factors. It is suitable for the preparation of protein samples for routine experiments such as Western Blot, IP and co-IP.

Components

Cat. No.	Component	Volume
LY004-A	NP40 Lysis Buffer	100 mL
LY004-B	Phosphatase Inhibitor	2*1 mL
LY004-C	PMSF	1 mL

Storage Conditions

Transport at ≤0℃. Store the lysis buffer at 4℃ and the other components at -20℃. The shelf life is one year.

Experimental Procedures

(I) Cultured Cell Samples

1. Thaw the NP40 Lysis Buffer and mix well. Take an appropriate volume of the lysis buffer and add PMSF a few minutes before use to a final concentration of 1 mM.
2. Cell lysis

(1）Adherent cells: Remove the culture medium and rinse the cells once with PBS, normal saline or serum-free medium (rinsing can be omitted if serum proteins cause no interference). Add the lysis buffer at a ratio of 150~250 μL per well of a 6-well plate. Pipette up and down several times to ensure full contact between the lysis buffer and cells. Cells are usually lysed 1~2 seconds after contact with the lysis buffer.

(2）Suspension cells: Collect the cells by centrifugation and add the lysis buffer at a ratio of 150~250 μL per well of cell culture from a 6-well plate, then mix well to lyse the cells thoroughly. No obvious cell pellet should be observed after complete lysis. For a large number of cells, aliquot the cell suspension into tubes with 0.5~1×10^6 cells per tube before

lysis. The dosage of NP40 Lysis Buffer can refer to Table 1.

3. After complete lysis, centrifuge at 10000~14000 rpm for 3~5 minutes, collect the supernatant, and proceed with subsequent experiments such as PAGE, Western Blot and immunoprecipitation.

(II) Tissue Samples

1. Cut the tissues into small pieces.
2. Thaw the NP40 Lysis Buffer and mix well. Take an appropriate volume of the lysis buffer and add PMSF a few minutes before use to a final concentration of 1 mM.
3. Add the lysis buffer at a ratio of 150~250 μL per 20 mg of tissue. Additional lysis buffer can be added if lysis is incomplete, and the dosage can be reduced if a high-concentration protein sample is required.
4. Homogenize with a glass homogenizer until the tissues are completely lysed.
5. After complete lysis, centrifuge at 10000~14000 rpm for 3~5 minutes, collect the supernatant, and proceed with subsequent experiments such as PAGE, Western Blot and immunoprecipitation.

Table 1. Dosage of NP40 Lysis Buffer

Sample Type	Sample Source	Harvested Cells	Dosage of NP40 Lysis Buffer	Number of Preparable Samples
Cell	6-well plate	$2.5*10^6$	150 ~250 μL	400 ~600
	60 mm culture dish	$5.2*10^6$	300 ~500 μL	200 ~300
	90 mm culture dish	$12.2*10^6$	500 ~1000 μL	100 ~200
	25 cm^2 culture flask	$5*10^6$	300 ~500 μL	200 ~300
	75 cm^2 culture flask	$2*10^7$	1000 ~2000 μL	50 ~100
Tissue	20 mg tissue block	-	150 ~250 μL	400 ~600