

# NP40 Cell Lysis Buffer

Catalog Number FNN0021

Pub. No. MAN0015953 Rev. 2.0 (30)

## Product Description

Cat. No.	FNN0021
Lot No.	See product label.
Quantity	100 mL
Description	NP40 Cell Lysis Buffer is suitable for the preparation of cell extracts to be analyzed by Antibody Bead Immunoassay, ELISA, and western blotting.
Buffer Formulation	<p>50 mM Tris, pH 7.4                  250 mM NaCl                  5 mM EDTA                  50 mM NaF                  1 mM Na<sub>3</sub>VO<sub>4</sub>                  1% Nonidet™ P40 (NP40)                  0.02% NaN<sub>3</sub></p> <p><b>Note:</b> This cell lysis buffer must be supplemented with 1 mM PMSF (not included) and protease inhibitor cocktail (not included) just prior to use.</p> <ul style="list-style-type: none"> <li>• For the PMSF addition, we recommend making a 0.3 M stock in DMSO and adding sufficient volume for a final concentration of 1 mM (i.e., 17 µL per 5 mL cell lysis buffer). PMSF is very unstable and must be added just prior to use, even if added previously.</li> <li>• For the protease inhibitor cocktail addition, we recommend Sigma-Aldrich™ Cat. No. P-2714. Reconstitute the protease inhibitor cocktail to a 10X concentrate, then add 500 µL per 5 mL cell lysis buffer. The stability of protease inhibitor-supplemented cell lysis buffer is 24 hours at 4°C.</li> </ul>
Instructions	<p>This protocol has been successfully applied to several cell lines. Some optimization may be required for each specific application.</p> <ol style="list-style-type: none"> <li>1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).</li> <li>2. Wash cells twice with cold PBS.</li> <li>3. Remove and discard the supernatant and collect the cell pellet.</li> <li>4. Lyse the cell pellet in cell lysis buffer for 30 minutes, on ice, with vortexing at 10-minute intervals. The volume of cell lysis buffer depends on the cell number and expression of target protein and level of phosphorylation. A suitable starting concentration is 10<sup>8</sup> cells per mL lysis buffer.</li> <li>5. Transfer the extract to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.</li> <li>6. Aliquot the clear lysate to clean microcentrifuge tubes. These samples are ready for assay. Lysates can be stored at -80°C. Avoid multiple freeze/thaws.</li> </ol>
Storage	Store at ≤-20°C. Thaw this buffer on ice. This buffer is stable for 2-3 weeks at 4°C or for up to 1 year when apportioned into working aliquots and stored at ≤-20°C.
Expiration	Expires one year from date of receipt when stored as instructed.



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