

# Cell Extraction Buffer

Catalog Number FNN0011

Pub. No. MAN0014666 Rev. 2.0 (30)

## Product description

Cat. No.	FNN0011
Lot No.	See product label.
Quantity	100 mL
Description	Cell extraction buffer suitable for use in ELISA and western blotting.
Buffer Formulation	<p>10 mM Tris, pH 7.4            100 mM NaCl            1 mM EDTA            1 mM EGTA            1 mM NaF            20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>            2 mM Na<sub>3</sub>VO<sub>4</sub>            1% Triton™ X-100            10% glycerol            0.1% SDS            0.5% deoxycholate</p> <p><b>Note:</b> This cell extraction 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 extraction 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, reconstituted according to the manufacturer's instructions, added at 250 µL per 5 mL cell extraction buffer. The stability of protease inhibitor-supplemented cell extraction 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 extraction buffer for 30 minutes, on ice, with vortexing at 10-minute intervals. The volume of cell extraction 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 extraction 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 2-8°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.



**Manufacturer:** Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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