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	10 mM Tris, pH 7.4 100 mM NaCl 1 mM EDTA 1 mM EGTA 1 mM NaF 20 mM $Na_4P_2O_7$ 2 mM Na_3VO_4 1% Triton™ X-100 10% glycerol 0.1% SDS 0.5% deoxycholate **Note:** This cell extraction buffer must be supplemented with 1 mM PMSF (not included) and protease inhibitor cocktail (not included) just prior to use. • 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. • 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.
Instructions	This protocol has been successfully applied to several cell lines. Some optimization may be required for each specific application. 1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent). 2. Wash cells twice with cold PBS. 3. Remove and discard the supernatant and collect the cell pellet. 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 10^8 cells per mL extraction buffer. 5. Transfer the extract to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C. 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.
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

The information in this guide is subject to change without notice.