



## Mild Cell Lysis PLUS

Cat. No.	size
E0293-01	50 ml
E0293-02	200 ml

**Storage and transport:** 4°C.

**Term of validity:** 12 months

**Content:** 50 mM Tris-HCl pH 7.6, 250 mM NaCl, 1 % detergent, 20 mM NaF, 5 mM EDTA, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 0.02 % NaN<sub>3</sub>

**Product Important Information:** Mild Cell Lysis PLUS does not contain protease inhibitors (1 mM PMSF or Protease Inhibitor Cocktail), which should be added directly before proteins extraction.

For extraction of nucleic and mitochondrial proteins it is recommended to use RIPA buffer (E0295).

Mild Cell Lysis PLUS contains phosphate inhibitors and is designed to lyse protein from adherent and suspension cultured mammalian cells and tissues, depending on nonionic detergent and recommended for cytoplasmic and membrane-bound proteins extraction. The buffer is complemented by NaF and Na<sub>3</sub>VO<sub>4</sub> and can be used to study the phosphorylated proteins.

### Procedure for Lysis of Monolayer-cultured Adherent Mammalian Cells:

- Carefully remove culture medium from adherent cells.
- Wash cells twice with chilled PBS.
- Add cold Mild Cell Lysis PLUS to the cells (1 mL of buffer per 10<sup>8</sup> cells - the volume might need optimization for different cell types and amount of the protein of interest).

Incubate on ice for 15 minutes, swirling the plate occasionally until the clear lysate is visible.

- Collect the lysate and centrifuge at ~14,000 × g for 15 minutes.
- Transfer supernatant to a new tube for further analysis or store at -80 °C.

### Procedure for Lysis of Suspension-cultured Mammalian Cells:

- Pellet the cells by centrifugation at 2500 × g for 5 minutes. Carefully remove and discard the supernatant.
- Wash cells twice in chilled PBS. Centrifuge at 2500 × g for 5 minutes. Carefully remove and discard the supernatant.
- Add cold Mild Cell Lysis PLUS to the cell pellet (1 mL of buffer per 10<sup>8</sup> cells - the volume might need optimization for different cell types and amount of the protein of interest). Pipette the mixture up and down to suspend the pellet.
- Shake mixture gently for 15 minutes on ice until the clear lysate is visible.
- Centrifuge samples at 14,000 × g for 15 minutes.
- Transfer supernatant to a new tube for further analysis or store at -80 °C.

### Procedure for Lysis of Tissues:

- Place the fresh tissue into chilled PBS and rinse several times. Mince the tissue into small pieces.
- Add cold Mild Cell Lysis PLUS to the tissue at 10:1 (10 mL Mild Cell Lysis PLUS per gram of tissue).
- Homogenize for several minutes at high speed until no tissue chunks remain.
- Incubate on ice for 3 minutes.
- Centrifuge at 10000 × g for 10 minutes
- Transfer supernatant to a new tube for further analysis or store at -80 °C.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039  
www.eurx.com.pl, orders@eurx.com.pl, tel. +48 58 524 06 97, fax +48 58 341 74 23