CelLytic™ M
Mammalian Cell Lysis/Extraction Reagent

Catalog Number C2978
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
Extraction of cell proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and/or reagent interference with protein immunoreactivity and biological activity.

The CelLytic™ M mammalian cell lysis/extraction reagent enables efficient and rapid cell lysis, and solubilization of proteins for both suspension and adherent cells such that adherent cells do not require scraping from the culture dish.

The CelLytic M extracted proteins can be used for reporter gene assays (β-gal, alkaline phosphatase, and CAT), immunoassays (Western blots, ELISA, and immunoprecipitation), kinase assays (PKC and tyrosine kinase) and phosphatase assays (general and tyrosine phosphatases). This buffer is compatible with Coomassie® Blue and silver staining of gels. Protein lysates can also be used for DNA-protein interaction assays (gel-shift assays).

The CelLytic M lysis/extraction reagent contains a low percentage of a mild detergent for minimal interference with protein interactions and biological activity. The detergent can be dialyzed out as needed and is supplied in a bicine buffer which is suitable for evaluation of biological activity.

CelLytic M reagent efficiency for protein extraction has been tested on, but not limited to, HeLa, CHO, COS, HL-60, Jurkat, A431, PC-12, and Bovine Aorta Endothelial Cells (BAEC).

For some applications, lysis at 4 °C and/or the addition of specific components might be advantageous. Components that may be added include protease or phosphatase inhibitor cocktails, reducing agents, chelators, or salts (may provide better results in immunoassays and better extraction of nuclear proteins).

Reagent
The CelLytic M mammalian cell lysis/extraction reagent is supplied ready-to-use. Sufficient reagent for the extraction of cells from 250 plates (100 mm diameter) is provided.

Reagents and Equipment Required but Not Provided
(Catalog Numbers are given where available)
• Protease Inhibitor Cocktail for mammalian cell and tissue extracts, Catalog Number P8340
• Test tubes
• Shaker
• Microcentrifuge (Eppendorf® 5417R, Catalog Number Z366013 or Z366021) or equivalent
• Dulbecco’s phosphate-buffered saline (DPBS), Catalog Number D8537

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage
Store at room temperature. Product may appear cloudy after extended period of storage. Product performance is unaffected and may be used, as is, without further filtration or clarification.

Procedure
The volume of CelLytic M lysis/extraction reagent to be added to the cells varies according to cell size and protein concentration required.

In general, 125 µl of CelLytic M is recommended for 10⁶–10⁷ cells. For adherent cells the plate size will dictate the amount of reagent covering the plate surface. Suggested working volumes are 500–1000 µl for a 100 mm plate and 200–400 µl for a 35 mm plate.

Protease Inhibitor Cocktail may be added to the CelLytic M reagent.
1. Wash cells and treat with CelLytic M.
   a. For adherent cells:
      Remove the growth medium from the cells to be assayed. Rinse the cells once with DPBS, being careful not to dislodge any of the cells and discard DPBS. Add appropriate volume of CelLytic M reagent.
   b. For cells in suspension:
      Collect cells in an appropriate centrifuge tube. Centrifuge for 5 minutes at 450 × g. Decant and discard the supernatant. Wash the cells once with DPBS and centrifuge for 5 minutes at 450 × g. Decant and discard supernatant. Resuspend the cell pellet in the recommended volume of CelLytic M reagent.

2. Incubate the cells for 15 minutes on a shaker.

3. Collect lysed cells.
   a. For adherent cells: remove cells from plates (cell scraping might increase total protein yield).
   b. For cells in suspension: go to step 4.

4. Centrifuge the lysed cells for 15 minutes at 12,000–20,000 × g to pellet the cellular debris.

5. Remove the protein-containing supernatant to a chilled test tube. 
   Note: Lysate preservation requires low temperatures. Therefore, for long term storage it is recommended to store lysate at −70 °C.

Related Products

- CelLytic B, Bacterial Cell Lysis/Extraction Reagent, Catalog Number B7435
- CelLytic B 2x, Bacterial Cell Lysis/Extraction Reagent, Catalog Number B7310
- CelLytic B 10x, Bacterial Cell Lysis/Extraction Reagent, Catalog Number C8740
- CelLytic MT, Mammalian Tissue Lysis/Extraction Reagent, Catalog Number C3228
- Mammalian Cell Lysis Kit, Catalog Number MCL1
- CelLytic NuCLEAR™ Protein Extraction Kit, Catalog Number NXTRACT

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