Product Information

CelLytic™ M

Cell Lysis Reagent, suitable for mammalian cell lysis and protein solubilization

C2978

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.

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). CelLytic™ M is compatible with Coomassie® Blue and silver staining of gels. Protein lysates can also be used for DNA-protein interaction assays (gel-shift assays).

CelLytic™ M 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. The product 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. Such components include protease or phosphatase inhibitor cocktails, reducing agents, chelators, or salts (which may provide better results in immunoassays and better extraction of nuclear proteins).

Several theses[1-3] and dissertations[4-22] have cited use of product C2978 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent

CelLytic™ M 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

(Cat. Nos. are given where available)

- Protease Inhibitor Cocktail suitable for mammalian cell and tissue extracts, such as Cat. Nos. P8340, PIC0002, or I3786
- Test tubes
- Shaker
- Microcentrifuge
- Dulbecco’s phosphate-buffered saline (DPBS), Cat. No. D8537

Storage/Stability

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

Procedure

General Notes

- The volume of CelLytic™ M 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 10^6-10^7 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
  - 200-400 µL for a 35 mm plate
- Protease Inhibitor Cocktail may be added to the CelLytic™ M reagent.

General Protocol

1. Wash cells and treat with CelLytic™ M.

   1.1. For adherent cells:

      1.1.1. Remove the growth medium from the cells to be assayed.

      1.1.2. Rinse the cells once with DPBS, being careful not to dislodge any of the cells.

      1.1.3. Discard DPBS.

      1.1.4. Add appropriate volume of CelLytic™ M reagent.

   1.2. For cells in suspension:

      1.2.1. Collect cells in an appropriate centrifuge tube.

      1.2.2. Centrifuge for 5 minutes at 450 × *g*.

      1.2.3. Decant and discard the supernatant. Wash the cells once with DPBS.

      1.2.4. Centrifuge for 5 minutes at 450 × *g*.

      1.2.5. Decant and discard supernatant.

      1.2.6. 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.

   3.1. For adherent cells: remove cells from plates. (Cell scraping might increase total protein yield.)

   3.2. 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.

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