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ProductInformation

CelLytic™ MT
Mammalian Tissue Lysis/Extraction Reagent

Product Code **C 3228**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Extraction of cell proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and interference with protein immuno-reactivity and biological activity.

The CelLytic™ MT mammalian tissue lysis/extraction reagent enables efficient extraction of tissue proteins for analysis. The lysis buffer consists of a dialyzable mild detergent at a low concentration (for minimal interference with protein interactions and biological activity), bicine (a buffer preferable for biological activity), and 150 mM NaCl. CelLytic MT can also be used for the efficient extraction of cell-line proteins that require salt in the lysis buffer. For cells not requiring salt in the lysis buffer, use CelLytic M, Product Code C 2978.

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

The CelLytic MT reagent has been used for protein extractions from rat (brain, liver, muscle, kidney, heart, and spleen) and mouse (brain, kidney, and muscle) tissues.

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

Reagent

Sufficient CelLytic MT reagent is supplied for extraction of 2.5 g (50 ml) or 25 g (500 ml) of tissue.

Reagents and Equipment Required but Not Provided

(Product Codes are given where available)

- Protease Inhibitor Cocktail, Product Code P 8340
- Test tubes
- Shaker
- Homogenizer
- Microcentrifuge (Eppendorf® 5417R, Product Code Z36,601-3 or Z36,602-1, or equivalent)
- Dulbecco's phosphate-buffered saline (DPBS), Product Code D 8537

Precautions and Disclaimer

Sigma's CelLytic MT mammalian tissue lysis/extraction reagent is for laboratory use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

A. Tissue protein extraction

For this procedure, a ratio of tissue to CelLytic MT reagent of 1:20 (1 gram of tissue/20 ml of reagent) is recommended. When a concentrated lysate is required, you can lyse the tissues in a lower volume of CelLytic MT reagent. The Protease Inhibitor Cocktail may be added to the CelLytic MT reagent.

1. Weigh tissue samples.
2. Add the appropriate amount of CelLytic MT to the tissue sample.
3. Transfer the sample (with lysis/extraction reagent) to a pre-chilled microhomogenizer and homogenize the tissue. Be aware that the homogenization procedure might be critical for the functional integrity of the target protein.

4. Centrifuge the lysed sample for 10 minutes at 12,000-20,000 × *g* to pellet the tissue debris.
5. Transfer the protein-containing supernatant to a chilled test tube.

Note: Lysate preservation requires low temperatures. For long term storage it is recommended to store the lysate at –70 °C.

B. Cellular protein extraction

The volume of CelLytic MT reagent to be added to the cells varies according to cell size and protein concentration required. In general, 125 µl of CelLytic MT is recommended for 10^6-10^7 cells. For adherent cells the plate size will dictate the amount of reagent used to cover the plate surface. Suggested working volumes are: 500-1000 µl for a 100 mm plate and 200-400 µl for a 35 mm plate.

1. Wash cells and lyse as below.
   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. Discard DPBS. Lyse cells with appropriate volume of CelLytic MT reagent.
   b. For suspension cells:
      Collect the cells into an appropriate centrifuge tube. Centrifuge for 5 minutes at 450 × *g*. Decant and discard the supernatant. Wash the cell pellets once with DPBS and centrifuge for 5 minutes at 450 × *g*. Decant and discard supernatant. Resuspend the cell pellet in CelLytic MT reagent
2. Incubate the cells for 15 minutes on a shaker.
3. Collect cell lysate.
   a. For adherent cells: collect cells (cell scraping might increase total protein yield).
   b. For cells in suspension: Skip to step 4.
4. Centrifuge the lysed cells for 10 minutes at 12,000-20,000 × *g* to pellet the cellular debris.
5. Transfer the protein-containing supernatant to a chilled test tube.

Note: Lysate preservation requires low temperatures. For long term storage it is recommended to store the lysate at –70 °C.

Related Products

- CelLytic B, Bacterial Cell Lysis/Extraction Reagent, Product Code B 3553
- CelLytic B II, Bacterial Cell Lysis/Extraction Reagent, Product Code B 3678
- CelLytic M, Mammalian Cell Lysis/Extraction Reagent, Product Code C 2978
- Mammalian Cell Lysis Kit, Product Code MCL-1
- Nu-Clear Protein Extraction Kit, Product Code N-XTRACT

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