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ProductInformation

Mammalian Cell Lysis Kit
Product Code **MCL1**

Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Extraction of cell proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and interference with the proteins' immunoreactivity and biological activity. The Mammalian Cell Lysis Kit can be used to prepare cell lysis (RIPA[1]) buffer and other modified buffers. The cell lysis buffer enables efficient solubilization of proteins for analysis and a low background in immunoprecipitation assays. Also, most antigens and antibodies are not adversely affected by the components of the cell lysis buffer (see Note).

The Mammalian Cell Lysis kit is composed of solutions to be mixed and used for lysis of adherent cells, non-adherent cells, and tissues. The cell lysis buffer has been tested for immunoreactivity on HeLa, CHO, COS, PC-12, Jurkat and Bovine Aorta Endothelial Cells (BAEC), using antibodies for nuclear, cytoplasmic, cytoskeletal and membrane proteins. In addition, the buffer has been tested on COS cells transfected with plasmid expressing FLAG-tagged recombinant protein (see Note). It has also been tested on mouse spleen, muscle and kidney tissues.

The Mammalian Cell Lysis kit contains buffer, detergents, NaCl and protease inhibitor cocktail solutions. This enables the researcher to change the buffer's components to obtain maximum efficiency for a specific protein.

Note: Some antigens can be denatured and some protein:protein complexes can be disrupted in the presence of the complete cell lysis buffer containing all three detergents. In such cases, the suitable detergent(s) must be carefully chosen. An example of this is the isolation or immunoprecipitation of FLAG-tagged proteins using the M2 monoclonal antibody. The FLAG-fusion proteins should be extracted with 1% Igepal CA-630 in buffered saline, without the SDS and DOC detergents.

Reagents Provided

Sufficient for extraction of 250 ml total, or extraction of cells from 250 plates (100 mm diameter)

- 5x Buffer, Product Code T8815 — 50 ml
  250 mM Tris-HCl, pH 7.5, 5 mM EDTA
- 5x NaCl, Product Code S4684 — 50 ml
  750 mM NaCl
- 5x SDS, Product Code L1787 — 50 ml
  0.5% Lauryl sulfate, sodium salt in deionized water
- 5x DOC, Product Code D4437, — 50 ml
  2.5% Deoxycholic acid, sodium salt in deionized water
- 5x Igepal CA-630, Product Code I2653 — 50 ml
  5% Igepal CA-630 in deionized water
- Protease inhibitor cocktail, Product — 2.5 ml
  Code P8340, containing 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), pepstatin A, bestatin, leupeptin, aprotinin and trans-epoxysuccinyl-L-leucyl-amido(4-guanidino)-butane (E-64)

Reagents and Equipment Required but Not Provided

(Sigma product numbers are given where available)

- Test tubes
- Shaker
- Microcentrifuge Eppendorf 5417R (Product Code Z366013 or Z366021) or equivalent
- Glass tissue homogenizer (grind tube with type B pestle)(optional)
- Cells scrapers, Product Code C2802
- Dulbecco's phosphate buffered saline (PBS), Product Code D8537

Precautions and Disclaimer
Sigma's Mammalian Cell Lysis Kit 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/Stability
The kit is shipped on dry ice and is stored at –20 °C. Kit components except for the protease inhibitor cocktail can be stored at 2–8 °C for several months. The Protease inhibitor cocktail must be kept at –20 °C and must be added to buffers immediately before use.

Preparation Instructions
Preparation of working solutions

- Before preparing the cell lysis buffer, all stock solutions should be equilibrated to room temperature. Be sure that the solutions are homogenous.
- Prepare the appropriate amount of cell lysis (RIPA) buffer. Prepare 1 ml cell lysis buffer for the extraction of 10^6–10^7 cells or 5–20 mg tissue.

  Preparation of 1 ml cell lysis buffer

5X Buffer (Tris-EDTA)	200 µl
5X NaCl	200 µl
5X SDS (lauryl sulfate)	200 µl
5X DOC (deoxycholic acid)	200 µl
5X Igepal CA 630	200 µl
Protease inhibitor cocktail	10 µl
Final volume	**1 ml**

  Note that the protease inhibitor cocktail is properly used at a 1:100 dilution in the cell lysis buffer. If one or two of the detergent solutions are to be omitted, use an equal volume of distilled deionized water to replace the detergent solution(s).

After preparing the cell lysis buffer, store at 2–8 °C.

Procedure
Perform all steps at 2–8 °C.

1. Wash cells/ tissue and treat with cell lysis buffer.
   a. For adherent cells:
   Remove the growth media from the cells to be assayed. Rinse the cells twice with PBS, being careful not to dislodge any of the cells. Discard PBS. Add cell lysis buffer (10^6–10^7 cells/ml).

   b. For cells in suspension:
   Collect the cells into an appropriate centrifuge conical test tube. Centrifuge for 5 minutes at 420 x *g*. Decant the supernatant and discard. Wash the cells twice by resuspending the cell pellets with PBS and centrifuge for 5 minutes at 420 x *g*. Decant supernatant and discard. Resuspend the cell pellet in cell lysis buffer (10^6–10^7 cells/ml).

   c. For tissues:
   Rinse the tissue at least twice with PBS. Discard the PBS from rinses. Add cell lysis buffer (5–20 mg tissue/ml).

2. Incubate the cells/tissue for 15 minutes on an orbital shaker.

3. Collect cell lysate.
   a. For adherent cells: Scrape and collect cells.

   b. For cells in suspension: Skip to step 4.

   c. For tissues: Transfer the sample (with cell lysis buffer) to a pre-chilled micro-homogenizer and homogenize the tissue. Be aware that the homogenization procedure used may affect the functional integrity of the target protein.

4. Centrifuge the lysed cells for 10 minutes at 12,000 x *g* to pellet the cellular debris. Alternatively, to prepare a protein solution using high-speed centrifugation, centrifuge for 45 minutes at 100,000 x *g*.

5. Remove the protein-containing supernatant to a chilled test tube. For immediate use, keep on ice. Otherwise, store the protein solution at –20 °C (or at –70 °C for improved stability).

Note: In special cases when a concentrated lysate is required, the cells can be lysed using a lower volume of lysis buffer. For adherent cells, the plate size will dictate the amount of buffer covering the plate surface. For cells in suspension, the volume can be decreased to a volume of 2X volume of packed cells.

References

1. Alternative names, **R**adio**i**mmuno **P**rotection **A**ssay or **R**adio**i**mmuno **P**recipitation **A**ssay

Related Products

- CelLytic™ M Cell Lysis Reagent, Product Code C2978
- RIPA Buffer, Product Code R0278
- CelLytic B Cell Lysis Reagent, for bacterial cell lysis, standard strength, Product Code B7435
- CelLytic B Cell Lysis Reagent, for bacterial cell lysis, 2X concentrate, Product Code B7310
- CelLytic B Cell Lysis Reagent, for bacterial cell lysis, 10X concentrate, Product Code C8740

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