Product Information

Mammalian Cell Lysis Kit

MCL1

Product Description

Storage Temperature: – 20 °C

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 buffer (such as RIPA buffer[1]) and other modified buffers. The cell lysis buffer enables efficient solubilization of proteins for analysis and low background in immunoprecipitation assays. Also, most antigens and antibodies are not adversely affected by the cell lysis buffer components (see Note 3 in the 'Preparation of Working Solutions' section).

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 that expresses FLAG®-tagged recombinant protein (see Note 3 in the 'Preparation of Working Solutions' section). 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 adjustment of the buffer's components to obtain maximum efficiency for a specific protein. Several theses[2-4] and dissertations[5-12] have cited use of product MCL1 in their 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.

Components

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

- 5× Buffer, 50 mL (Component T8815): 250 mM Tris-HCl (pH 7.5), 5 mM EDTA
- 5× NaCl, 50 mL (Component S4684): 750 mM NaCl
- 5× SDS, 50 mL (Component L1787): 0.5% Lauryl sulfate, sodium salt (SDS) in deionized water
- 5× DOC, 50 mL (Component D4437): 2.5% Deoxycholic acid, sodium salt in deionized water
- 5× Igepal® CA-630, 50 mL (Component I2653): 5% Igepal® CA-630 in deionized water
- Protease inhibitor cocktail, 2.5 mL (Component P8340): contains AEBSF [4-(2-aminoethyl) benzenesulfonyl fluoride], Pepstatin A, Bestatin, Leupeptin, Aprotinin, and E-64 [trans-epoxysuccinyl-L-leucyl-amido(4-guanidino)-butane]

Reagents and Equipment Required

(Not provided)

- Test tubes
- Shaker
- Microcentrifuge
- Glass tissue homogenizer (grind tube with type B pestle; optional)
- Cell scrapers, such as Cat. No. CLS3010
- Dulbecco’s phosphate buffered saline (PBS), Cat. No. D8537

Storage/Stability

This kit is shipped on dry ice, and is stored at –20 °C.

After receipt, 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.
    - For example, for the extraction of 10^6 – 10^7 cells, or of 5-20 mg tissue, prepare 1 mL of cell lysis buffer, such as follows:

Component	Volume
5× Buffer (Tris-EDTA)	200 µL
5× NaCl	200 µL
5× SDS (lauryl sulfate)	200 µL
5× DOC (deoxycholic acid)	200 µL
5× Igepal® CA 630	200 µL
Protease Inhibitor Cocktail	10 µL

Notes

1. 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).
2. After preparing the cell lysis buffer, store the buffer at 2-8 °C.
3. Some antigens can be denatured and some protein:protein complexes can be disrupted in the presence of the complete cell lysis buffer that contains 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 instead be extracted with 1% Igepal® CA-630 in buffered saline, without the SDS and DOC detergents.

Procedure

Perform all steps at 2-8 °C.

Wash cells/tissue and treat with cell lysis buffer.

For adherent cells

1. Remove the growth media from the cells to be assayed.
2. Rinse the cells twice with PBS, being careful not to dislodge any of the cells.
3. Discard the PBS.
4. Add cell lysis buffer (10^6 – 10^7 cells/mL).

For cells in suspension

1. Collect the cells into an appropriate centrifuge conical test tube.
2. Centrifuge for 5 minutes at 420 × g.
3. Decant the supernatant. Discard the supernatant.
4. Wash the cells twice by resuspending the cell pellets with PBS.
5. Centrifuge for 5 minutes at 420 × g.
6. Decant supernatant. Discard the supernatant.
7. Resuspend the cell pellet in cell lysis buffer (10^6 – 10^7 cells/mL).

For tissues

1. Rinse the tissue at least twice with PBS.
2. Discard the PBS from the rinses.
3. Add cell lysis buffer (5-20 mg tissue/mL).
4. Incubate the cells/tissue for 15 minutes on an orbital shaker.
5. Collect cell lysate.

For adherent cells

Scrape and collect cells.

For cells in suspension

Skip to For Tissues section, Step 3.

For tissues

1. Transfer the sample (with cell lysis buffer) to a pre-chilled micro-homogenizer.
2. Homogenize the tissue. Be aware that the homogenization procedure used may affect the functional integrity of the target protein.
3. Centrifuge the lysed cells for 10 minutes at 12,000 × g to pellet the cellular debris.
    - Alternatively, to prepare a protein solution using high-speed centrifugation, centrifuge for 45 minutes at 100,000 × g.

4. Remove the protein-containing supernatant to a chilled test tube.
    - For immediate use, keep on ice. Otherwise, store the protein solution at –20 °C.
    - The protein supernatant may also be stored 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 2× 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.
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