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Product Information

CelLytic™ MEM Protein Extraction Kit

Catalog Number **CE0050**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The CelLytic™ MEM Protein Extraction Kit offers a fast and convenient method to isolate hydrophobic and raft (glycosphingolipids and cholesterol rich membrane microdomains) associated proteins from cells. The method, based on phase separation, does not require cell membrane isolation. Hydrophobic and raft associated proteins are concentrated in the hydrophobic phase and may be used for further experiments. The kit is compatible with the following:

- Immunoprecipitation
- Gel electrophoresis – Coomassie® Brilliant Blue and Silver staining
- Dot blot and Western blot

The kit has been tested with the following cell lines: HeLa, HEK-293, NIH 3T3, COS, and CHO.

Components

Lysis and Separation Buffer Catalog Number L2417	50 ml
Wash Buffer for CelLytic Kit Catalog Number W2514	50 ml
Sodium Chloride, 4 M solution Catalog Number S2568	1.5 ml
Protease Inhibitor Cocktail for mammalian cell and tissue extracts - Catalog Number P8340	1 ml

Reagents and Equipment Required but Not Provided

- Tissue culture centrifuge tubes,15 ml conical
- Microcentrifuge tubes
- Serological centrifuge
- Microcentrifuge, refrigerated
- Dulbecco's Phosphate Buffered Saline (PBS) (Catalog Number D8537)
- PBS containing 1 mM EDTA
- Cell scraper (Catalog Number CLS3010)

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/Stability

The kit ships on wet ice and it is recommended to store the unopened kit at –20 °C. After opening the kit, store the Protease Inhibitor Cocktail for mammalian cell and tissue extracts at –20 °C. Store the other components at 2–8 °C.

Preparation Instructions

<u>Preparation of the Lysis and Separation Working Solution</u>

Always store the Lysis and Separation Buffer refrigerated or on ice. Mix the Lysis and Separation Buffer before use. Prepare the Lysis and Separation Working Solution fresh daily by adding 6 µl of the Protease Inhibitor Cocktail for mammalian cell and tissue extracts to 600 µl of the Lysis and Separation Buffer.

<u>Modifications of the Lysis and Separation Working Solution and the Wash Buffer</u>

- The Lysis and Separation Buffer can be diluted with the Wash Buffer (1:1) in order to decrease the volume of the hydrophobic phase, resulting in a more concentrated hydrophobic protein sample. The Lysis and Separation Working Solution, prepared with Lysis and Separation Buffer diluted with the Wash Buffer, does not significantly affect the protein yield in the hydrophobic phase.

- The Lysis and Separation Buffer and the Wash Buffer contain 150 mM NaCl. Some hydrophobic proteins require a higher salt concentration for extraction into the hydrophobic phase. Use the supplied 4 M NaCl solution to adjust the NaCl concentration to the required level for the protein(s) of interest.

Procedure
This procedure is suitable for extraction of 10^6 to 10^7 cells. If a larger number of cells are to be used or multiple extractions are run in parallel, adjust the procedure accordingly.

A. Cell collection
For adherent cells steps 1a–3a are required. For cells growing in suspension, begin with step 3a.

1a. Aspirate the growth medium from the tissue culture vessel and wash the cells with Dulbecco's PBS.

2a. Add 0.1 ml of PBS containing 1 mM EDTA solution per each cm^2 of culture area. Incubate until the cells detach. Alternatively, add PBS and scrape the cells using a cell scraper.

3a. Transfer the cell suspension into a 15 ml conical tube and collect the cells by centrifugation at 600 × *g* for 5 minutes. Aspirate the supernatant and then store the pellet on ice.

B. Separation of hydrophobic and hydrophilic proteins

1b. Mix the prepared Lysis and Separation Working Solution containing the Protease Inhibitor Cocktail before use. Resuspend 10^6–10^7 cells in 600 µl of ice-cold Lysis and Separation Working Solution. Mix gently by pipetting up and down, and vortex briefly. Transfer the suspension to a microcentrifuge tube.

2b. Incubate the cell suspension on ice for 10 minutes.

3b. Centrifuge the cell lysate in a pre-cooled (4 °C) microcentrifuge at 10,000 × *g* for 5 minutes. Transfer the clarified lysate to a new micro-centrifuge tube. A 30–50 µl aliquot of the total protein lysate may be saved for further analysis.

4b. Incubate the lysate at 30 °C for 3–5 minutes. During the incubation, mix once by inverting the tube. The lysate will turn cloudy during the incubation.
Note: Incubation at 30 °C is preferable; however, incubation at temperatures up to 37 °C is possible.

5b. Centrifuge the tube in a microcentrifuge at room temperature at 3000 × *g* for 3 minutes. Ensure the centrifuge temperature is higher than 20 °C. **Do not return the tube to ice after centrifugation.** Transfer the upper hydrophilic phase containing hydrophilic proteins to a new microcentrifuge tube. The lower hydrophobic phase is greatly enriched with hydrophobic and raft associated proteins.

6b. In order to remove residual hydrophilic proteins from the hydrophobic phase, the hydrophobic phase may be washed with the Wash Buffer. Add 400 µl of the Wash Buffer to the hydrophobic phase. Mix gently and incubate the tube on ice for 10 minutes. Repeat steps 4b and 5b.

C. Downstream applications

1c. SDS-PAGE electrophoresis
Samples from the hydrophilic and hydrophobic phases can be used directly for acrylamide gel electrophoresis. The dye front may appear diffuse but the final protein pattern is not affected. However, some PAGE systems will require the samples to be diluted 5–10 fold to obtain good resolution.

Alternatively, the samples can be precipitated with TCA to obtain more concentrated samples. For comparative analysis of protein separation between the phases, it is recommended to normalize the samples loaded on the gel.

2c. Dot blot
For fast analysis of an extraction for a specific protein, a dot blot can be performed using 1–2 µl samples.

3c. Immunoprecipitation
Before adding the immobilized antibody, dilute the hydrophobic phase 10 to 12-fold with Wash Buffer to make the solution compatible with antibody binding.

4c. Other applications
For applications requiring low salt concentrations, the sample may be dialyzed.

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