ab65399

Mammalian Cell Extraction Kit

Instructions for Use

For the rapid and sensitive extraction of Mammalian proteins from cultured cells and tissue samples.

This product is for research use only and is not intended for diagnostic use.

Version 2b Last updated 23 April 2020

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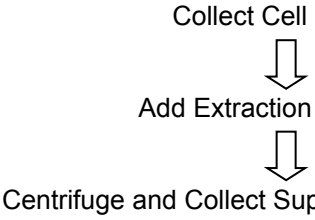
1. Overview

Abcam's Mammalian Cell Extraction Kit provides optimized cell extraction buffer, protease inhibitor cocktail, and DTT for convenient extraction of mammalian proteins from cultured cells and tissue samples, under non denaturing conditions.

Cell lysate prepared using the kit can be used in a variety of applications, such as enzyme activity assays (e.g., caspase activity assays), Western blot analysis, and others. The entire procedure takes less than 20 minutes.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Extraction Buffer	100 mL
Protease Inhibitor Cocktail (Lyophilized)	1 vial
DTT (1 M)	110 µL

Store kit at -20°C. After opening the kit, store Cell Extraction Buffer at +4°C. Store Protease Inhibitor Cocktail and DTT at -20°C. Read the entire protocol before beginning the procedure.

PROTEASE INHIBITOR COCKTAIL: To reconstitute, add 100 µl DMSO to the vial, pipette several times to dissolve all powder. This makes 500X concentrated Protease Inhibitor Cocktail solution.

Be sure to keep the Extraction Buffer Mix on ice at all times during the experiment.

EXTRACTION BUFFER: Before use, add 2 µl of DTT and 2 µl of Protease Inhibitor Cocktail to 1 ml of Extraction Buffer (The mixture is referred as Extraction Buffer Mix).

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- 96-well plate
- Orbital shaker

4. Assay Protocol

The following protocol is described for extraction of ~2 x 10^6 cells and should generate ~100-300 µg of cell lysate. If large amounts of cell lysate are desired, scale up the volumes proportionally.

1. Collect cells by centrifugation at 600 x g for 5 minutes at +4°C.

Note:

For adherent cells, scrape cells into PBS and spin down to pellet cells.

2. Re-suspend cells in 100 µl of the Extraction Buffer Mix. Pipette up and down several times.

Note:

For tissue samples, homogenize tissues in 2-3 volume of the Extraction Buffer Mix, until it is completed lysed.

3. Incubate on ice for 10 minutes, then vortex for 5 seconds.

4. Centrifuge in a microcentrifuge at top speed for 3 minute.

5. Collect the supernatant (Cell Lysate) and discard the pellet.

6. Store cell lysate at -80°C for further studies.

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