Mammalian Whole Cell Protein Extraction Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K269
Description	The 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 nondenaturing 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.
Quantity	500 isolations

Kit Components

Components	Quantity	Cap code
Cell Extraction Buffer	100 ml	NM
Protease Inhibitor Cocktail	1 vial*	Red
DTT (1 M)	110 µl	Blue

*Reconstitute in 100 µl DMSO

Applications / Assay Protocol

A. General Consideration and Reagent Preparation:

- After opening the kit, store Cell Extraction Buffer at +4°C. Store Protease Inhibitor Cocktail and DTT at –20°C.
- The Protease Inhibitor Cocktail is provided in lyophilized form. To reconstitute, add 100 µl DMSO to the vial and pipet several times to dissolve all powder (this yields 500X concentrated Protease Inhibitor Cocktail).
- Before use, add 2 µl of DTT and 2 µl of Protease Inhibitor Cocktail to 1 ml of Cell Extraction Buffer (the mixture is referred as *Extraction Buffer Mix*).
- Be sure to keep the *Extraction Buffer Mix* on ice at all times during the experiment.
- The following protocol is described for extraction of ~2 x 10^6 cells and should generate ~100-300 µg of cell lysate. If larger amounts of cell lysate are desired, scale up the volumes accordingly.

B. Mammalian Protein Extraction Protocol:

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. Resuspend cells in 100 µl of the *Extraction Buffer Mix*. Pipet 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 minutes.
5. Collect the supernatant (cell lysate) and discard the pellet.
6. Store cell lysate at –70°C for further studies.

Storage & Stability

Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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