Cytoplasmic and Nuclear Protein Extraction Set, (A+B)

Protein Chemistry

Catalogue Number: MP016

Description: The Cytoplasmic and Nuclear Protein Extraction Set is designed for rapid stepwise isolation of native, non-denatured cytoplasmic and nuclear fractions from cultured cells or tissue samples. The proteins are ready for direct use in many applications such as 1-D and 2-D electrophoresis, immunoprecipitation, affinity purification followed by assays including Western blotting, electrophoretic mobility shift assays (EMSA) proteomic analysis, enzymatic activity assays, and reporter gene assays. Cell extracts prepared using this set are compatible with quantification assays such as Bradford, Lowry and the BCA. The EZ-protocol, based on lysis with mild detergent conditions, provides our researchers with a rapid and simple method.

Pack Size: 1 set

Storage: 4 ºC

Application: Preparation of native, non-denatured cytoplasmic and nuclear protein extracts from mammalian cultured cells or tissue samples.

Components of the Kit: The kit contains sufficient reagents for extraction of cytoplasmic and nuclear fractions from 50 samples of cells cultured in 10-cm dish or up to 100 mg of tissue.

Components	Name	Volume	Add freshly:
A	CytoEx	100 mL	DTT, 1mM
B	NuEx	10 mL	Proteinase inhibitor

EZ-protocol (for cells in 10-cm culture dish):

1) Collect cells by trypsinization.
2) Add 1mL ice-cold PBS (cat #: CC006) and transfer cells into a 1.5 mL eppendorf tube.
3) Pellet cells by gentle spin (1000 x g for 5 minutes).
4) Wash the cell pellet twice with 1 mL of ice-cold Ca^{2+} and Mg^{2+}-free PBS (cat #: CC008) and remove PBS carefully after last wash.
5) Add 500 uL of CytoEx and resuspend cell pellet by gentle pipetting.
6) Lyse cells in CytoEx buffer on ice for 15 minutes.
7) Spin down at 5000 x g for 5 minutes and collect the supernatant (this is the cytoplasmic fraction).
8) Wash pellets twice with 500 uL of CytoEx buffer.
9) Extract nuclear proteins with 50-200 uL (2-3 pellet volume) of NuEx buffer on ice 30 minutes.
10) Remove debris by centrifugation at 12,000 x g for 5 minutes and collect the supernatant (this is the nuclear fraction).