PRODUCT DATA SHEET

Bioworld Technology CO., Ltd.

Bioepitope Nuclear and Cytoplasmic Extraction Kit

Cat No.: BD0030

Introduction

For research of cell protein, the separation of cytosol and nucleus protein attracted researchers' attention. This requires effective reagents to separate cytoplasmic and nuclear extracts from cultured mammalian cells. The kit enables stepwise proteins: Reagent A disrupts cell membrane while not acts on nuclear. Reagent B lyses the nuclei. It is effective to separate cytoplasmic and nuclear proteins in short time with this kit. The extracts are suitable for WB, co-IP and purification of proteins.Due to the reagents in this kit, BCA Protein Assay method is recommended to quantify the protein concentrations of extracts. There are no protease inhibitors in this kit, prepare protease inhibitors before use.

Reagents

Cytoplasmic Extraction Reagent A: 50ml

Nuclear Extraction Reagent B: 10ml

Application

Nuclear and Cytoplasmic Extraction

Storage & Shelf life

Stored at 4℃, recommend to aliquot before stored at -20℃.

Procedure

1) For adherent cells(1-10*106 cells): the cells were harvested with trypsin-EDTA, the centrifuged at 500g for 3min, 4℃; For suspension cells(1-10*106 cells): the cells were centrifuged at 500g for 3min. Resuspend and wash the cells with PBS in a 1.5 ml tube. Then centrifuge the cells at 500g for 3min, remove and discard the supernatant as much as possible.

2) Add 800-1000μl reagent A (for 1-10*106 cells) with protease inhibitors to the precipitate; Resuspend the precipitate and vortex the tube for 15s.

3) Place the tube on ice for 20min. During this period, vortex the tube for 15s to resuspend the precipitate each 5min.

4) Centrifuge the tube at 3000g for 10min, 4℃. Transfer the supernatant (cytoplasmic extracts) to a new tube. Store at 4℃ (for short term) or -80℃ (for long term).

5) Add 500μl reagent A with protease inhibitors to the precipitate (left in the tube during step 4), resuspend and wash the precipitate. Centrifuge at 3000g for 10min. Remove and discard the supernatant. (May be omitted if you can almost transfer the cytoplasmic extracts completely in step 4.)

6) Add 150-200μl reagent B with protease inhibitors to the precipitate (left in the tube during step 4 or 5). Resuspend the precipitate and vortex the tube for 15s. Place the tube on ice for 10min. Then vortex the tube for 15s to resuspend the precipitate every 5min, for total 20min.

7) Centrifuge the tube at 12000g for 20min, 4℃. Transfer the supernatant (nuclear extract) to a new tube. Store at 4℃ (for short term) or -80℃ (for long term).

NOTE

1) Avoid freeze-thaw. Recommend to aliquot before stored at -20℃ or -80℃.

2) This kit doesn't contain protease inhibitors, prepare protease inhibitor before use.

3) Resuspend the precipitate with reagent B in the tube as you can as possible, but there might be flocculent fragments. That causes no impact on the nuclear extract.

4) All steps on ice.

Research Use

For research use only, not for use in diagnostic procedures.

MADE IN CHINA

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