Product Information Sheet

Cytoplasmic and Nuclear Protein Extraction Kit

Catalog No. AR0106
Size Sufficient for performing up to 50-100 assays.
Storage At 4℃ for one year.
Kit Component Cytoplasmic Extraction Reagent (CER) 30ml
Nuclear Extraction Reagent (NER) 5ml

Introduction

This kit supplies a complete set of lysis reagents that enable the separation of nuclear protein and cytoplasmic fractions from cultured cells and tissue. The optimized reagents and protocol allow non-denatured, active proteins to be purified in less than two hours. It is sufficient for extracting 50-100 protein samples, the quantity for each sample is (5-10) x 108 cells (about 50mg).

Protocol

1. **Anchorage-dependent cell：** remove the nutrient solution with PBS buffer, or deal it with EDTA solution to loose cells, then stroke cells in pipette (digesting with pancreatin is not recommended, it may degrade the protein). Centrifuge at 500xg for 2～3min, then collect cells. Try to separate sediment from supernatant for stand-by.
2. **Suspending cell**: Wash with PBS buffer, Centrifuge at 500xg for 2～3min, then collect cells. Try to separate sediment from supernatant for stand-by.
3. **Solution preparation:** pipette proper volume of Cytoplasmic Extraction Reagent (CER) and Nuclear Extraction Reagent (NER) on ice. Before use, add PMSF to make its final concentration to be 1mM (End users can determine whether add PMSF or not).
4. Re-suspend the sedimentary cell into as 5-10 times volume of CER, shake and vortex strongly for 15 seconds to make the cell suspending and separate completely (Prolong the shaking time if the cell is not suspending and separate completely) . Incubate for 10-30mins on ice.
5. Shake at high speed for 5 seconds, centrifuge at 12000～16000xg for 10 minutes at 4℃。
6. Pipette supernatant into a precooling plastic tube, the sediment is cytoplasmic. End users can analyze it in WB, Immunoprecipitation/IP or Co-Immunoprecipitation. Or store it at -70℃. (Do not touch sediments.)
7. Remove the remanent supernatant completely, add NER as 2 times volume as it. Avoid to pollute cytoplasmic protein, please make sure the supernatant was removed completely.
8. Shake at high speed for 15 seconds until cell suspending and separate completely. Then place it in ice-bath incubate for 30min. Shake at high speed for 10-20 seconds , centrifuge at 12000～16000xg for 10 minutes at 4℃ at each 5 min interval.
9. Pipette supernatant to a precooling plastic tube, the sediment is nuclear protein. Now you can move on to next step or store it at -70℃.

Tissue Sample:

A. Cut sample into slices as small as you can. Homogenate with PBS buffer to cell suspension. centrifuge at 500Xg for 2～3 minutes. Collect cells and remove supernatants, estimate the sediment volume. Then follow the step 4.

B. After weight tissue sample, cut it into slices as small as you can. Add CER at the ratio 1:10. After homogenate, pipette it into plastic centrifuge tube. Then follow the step 5.

To reorder contact us at:

Antagene, Inc.

Toll Free: 1(866)964-2589

Tel: (650) 964-2589

Fax: (650) 964-2519

email: Info@antageneinc.com