Hytra Nuclear/Cytosol Protein Extraction Kit

Product No. **W-7883 / 100 rxns**

Introduction

Hytra Nuclear/Cytosol Protein Extraction Kit provides for efficient cell lysis and extraction of separate cytoplasmic and nuclear protein fractions in less than two hours. It can efficiently solubilize and separate cytoplasmic and nuclear proteins into fractions with minimal cross-contamination or interference from genomic DNA and mRNA. The isolated proteins can be used for further analysis, such as mobility shift assays (EMSA), co-immunoprecipitation (Co-IP) and pull-down assays.

Storage

This product is stable for one year at 4℃.

Protocol

- ✧ Prepare 4℃ centrifuge.
- ✧ Keep all samples on ice during operation.
- ✧ 1 reaction is used for $5X10^6$ cells or 10cm dish.
- ✧ If desired, add protease inhibitors and/or phosphatase inhibitor to reagent 1 and reagent 2 just before use.

Procedure for adhesion Cells

1. Before using, thawed the 100 X reagent 1 in room temperature. And prepare 1 X working solution by diluting with sterilized H_2O. (e.g. 10 μl + 990μl H_2O)
2. Carefully remove culture medium from adherent cells.
3. Wash cells twice in wash buffer (e.g., PBS).
4. Discard PBS.
5. Add 600 μl reagent 1. Collect cells by scraping and transfer the cells lysate to a microcentrifuge tube.
6. Vortex for 5 seconds then put on ice for 5 minutes.
7. Vortex for 5 seconds again, and centrifuge the sample at 2000 rpm (400 x g) for 2 minutes.
8. Discard the supernatant part.

※ If necessarily, collect the supernatant from step 7 and centrifugation at 8000 rpm for 8 minutes. The supernatant is cytosol fraction.

9. Add 600 μl reagent 2 to pellet from step 7. Vortex it until it become homogeneous solution.
11. Centrifuge at 8000 rpm (6000 x g) for 8 minutes.
12. Discard the supernatant.
13. Add 400 μl reagent 3 to the pellet of step 11. Vortex it until the condense pellet become homogeneous solution.
13. Centrifuge at 13000 rpm (16000 x g) for 12 minutes.
14. Collect the supernatant (nuclear fraction) to new microcentrifuge tube, and continue with downstream analysis.

Procedure for suspension cells

1. Before using, thawed the 100 X reagent 1 in room temperature. And prepare 1 X working solution by diluting with sterilized H_2O. (e.g. 10 μl + 990μl H_2O)
2. Collect the cells ($5X10^6$ cells) into an appropriate centrifuge tube.
3. Centrifuge for 5 minutes at 450 × g.
4. Decant and discard the supernatant.
5. Wash the cell pellets once with PBS and centrifuge for 5 minutes at 450 × g for 5 minutes. Decant and discard supernatant.
6. Add 600 μl reagent 1.
7. Vortex for 5 seconds then put on ice for 5 minutes.
8. Vortex for 5 seconds again, and centrifuge the sample at 2000 rpm (400 x g) for 2 minutes.
9. Discard the supernatant part.

※ If necessarily, collect the supernatant from step 8 and centrifugation at 8000 rpm for 8 minutes. The supernatant is cytosol fraction.

10. Add 600 μl reagent 2 to pellet from step 8. Vortex it until it become homogeneous solution.
11. Centrifuge at 8000 rpm (6000 x g) for 8 minutes.
12. Discard the supernatant.
13. Add 400 μl reagent 3 to the pellet of step 11. Vortex it until the condense pellet become homogeneous solution.
13. Centrifuge at 13000 rpm (16000 x g) for 12 minutes.
14. Collect the supernatant (nuclear fraction) to new microcentrifuge tube, and continue with downstream analysis.

Related products

Product No.	Description	Size
W-7849	*Hycell* RIPA Buffer	500 ml
W-7850	*Hytra* Cell Protein Extraction Reagent 500ml	500 ml
W-7851	*HyTra* Tissue Protein Extraction Reagent	500 ml
W-7852	*Hytra* Mitochondria Protein Extraction Kit	100 rxns
W-8982	*Hytra* Membrane Protein Extraction Reagent	100 rxns
HC 100-007	100X Protease Inhibitor Cocktail	2 ml
HC 100-008	100X Phosphatase Inhibitor Cocktail	2 ml

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