Nuclear Protein Extraction Kit

Cat.No: PE002-1 50T
PE002-2 100T

PE002

I. Introduction

The Nuclear Protein Extraction Kit provides the simple method for extracting nuclear and cytoplasmic proteins from mammalian tissues, suitable for quick preparation of nuclear extracts from all kinds of primary cells , passage cell and tissue samples: such as brain, spinal cord , ganglion or fibre ,fat, liver, alimentary canal, kidney ,heart, muscle, vessel, connective tissue and so on. The whole procedure could be completed within 1 hour and could get high purity of nuclear and cytoplasmic proteins with natural activity. Cross -contamination is very seldom. The kit could be used for a variety of applications including western blotting, transcriptional activity, Gel shift assays, co-immunoprecipitation, nuclear enzyme assays and others assays for protein.

II. Kit contents:

Cat.No	PE002-1	PE002-2
Extration Solution A	10ml	20ml
Extration Solution B	10ml	20ml
Protease Inhibitor Cocktail	250μl	500μl
Phosphatase Inhibitor Cocktail	250μl	500μl

1. Protein Extraction Solution: includes some effective components, which could release the nuclear and cytoplasmic proteins completely and could combine the released protein to protect the precipitation.

2. Protease Inhibitor Cocktail: includes 7 independent protease inhibitor, AEBSF、Aprotinin、Leupeptin、Pepstatin A、Bestatin、E-64、EDTA.

Note:

1. The Protease Inhibitor Cocktail should be avoid repeated freeze / thaw cycles.
2. If the kit could not be used up in one time, please do not add all the Protease Inhibitor cocktail into the extraction solution.

III. Storage

1. Storage the Protease Inhibitor Cocktail at -20℃.
2. Storage the extraction solution and Phosphatase Inhibitor Cocktail at 2-8℃.

IV. Period of validity

One Year

V. Additional Materials Required

Pipettor and Tips, Centrifuge and centrifugal tube, Vortex, Refrigerator and Ice box

VI. Protocol:

A. Extraction of cell protein

1. Preparation of extraction solution: Respectively add 2ul Protease Inhibitor

Cocktail and Phosphatase Inhibitor Cocktail to 200ul cold Extration Solution A and B, shake up then put them on ice bath;

2. Take 5-10×10^6 cells①, centrifugate them at 4℃,500 Xg for 2-3 mins, draw the medium carefully and dry, then collect the cells;
3. Wash the cells twice using cold PBS. Carefully remove and discard the supernatant each time;
4. Add 200ul cold solution A to 20ul cell sample, oscillate it on Vortex for 15s then put on ice bath for 10 mins;
5. Then shake it again in the vortex for 5s , Centrifuge tubes at 16000 Xg for 5 mins at 4°C;
6. Transfer supernatant to new tubes ,that is the cytoplasmic proteins and store them on ice bath or -80℃ refrigerator;
7. Add 200ul cold solution B to the precipitation, oscillate it on Vortex for 15s.
8. Put it on ice bath for 40 mins and oscillate it on Vortex for 15s every 10 mins;
9. Centrifuge them at 16000 Xg for 10 mins at 4°C;
10. Transfer supernatant quickly to new tubes and get the nuclear protein;
11. Quantitation the extraction② and package it ,then store at -80℃ refrigerator or use it for the following experiments directly③.

Note:

① Adjust the quantity of cell according to the experiment, the usage of lysis buffer is not the same every time, so please adjust it .

②Using BCA method to quantitation the protein.

③The protein sample is also available after store at －80℃ for one year. Keep it away from protease and bacteria.

B. Extraction of tissue protein.

1. Preparation of extraction solution: Respectively add 2ul Protease Inhibitor Cocktail to 200ul cold Solution A and B, shake up then put them on ice bath;
2. Take proper quantity of tissue sample and cut it into layers, add cold PBS, homogenate it with Tissue Grinders① till there is no solid could be seen(or grinding it with Liquid nitrogen),then put it on ice bath for 5 mins,
   Carefully transfer the homogenate to another pre-cooling clean centrifugal tube.
3. Centrifugate the tube at 4℃②,500 Xg for 2-3 mins, discard the supernatant.
4. Add 200ul cold solution A to 20ul cell sample, oscillate it on Vortex for 15s then put on ice bath for 10 mins;
5. Then shake it again in the vortex for 5s , Centrifuge tubes at 16000 Xg for 5 mins at 4°C;
6. Transfer supernatant to new tubes ,that is the cytoplasmic proteins and store them on ice bath or -80℃ refrigerator;
7. Add 200ul cold solution B to the precipitation, oscillate it on Vortex for 15s;
8. Put it on ice bath for 40 mins and oscillate it on Vortex for 15s every 10 mins;
9. Centrifuge them at 16000 Xg for 10 mins at 4°C;
10. Transfer supernatant quickly to new tubes and get the nuclear protein;
11. Quantitation the extraction② and package it ,then store at -80℃ refrigerator or

use it for the following experiments directly[③].

Note:

① Liquid nitrogen grinding method is better;

② Using BCA method to quantitation the protein.

③The protein sample is also available after store at －80℃ for one year. Keep it away from protease and bacteria.