Filamentous Fungi Nuclear Protein Extraction Kit (Pull-down, co-IP, etc.)

Cat: EX1821
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Filamentous Fungal Nucleoprotein Extract A	50mL	100mL	2-8℃
Component B: Filamentous Fungal Nucleoprotein Extract B	10mL	20mL	2-8℃
Component C: Protease Inhibitor Mixture C	100μL	200μL	-20℃

Note:

1. Protease inhibitors can also be stored at 2-8℃ before use without open lid. Store at -20℃ after opening the lid for use.
2. The protease inhibitor is solid at 2-8℃. Take it out of the refrigerator and return to room temperature or 37℃ water bath for a short time. When it becomes liquid, centrifuge it to the bottom of the tube and then open the lid.
3. Please use the reagent as soon as possible after unpacking!

Introduction:

Filamentous fungi nuclear protein extraction kit is a fast and efficient high-yield nuclear protein extraction kit. The fungal nucleoprotein extraction kit provides a complete set of reagents suitable for extracting nuclear protein from various filamentous fungi. The optimized lysate formula can fully release a variety of membrane and non-membrane mycelium proteins as well as a variety of mycelium metamorphosis nucleoproteins. The extraction process is simple and convenient, and can be completed within 1 hour. This kit can not be used for yeast nuclear protein extraction.

This kit contains a unique formula that effectively dissolves filamentous fungal nuclear components. The kit contains a protease inhibitor mixture that prevents the protease from degrading the protein and ensures the extraction of high purity proteins.

The protein extracted from this kit can be used for Pull-Down, IP and other protein interaction studies, as well as WB, protein electrophoresis, ELISA, transcriptional activity analysis, Gel shift gel blocking experiment, enzyme activity determination and other downstream protein research experiments.

The proteins extracted by this kit are active proteins with natural protein conformation, which can be used for different downstream applications.

EDTA is not contained in this kit and is compatible with metal chelates and chromatography, among others.

Self-prepared Reagents and Instruments:

Centrifuge, oscillator, homogenizer/homogenizer, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves.

Product Features:

1. Easy to use.
2. Contains protein stabilizer, the extracted protein is stable.
3. The background interference is low when the protein concentration is detected by UV.
4. Protease inhibitors inhibited protein degradation, and the formulation of protease inhibitors was optimized. The protease inhibitor mixture consists of 6 separate protease inhibitors, each of which specifically inhibits one or several protease activities. The optimized composition of this mixture allows it to inhibit almost all important protease activities, including serine protease, cysteine protease, aspartate protease, alanyl-aminopeptidase, etc.

Protocols:

First, use precautions

1. Before the formal experiment, please select several samples to do pre-experiment, in order to optimize the experimental conditions and achieve the best experimental results.

2. Centrifuge the reagent in the screw cap microreagent tube briefly before opening the cap, and centrifuge the liquid on the cap and inside wall to the bottom of the tube to avoid reagent loss when opening the cap.
3. All reagents in the process of the experiment must be pre-cooled; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
4. If the solution of protease inhibitor is precipitated during storage, it will not affect the use, and it should be used normally after dissolution.
5. If the kit cannot be used up in a short time, the protease inhibitor mixture should not be added to the extraction solution all at once.
6. Other protease inhibitor products can be added as needed for your own experiment.
7. In the downstream experiment, if the enzyme activity of specific protease or phosphatase is detected, the extract can be without protease or phosphatase inhibitors. Pay attention to the low temperature operation during the extraction process to shorten the centrifugation time.
8. It is prohibited to mix with other brands of reagents, otherwise the effect will be affected.
9. Contamination of samples or reagents with bacteria or fungi or cross-contamination of reagents may lead to false results.

Second, filamentous fungi nuclear protein extraction

1. Extraction solution preparation:
   Add 2μL protease inhibitor mixture into every 200μL extract solution B, mix well and put on ice for use.
2. Take 100-200mg filamentous fungus sample and wash it with PBS.
3. Add 500μL-1mL of extract solution A and fully homogenize with a tissue homogenizer/homogenizer.
4. Oscillate the homogenate at 2-8°C for 30min.
5. Centrifuge the extract at 1000×g at 4°C for 5min, discard the supernatant and leave the precipitation.
6. Add 200μL of cold extract B to the precipitation and swirl at high speed for 5s.
7. Oscillate at 2-8°C for 40min to 1h.
8. Centrifuge for 10min at 4℃, 12000-14000×g.
9. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the nuclear protein.
10. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

Analysis of Common Problems:

1. Low protein concentration?

Some tissue samples may not be fully cleaved when processed, resulting in low protein concentrations. Just extend the processing time of reagents A and B appropriately. It is best to handle under the condition of continuous oscillation, and it can be mixed with a suction head at intervals of several minutes without an oscillator.

2. How to quantify protein?

The BCA method is recommended. The Bradford method is not suitable because reagent A contains components that interfere with the Bradford method, resulting in inaccurate quantification. If dialysis has been performed or the buffer system has been replaced with a desalting column, the Bradford method can be used for quantification.

3. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the protein structure, does not destroy the original interaction between proteins, The proteins maintain their natural conformation and activity.

Note:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware must be washed and thoroughly removed of residual cleaners before use.
3. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
4. Avoid skin or mucous membranes coming into contact with the reagent.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.