Filamentous Fungi Cytoplasmic Protein Extraction Kit

Cat: EX1810
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Fungal Cytoplasmic Protein Extract A	25mL	50mL	2-8℃
Fungal Cytoplasmic Protein Extract B	10mL	20mL	2-8℃
Protease Inhibitor Mixture C	100μL	200μL	-20℃

Note:

1. Protease inhibitors can also be stored at 2-8°C before use without open lid. Store at -20°C after opening the lid for use.
2. The protease inhibitor is solid at 2-8°C. Take it out of the refrigerator and return to room temperature or 37°C 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 cytoplasmic protein extraction kit provides a full set of reagents, suitable for extracting cytoplasmic protein from various filamentous fungi samples. The extraction process is simple and convenient. The prepared cytoplasmic protein not only has high purity and natural activity, but also has little cross contamination.

The kit contains a protease inhibitor mixture, which prevents the protease from degrading the protein and provides a guarantee for the extraction of high purity protein.

The proteins extracted from this kit can be used for downstream protein research experiments such as Western Blotting, protein electrophoresis, immunoprecipitation, ELISA, transcriptional activity analysis, Gel shift gel blocking assay, and enzyme activity determination.

The proteins extracted by this kit are active proteins with natural protein conformation.

EDTA is not present in this kit and is compatible with downstream applications such as metal chelation and chromatography.

The protein samples extracted by this kit contain high concentration of salt components and cannot be directly used for 2D electrophoresis. The obtained protein samples need to be desalted by desalting column before being used for 2D electrophoresis.

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, the extracted protein is stable.
2. Containing 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. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents in the process of the experiment must be pre-cooled; All appliances must be pre-cooled in the -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
3. If the solution of protease inhibitor precipitates during storage, it will not affect the use, and it will be used normally after dissolution.
4. If the kit can not be used up in a short time, the protease inhibitor mixture can not be added to the extraction solution at one time.
5. You can add other protease inhibitor products according to your own experimental needs.
6. The experiment such as the downstream fruit is to detect the enzyme activity of specific proteases, the extract can be without protease inhibitors, pay attention to the extraction process. Keep the operation at low temperature to shorten the centrifugation time.

Second, extraction of cytoplasmic protein from filamentous fungi

1. Extraction liquid preparation:
   Every 500μL extract A, add 2μL protease inhibitor mixture, mix well and put on ice for use.

[Note]:

(1) Prepare the protein extract solution according to the number of samples needed to be processed. The protease inhibitor mixture can not be added to the extract at one time.

(2) If the extract with protease inhibitor is not used completely within a week, it is necessary to add protease inhibitor again before using again.

(3) The protein extract used in the following steps is an extract containing the protease inhibitor prepared for this step.

2. Take a 100-300mg fungal tissue sample and wash it once with PBS. Centrifuge to collect thallus precipitates.
3. Add 500μL of extract A to the cell precipitate and fully homogenize with a homogenizer/homogenizer.

[Note]:

(1) If possible, put the bacteria in the mortar with liquid nitrogen to fully grind, after grinding add 500ul of cold extract A.

(2) If there is no liquid nitrogen grinding condition, 500ul extract A can be added directly to the bacteria and ground directly on the ice.

4. Centrifuge the homogenate at 500×g for 5min, discard the precipitation and collect the supernatant.
5. Add 100μL of extract B to the supernatant and mix well.
6. Oscillate the extract at 2-8℃ for 30-40min.

[Note]:

(1) Using the low speed of the oscillator/shaker, the extraction liquid can be slightly shaken.

(2) No vibration conditions can also not oscillate, slightly extend the extraction processing time, in the middle of every few minutes with pipette blow evenly.

7. Centrifuge the extraction solution at 4℃, 12000×g, for 10min, and discard the precipitation.
8. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain cytoplasmic protein.
9. The protein extract was quantified and divided into -80℃ refrigerator for reserve or directly used in downstream experiment.

[Note]:

(1) It is recommended to use BCA method for protein quantification.

(2) The protein sample is stored at -80℃ for one year without problems. Be careful not to be hydrolyzed away by protease and not to be contaminated by bacteria.

Analysis of Common Problems:

1. Low protein concentration?

Some samples may not be fully lysed when processed, resulting in low protein concentration. As long as the processing time of reagents A and B is extended appropriately. It is best to handle under the condition of continuous oscillation, and no oscillator can also be blown and mixed with a suction head at intervals of several minutes.

2. What is the method of quantifying the protein?

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 disrupt the original interaction between the proteins, and the proteins maintain their natural conformation and activity.

Note:

1. Before the formal experiment, please select several samples for pre-experiment to optimize the experimental conditions and achieve the best experimental results.
2. The reagent in the screw cap trace reagent tube should be centrifuged briefly before opening the cap, and the liquid on the cap and inner wall should be centrifuged to the bottom of the tube to avoid the loss of the reagent when opening the cap.
3. It is prohibited to mix with other brands of reagents, otherwise it will affect the use effect.
4. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to wrong results.
5. It is best to use disposable suction heads, tubes, bottles or glassware, reusable glassware must be cleaned before use. And thoroughly remove residual cleaners.
6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.