Yeast nuclear protein extraction kit

Item No. : EX2220

Specification: 50T/100T

Validity: 2-8℃ storage, valid for one year.

Product content:

Name	50T	100T	Storage conditions
Yeast nucleoprotein extract A	20mL	40mL	Store at 2-8°C
Yeast nucleoprotein extract B	25mL	50mL	Store at 2-8°C
Yeast nuclear protein extract C	20mL	40mL	Store at 2-8°C
Yeast nuclear protein extract D	15mL	30mL	Store at 2-8°C
Protease inhibitor mixture	100μL	200μL	Store at -20°C

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℃. 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!

Product Introduction:

Yeast nuclear protein extraction kit provides a complete set of reagents, suitable for extracting nuclear protein from various yeast. The extraction process is simple and convenient. The prepared nuclear protein has high purity, natural activity and little cross contamination.

The kit contains a protease inhibitor mixture, which prevents protease from degrading the protein and provides a guarantee for extracting 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.

Bring your own reagents and instruments:

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

Product Features:

1、Easy to use, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.

2、The time of protein extraction is reduced to 30 minutes to 1 hour.

3、Containing protein stabilizer, the extracted protein is stable.

4、The background interference is low when the protein concentration is detected by UV.

5、The protein extract contains a variety of effective components, which can fully release cytoplasmic protein and nuclear protein, and can bind the released protein to prevent precipitation.

6、Protease inhibitor inhibits the degradation of protein, and the formula of protease inhibitor is optimized. The protease inhibitor mixture consists of 6 independent protease inhibitors AEBSF, Aprotinin, Leupeptin, Pepstatin A, Bestatin, E-64, each of which can specifically inhibit 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.

How to use:

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, protease inhibitor at 2-8℃ is a solid state, from the refrigerator to return to room temperature or 37℃ for a short time water bath, into a liquid state, centrifuge to the bottom of the tube and then open the lid.

Second, operation steps

1. Preparation of extraction liquid:

Add 2μL protease inhibitor mixture into every 200μL protein extract D, mix well and put on ice for later use.

2. The yeast culture was centrifuged at 1000×g at 4℃ for 5-10min. The medium was carefully absorbed and dried as much as possible to collect yeast precipitates.
3 Wash the yeast twice with PBS, sucking up as much supernatant as possible after each wash.
4. Add 200μL yeast nuclear protein extract A to every 100μL volume of yeast sediment, mix well, and keep warm at 30℃ for 15 minutes.
5. Centrifuge at 1000×g for 5-10 minutes, discard the supernatant, and collect the yeast precipitate.
6. Wash the yeast once with 500μL PBS and centrifuge to collect the bacteria.
7. Add 300-500μL yeast protein extract B to the yeast sediment and mix thoroughly.
8. Gently oscillate at 37℃ or room temperature for 45-60 minutes.
9. Centrifuge at 1000×g for 5-10 minutes, collect precipitation and discard supernatant.

10. Wash the precipitate with 500μL PBS. Centrifuge to collect the precipitate.

11. Add 400μL yeast core extract C to the precipitate, swirl at high speed for 15 seconds to mix, and then oscillate on an oscillator for 15-30 minutes.

12. Vortex again at high speed for 5 seconds, then centrifuge at 4℃, 2000×g for 5 minutes, discard the supernatant and leave the precipitation.

13. Add 200μL of cold nuclear protein extraction solution D to the precipitation and mix thoroughly.

14. Oscillate on the oscillator for 30-40 minutes.

15. Centrifuge at 4℃, 12000×g, for 10 minutes.

16. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain yeast nuclear protein.

17. 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?

Processing part of the yeast sample may not have fully cracked, resulting in low protein concentrations. Just extend the treatment time of reagent BC appropriately. It is best to deal with it under the condition of continuous oscillation, and it can be mixed with a suction head at intervals of several minutes without an oscillator.

Yeast nucleoprotein abundance is low, in the case of conditions, increase the yeast loading amount.

2. What method is used to quantify 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.

4. Is there a gelatinous precipitate during extraction?

A small amount of transparent glue sometimes appears in the treatment products of protein extract, which is a normal phenomenon. The transparent glue is a complex containing genomic DNA, etc. Without detecting specific proteins that bind particularly closely to genomic DNA, the supernatant can be directly centrifuged for subsequent experiments. If it is necessary to detect the protein closely bound to the genome, it can be treated by ultrasound, 300w/ 10sec interval of 10 seconds, ultrasound for 3 minutes, and then centrifuge the supernatant for follow-up experiment.

Points to 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.