Yeast Histone Extraction Kit

Cat: EX1550
Size: 50T/100T
Storage: 2-8℃, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Protein Extract Solution A	25mL	50mL	2-8℃
Component B: Protein Extract B	6mL	12mL	RT
Component C: Reagent C	5mL	10mL	RT
Component D: Protease Inhibitor Mixture	100μL	200μL	-20℃
Component E: Protein Extract E	25mL	50mL	2-8℃

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:

Yeast histone extraction kit is suitable for extracting histone from various insect tissue samples. The extraction process is simple and convenient, avoiding the destruction of yeast cells by grinding method, ultrasonic method or pressing method with poor repeatability, avoiding the destruction of the target protein caused by oxidation and heat increase caused by intense mechanical treatment.

The extracted proteins can be used for Western Blotting, protein electrophoresis, histone modification experiments such as acetylation, methylation, sumoylation and other downstream protein studies.

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, extract protein from cells and tissues without grinding, repeated freeze-thaw, ultrasonic crushing and other pre-treatment.
2. The extraction process is simple and convenient.
3. Containing protein stabilizer, the extracted protein is stable.
4. The background interference is low when the protein concentration is detected by UV.
5. 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 the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.

Second, yeast histone extraction

1. Preparation of extract solution: Add 2μL protease inhibitor mixture into every 500μL protein extract solution A, mix well and put on ice for later use.
2. Collect the yeast culture, centrifuge it at 1000×g at 4℃ for 5-10min, drain the supernatant, and collect the yeast cells.
3. Wash the yeast cells twice with cold PBS, sucking up as much supernatant as possible after each wash.
4. Add 250-500μL of cold yeast protein extract E to every 100-200mg of wet weight yeast cells (or 100-200μL of yeast precipitation volume) and mix thoroughly.
5. Oscillate at room temperature or 37℃ for 30 minutes to 2 hours.
6. Centrifuge at 4℃, 2000×g, for 5-10min, discard the supernatant and leave the precipitation.
7. Add 500μL protein extract A to the precipitation, mix well, and gently oscillate at 4℃ for 20-40min.
8. Centrifuge at 4℃, 16000×g for 15min, discard the supernatant and leave the precipitation.
9. Add 100μL histone extract B to the precipitation.
10. Use 200μL gun tip to blow and mix repeatedly or mix with full vortex oscillation.
11. Store in 4℃ refrigerator overnight.
12. Centrifuge at 12000×g for 10min to collect the supernatant.
13. Add 10-25μL reagent C to the supernatant and mix thoroughly.
14. Add the sample buffer and mix well, then boil, and store in -80℃ refrigerator for later use or directly for downstream experiment.

Analysis of Common Problems:

1. Low protein concentration?

Some samples may not be fully cleaved when processed, resulting in low protein concentrations. Just extend the processing time of reagents E and A 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. What method is used to quantify the protein?

The Bradford method is recommended. The BCA method is not recommended.

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.