Insect Histone Extraction Kit

Cat: EX1540
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	2-8℃
Component C: Reagent C	5mL	10mL	2-8℃
Component D: Protease Inhibitor Mixture	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:

Insect histone extraction kit is suitable for extracting histone from various insect tissue samples. The extraction process is simple and convenient.

The yield of histone extraction products is about 0.4mg per 10^7 cells or 100mg tissue, and the yield varies greatly among different cells and tissues.

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. The reagent in the screw cap microreagent 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 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°C 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, extraction of histone from insect cells

1. Extraction solution preparation:
   Add 2μL protease inhibitor mixture into every 500μL protein extract solution A, mix well and put on ice for later use.
2. Collect 5-10×10^6 cells and centrifuge at 4°C, 1000×g, for 5-10min, carefully absorbing the medium and draining it as dry as possible.
3. Wash the cells twice with cold PBS, sucking up as much supernatant as possible after each wash.
4. Add 500μL of cold protein extract A to every 5×10^6 cells, mix well, and gently shake at 4℃ for 20-30min.
5. Centrifuge at 4℃, 16000×g for 15min, discard the supernatant.
6. Add 100μL histone extract solution B to the precipitation.
7. Use 200μL gun head to blow and mix repeatedly or mix with full vortex oscillation.
8. Store in 4℃ refrigerator overnight.
9. Centrifuge at 16000×g for 10min and collect the supernatant.
10. Add 10-25μL of reagent C to the supernatant and mix thoroughly.
11. Add the sample buffer and mix well, then boil, and store in the refrigerator at -80℃ for further use or directly for downstream experiment.

Third, extraction of histone from insect tissue

1. Extraction solution preparation:
   Add 2μL protease inhibitor mixture into every 500μL protein extract solution A, mix well and put on ice for later use.
2. Take 100mg tissue sample and cut it into pieces, add protein extract solution A, and fully homogenize it with a tissue homogenizer/homogenizer until there are no significant large solids.
3. Inhale the tissue homogenate into a pre-cooled clean centrifuge tube and gently oscillate at 4°C for 20-30min.
4. Centrifuge at 4℃, 16000×g for 15min, discard the supernatant.
5. Add 100μL histone extract solution B to the precipitation.
6. Use 200μL gun tip to blow and mix repeatedly or mix with full vortex oscillation, and store in 4℃ refrigerator overnight.
7. Centrifuge at 16000×g for 10min and collect the supernatant.
8. Add 25μL of reagent C into the supernatant and mix thoroughly.
9. Add the sample buffer and mix well, bring to boil, and store in the refrigerator at -80℃ for later use or directly for downstream experiment.

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.