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Bone tissue Protein Extraction Kit

Cat: EXP0184
Specification: 50T/100T
Storage: Storage at 2-8℃, valid for 1 year.

Product composition:

Components	50T	100T
Reagent A：Bone Tissue Protein Extraction Solution A	25mL	50mL
Reagent B：Protein Stabilizer B	100μL	200μL
Reagent C：Protease Inhibitor Mixture C	100μL	200μL

Note before using:

1．Protease inhibitors can also be stored at 2-8℃ before use without lid, and at -20℃ after use with lid open.

2．Protease inhibitor at 2-8℃ low temperature is a solid state, removed from the refrigerator to return to room temperature or 37℃ for a short time water bath, into a liquid state, cover and tube wall trace liquid centrifuge to the bottom of the tube, then open the cover for use.

3．Please use the reagent as soon as possible after unpacking!

Product introduction:

Bone tissue Protein Extraction Kit is suitable for extracting total protein from a variety of animal dense bone, loose bone and cartilage tissue samples. The kit can be used for fresh bone tissue samples or decalcified bone tissue samples. The extraction process is simple and convenient and can be completed within 1 hour. The protein extraction solution in this kit can fully lyse osteoblasts, osteoclasts, and osteocytes. The protease inhibitor cocktail contained in it prevents protease degradation of proteins, ensuring the extraction of high-purity proteins.

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

The proteins extracted by this kit are active proteins with natural protein conformation. The downstream application range is wide. The lysis capability of the extraction solution is relatively mild, and the lysis time needs to be optimized according to the actual sample conditions.

Equipment supplies and reagent:

centrifuge, oscillator/mixer, homogenizer/pestle, ice box, pipette, centrifugal tube, suction head, PBS buffer, protein quantification kit, disposable gloves.

Product Features:

1．Convenient to use, reducing the protein extraction time to 30 minutes to 1 hour.

2．Contains protein stabilizers, ensuring the stability of the extracted proteins.

3．Low background interference when detecting protein concentration using UV.

4．Protease inhibitors prevent protein degradation, with an optimized protease inhibitor formula. The protease inhibitor cocktail comprises 7 independent protease inhibitors; each inhibitor can specifically inhibit the activity of one or several proteases. The optimized

composition of this mixture enables it to inhibit the activity of almost all important proteases, including serine proteases,
cysteine proteases, aspartic proteases, and aminopeptidases.

Procedure: (for reference only)

I. Precautions for Use:

1. For reagents in screw-cap microcentrifuge tubes, please briefly centrifuge the tubes before opening the caps to spin down any liquid from the cap and the inner walls of the tube to the bottom, avoiding reagent loss when opening the cap.

2. All reagents must be pre-cooled during the experiment, and the sample must be kept at a low temperature during the whole process.

3. If precipitates appear in the protease inhibitor solution during storage, this does not affect its use. The solution can be dissolved and used normally.

4. You can add other individual protease inhibitors according to your experimental needs.

II. Operating Procedures

1. Preparation of Extraction Solution: Based on the number of samples, add 2μl of protease inhibitor and 2μl of protein stabilizer to every 500μl of cold Bone Tissue Protein Extraction Solution A. Mix well and store at 2-8℃ for later use.

【Note】

● Prepare the protein extraction solution according to the number of samples to be processed. The protease inhibitor mixture should not be added to the extraction solution all at once.

● If the extraction solution to which protease inhibitors have been added is not used up within a week, protease inhibitors need to be added again before reuse.

● The protein extraction solution used in the following steps is the extraction solution prepared in this step, which contains protease inhibitors.

2. Take fresh bone tissue samples and soak them thoroughly in pH 7.4 PBS buffer or physiological saline at 4℃. Replace with fresh physiological saline/PBS and soak thoroughly again, then rinse with pure water to remove blood and red blood cells.

3. Cut the bone tissue into small pieces, weigh them, and place them into a mortar containing liquid nitrogen. Grind the bone tissue into a powder while ensuring that the liquid nitrogen does not completely evaporate.

【Note】

● If it is inconvenient to grind with liquid nitrogen, you can also add the extraction solution and then grind.

● When the tissue block is small and difficult to grind, you can wrap it with aluminum foil, place it in a small amount of liquid nitrogen, and strike it quickly. Be sure to control the force and try to avoid breaking the aluminum foil. Repeat this process several times.

● For softer small bone tissues, you can directly add extraction solution A and then homogenize using a small homogenizer/homogenizing machine.

4. Transfer the bone tissue powder into a centrifuge tube and add 500μl of protein extraction solution for every 200mg of bone tissue.

5. Mix thoroughly and incubate at 4℃ with shaking for 30 minutes.

【Note】

● Use a lower speed on the oscillator/shaker so that the extraction solution can just barely move.

● If you don't have an oscillator, you can skip it. Just extend the processing time of the extraction solution a little bit. Every few minutes, use a pipette to blow and mix it.

6. Subject the mixture to 20 cycles of sonication at 80-100W, with each cycle consisting of 5 seconds of sonication followed by a 5-second interval, all performed in an ice bath.

7. Centrifuge at 12,000×g for 10 minutes at 4℃.

8. Transfer the supernatant to another pre-cooled, clean centrifuge tube. This supernatant contains the total protein extracted from the bone tissue.

9. The extracted protein can be used directly for downstream experiments or aliquoted and stored at -80℃ for future use.

【Note】

● It is recommended to use the BCA method for protein quantification.

● Protein samples can be stored at -80℃ for up to one year without any issues. Be sure to avoid protease digestion and bacterial contamination.

Analysis of Common Issues

1. Slow extraction speed?

To fully ensure the activity of the extracted proteins, the extraction solution uses a unique formula to protect proteins, with a mild lysis capability, which ensures a wide range of downstream applications. Simply extend the lysis time appropriately.

2. Low protein concentration?

Incomplete lysis of some tissue samples may result in low protein concentration. Just extend the treatment time with Reagent A appropriately. It's best to process under continuous shaking conditions. If a shaker is not available, mix by pipetting every few minutes with a pipette tip.

3. What method should be used for protein quantification?

It is recommended to use the BCA method. The Bradford method is not suitable because Reagent A contains components that interfere with the Bradford method, leading to inaccurate quantification. If dialysis has been performed or the buffer system has been changed using a desalting column, the Bradford method can be used for quantification.

4. Is the extracted protein active?

This kit does not contain ionic detergent components, which do not disrupt the protein structure or the original interactions between proteins. The proteins maintain their native conformation and activity.

Notes:

1. Before conducting the formal experiment, please select a few samples for a preliminary experiment to optimize the experimental conditions and achieve the best results.

2. For reagents packaged in screw-cap microcentrifuge tubes, please briefly centrifuge before opening the cap to spin down any liquid from the cap and the inner walls of the tube to the bottom, avoiding reagent loss when opening the cap.

3. Do not mix with reagents from other brands, as this may affect the effectiveness of use.

4. Contamination of samples or reagents by bacteria or fungi, or cross-contamination of reagents, may lead to erroneous results.

5. It is best to use disposable pipette tips, tubes, bottles, or glassware. Reusable glassware must be cleaned and thoroughly rinsed to remove any residual cleaning agents before use.

6. After the experiment is completed, all samples and the vessels that have come into contact with them should be disposed of according to the prescribed procedures.