

Whole Blood Protein Extraction Kit

Cat: EX1200

Size: 50T/100T

Storage: 2-8°C, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Reagent A: Protein Extract Solution A	20mL	40mL	2-8°C
Reagent B: Protease Inhibitor Mixture B	100μL	200μL	-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°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:

Whole Blood protein extraction kit is suitable for extracting total protein from various animal anticoagulant/non-anticoagulant whole blood samples.

This kit contains a unique formula that effectively dissolves cell membrane components, including the plasma membrane, the nuclear membrane, and various organelle membranes. The kit contains a protease inhibitor mixture that prevents the protease from degrading the protein and ensures the extraction of high purity proteins.

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

The protein extracted by this kit is the active protein 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 from this kit contain a high concentration of salt components and cannot be used directly for 2D electrophoresis. The final sample can also be demineralized with a column and then used for 2D electrophoresis.

Self-prepared 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, the protein extraction time is reduced to 30min.
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. 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°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, whole blood protein extraction

1. Extraction solution preparation:

Add 2 μ L protease inhibitor mixture into every 500 μ L cold total protein extract, mix well and put on ice for later use.

[Note]:

- (1) Prepare the protein extract according to the number of samples to be processed, the protease inhibitor mixture cannot be added to the extract all at once.
- (2) If the extract with a protease inhibitor is not used up within a week, the protease inhibitor should be added again before being used again.
- (3) When protein samples are used to determine some intracellular protease, phosphatase activity and other downstream experiments, pay attention to adjusting whether the inhibitor mixture is added according to the actual situation.
- (4) The protein extract used in the following steps is an extract prepared for this step that contains protease inhibitors.

2. Take a 300 μ L whole blood sample, add 300 μ L protein extract, mix well, oscillate at 4°C for 20-40min.

[Note]:

- (1) Use the lower speed of the oscillator/shaker, so that the extract can shake slightly.
 - (2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with the pipette blow mixing can be.
3. Centrifuge at 4°C, 14000 \times g, for 10min.
 4. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the whole blood protein.

[Note]:

- (1) Generally, the precipitation is just a thin layer at the bottom of the centrifugal tube. There are no obvious clumps.
 - (2) The whole red liquid is the whole blood protein supernatant.
5. The protein extract was quantified and divided into -80°C refrigerator for reserve or directly used in downstream experiments.

[Note]:

- (1) BCA method is recommended for protein quantification.
- (2) The protein sample is stored at -80°C for one year without problem. Be careful not to be hydrolyzed off by protease and not to be contaminated by bacteria.
- (3) Whole blood samples have higher protein concentrations and need to be diluted for downstream experiments.

Analysis of Common Problems:

1. What methods are used to quantify 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.

2. 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. 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.

