# Blood Monocyte Protein Extraction Kit

**Cat:** EX1790
**Size:** 50T/100T
**Storage:** 2-8℃, valid for 1 year.

**Kit Components:**

| Kit Components | 50T | 100T | Storage |
|---|---|---|---|
| Component A: Cell Extract Solution A | 100mL | 200mL | 2-8℃ |
| Component B: Cell Extract Solution B | 20mL | 40mL | 2-8℃ |
| Component C: Protein Extract C | 20mL | 40mL | 2-8℃ |
| Component D: Protease Inhibitor Mixture | 100μL | 200μL | -20℃ |

**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:**

Blood monocyte protein extraction kit is suitable for extracting total protein from monocytes in all kinds of whole blood samples. The extraction process is simple and convenient, and can be completed within 1h. The kit contains a mixture of protease inhibitors, which prevents the protease from degrading the protein, providing a guarantee for the extraction of high purity protein.

The protein 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, enzyme activity determination, etc.

The proteins extracted by this kit are active proteins with natural protein conformation, which can be used for different downstream applications.

EDTA is not contained in this kit and is compatible with metal chelates and chromatography, among others.

**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, shorten the time of protein extraction to 30min to 1h.
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. 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℃ 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.
10. Follow the steps below for fresh whole blood samples, starting with step 10 for extracted monocyte samples.

**Second, monocyte protein extraction**

1. Extraction solution preparation:

According to the number of samples to be extracted, add 2μL protease inhibitor mixture into every 400μL cold protein extract solution C, mix well and put on ice for use.

2. Take 1.5mL of fresh anticoagulant blood and let it stand to absorb the upper plasma.
3. Add 1mL of cell extract solution A, gently blow and mix well, and leave on ice for 5min. Shake occasionally and gently during the process to mix well.
4. Centrifuge at 4℃, 400-500×g for 5min, discard the supernatant, and collect the bottom cell precipitation.
5. Add 1mL of cell extract solution A to the cell precipitate, gently blow and mix well.
6. Leave on ice for 5min, shaking occasionally and mixing well.
7. Centrifuge at 4℃, 400-500×g for 5min, discard the supernatant, and collect the bottom cell precipitation.

8. Add 400μL cell extract solution B to the cell precipitate, gently blow and mix well.
9. Centrifuge at 4℃, 1000×g, for 5min, discard the supernatant, and collect the bottom cell precipitates.
10. Add 100-200μL of cold protein extract solution C to the cell precipitation and mix thoroughly.
11. Oscillate at 4°C for 20-30min.
12. Centrifuge at 4℃, 12000×g, for 15min.
13. Inhale the supernatant into another pre-cooled clean centrifuge tube to obtain the total monocyte protein.
14. 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?

Some samples may not be fully cleaved when processed, resulting in low protein concentrations. Just extend the processing time of reagent 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. Slow cell lysis rate?

In order to fully ensure the activity of the extracted protein, the extract adopts a unique formula of protective protein, with mild cracking ability and wide range of downstream applications. The cracking time can be extended appropriately

3. What method is used to quantify protein?

The 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.

4. Is the extracted protein active?

This kit does not contain ionic detergent components, does not destroy the structure of the protein, does not destroy 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.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.