Collection and treatment of common samples for biochemical detection

(For reference only)

1. Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the upper light yellow clarified liquid layer which is the serum and preserve it on ice for detection. If it can’ t be tested on the day, it can be stored at -80℃ for a month.

2. Plasma

Collect fresh anticoagulant blood (Heparin is used as anticoagulant and concentration of heparin is 10-12.5 IU/mL blood), centrifuge at 700-1000 g for 10 min at 4℃ . Take the upper light yellow clarified liquid layer which is the plasma (don't take white blood cells and platelets in the middle layer) and preserve it on ice for detection. If it can’t be tested on the day, it can be stored at -80℃ for a month.

3. Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant and preserve it on ice for detection. If it can’t be tested on the day, it can be stored at -80℃ for a month.

4. 10% Tissue homogenate

Take 0.02-1g fresh tissue and wash with 2-8℃ PBS (0.01 M, pH 7.4) to remove blood cells, absorb the water with filter paper, then weigh and homogenized in 2-8℃ homogenized medium on ice, the volume of homogenized medium (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged for 10 min at 1500 g at 4℃ . Take the supernatant and preserve it on ice for detection. If it can’t be tested on the day, it can be stored at -80℃ for a month.

5. Cells

Suspension cells: centrifuge at 1000-2000 g for 10 min at 4℃ to collect cells and resuspend the cells with 2-5 mL 2-8℃ PBS (0.01 M, pH 7.4), centrifuge at 1000-2000 g for 10 min at 4℃ and discard supernatant. Add homogenized medium into the sediment according to the ratio of cells number (10^6): homogenized medium (μL) =1: 300-500. Mechanically homogenize the cells to break the cells fully (There is no obvious sediment, which can be observed with microscope). Then centrifuge at 1500 g for 10 min at 4℃ . Take the supernatant and preserve it on ice for detection. If it can’t be tested on the day, it can be stored at -80℃ for a month.

Adherent cells: discard the culture medium and wash the cells with PBS (0.01 M, pH 7.4) for once. Collect the cells with a cell scraper (it can't be treated with trypsin or EDTA) and then add 2-5 mL PBS and collect the cell suspension. Then see the operation steps of suspension cells.

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Note:

1. Homogenized medium

20 mM Hepes-KOH(pH 7.2), contain 1 mM EGTA, 210 mM mannitol, 70 mM sucrose.

2. Homogenized method

(1) Hand-operated: weigh the tissue and mince to small pieces (1 cm^3), then put the tissues to glass homogenized tube, add homogenized medium, place the tube in the ice bath with left hand, insert the glass tamping rod vertically into the homogenized tube with right hand and grind up and down for 6-8 min. Or put the tissue into the mortar, add liquid nitrogen and grind fully, then add the homogenized medium after grinding, and absorb the prepared homogenate into the EP tube for detection.

(2) Mechanical homogenate: weigh the tissue and put into EP tube, add the homogenized medium and homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. For skin, muscle and plant tissue, it can be properly prolonged the time of homogenization.

(3) Ultrasonication: the ultrasonic generator was used to treat the cells for 30 s with the amplitude of 14 μm inthe ice bath. Or treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

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