ELISA Sample Preparation Protocol

Elisa is a qualitative and quantitative analysis method with high sensitivity and accuracy. Sample preparation plays an central role in successfully performing Elisa test and should gently collect target proteins due to denaturation and degradation. Prepared samples can be sub-packed and sealed. It's very important to avoid repeated free-thaw cycles during the storage. Suggested storage conditions: 2~8℃ for 5 days; -20℃ for 6 months; -80℃ for 2 years. Otherwise, samples should be cryopreserved in the liquid nitrogen for a longer time. Cryopreserved samples are rapidly thawed in a water bath at 15-25℃ to decrease the damage of ice crystal (0℃) to samples. Thawed samples can be used for a detection or temporarily stored at 2~8℃.

Sample types for Elisa test include: blood(serum, plasma); tissue homogenates; cell lysate; cell culture supernatant; urine; stool; bronchoalveolar lavage fluid (BALF); saliva; cerebrospinal fluid; pleural effusion; prostatic fluid; semen; vaginal discharge etc. Common sample preparation protocols are specified below for reference.

A. Blood Sample

1. Serum

1) Let the whole blood sample collected from serum separator tube stand for 2 hours at room temperature or overnight at 2-8℃. (This is a natural blood coagulation procedure. The coagulation process is slower with the drop of temperature. Blood clotting accelerant can be added according to assay requirements.);

2) Centrifuge the sample for 20 minutes at 1000×g and collect the supernatant;

3) Perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃.

2. Plasma (Citric Acid, EDTA, Heparin)

1) Plasma sample requires for anticoagulation. Collect the whole blood into tube containing anticoagulant and mix gently;

2) Centrifuge the sample for 15 minutes at 1000×g at 2-8℃ within 30 min of collection;

3) Perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃.

Anticoagulant Types and Selection

Select different anticoagulants suitable for the property of target samples to be tested;

Citrate(sodium citrate): Ca2+ has the coagulant effect. Citrate can form soluble chelate with Ca2+ in the blood to prevent blood coagulation. The general anticoagulant working concentration of citrate is 0.25%. (The proportion between anticoagulant and blood is 1:16 when using 4% anticoagulant.)

Advantages: Protect coagulation factor well. Most coagulation tests can use the citrate for anticoagulation.

Disadvantages: Low solubility in the blood, weak anticoagulation.

Ethylenediamine Tetraacetic Acid (EDTA): Form coordination compounds by binding with Ca2+ in the blood to prevent blood coagulation. The anticoagulant working concentration of EDTA: 10ml blood contains 12mg EDTA.

Advantages: Small influence on erythrocyte and leukocyte morphology;

Disadvantages: Interference with platelet aggregation. Unsuitable for coagulation tests and platelet functional detection.

Heparin: Enhance effects of antithrombin by binding with antithrombin III(AT-III). Inactivate serine protease to prevent the formation of thrombin, platelet aggregation and blood coagulation. Pure 10mg heparin the anticoagulant effect in 65~125 ml blood.

Advantages: Strong anticoagulation, no effects on blood cell volume, difficult hemolysis, thermotolerant

Disadvantages: Cause leucocyte aggregation. Heparin anticoagulant should be used in a short time. Otherwise, blood will be coagulated again after a longer time.

Selection and Differences between Serum and Plasma

Serum is the plasma without fibrinogen after the blood is coagulated. Thus, except fibrinogen and anticoagulant, other components of plasma are equivalent to serum.

Plasma sample is recommended to detect coagulation factors due to lack of many coagulation factors in the serum containing coagulation products.

Serum sample is recommended if fibrinogen in the plasma has an effect on the target to be tested.

In most situations, both serum and plasma can be selected.

B. Tissue Samples

Tissue samples are processed into tissue homogenates. Detailed steps are listed below:

1) Use a clean tool to dissect the target tissue and quickly put it on the ice to prevent protease from degradation. (Put the temporarily unused tissue into circular microcentrifuge tube and rapidly immerse the tube into liquid nitrogen for cryopreservation. Store the sample at -80℃ for next time usage.);

2) Wash tissues with pre-cooling PBS buffer(0.01M, pH=7.4) to remove residual blood. Then weigh for usage. Cut larger tissues into fragments first;

3) Use the grinding buffer on the ice. PBS buffer is commonly used. It's suggested to integrally use 50mM Tris+0.9%NaCL+0.1%SDS,pH7.3 or RIPA lysis buffer(medium). Notes: RIPA lysis buffer requires for pH7.3. Components containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentration are not recommended due to the interfering with antigen-antibody reaction. Grinding tissue homogenate: The volume of added grinding buffer depends on the tissue weight. Usually, 9ml grinding buffer is appropriate for 1g tissue fragment. Add some protease inhibitors (e.g. 1mM PMSF) into PBS buffer. The processed homogenate can be subject to ultrasonic disruption or free-thaw cycles. (Ice bath cooling is required for ultrasonic disruption. Free-thaw cycles can be repeated twice.);

4) Centrifuge the processed homogenate for 5 minutes at 5000×g. Then collect the supernatant to perform the assay or sub-pack for cryopreservation at -20℃ or -80℃;

5) Tissue homogenate samples can quantify the total protein to get statistical analysis data according to requirements of the assay. Usually, the concentration of total protein is within 1-3mg/ml. Some tissue samples(e.g. liver, kidney, pancreas) containing endogenous peroxygenase with higher concentration may react with TMB substrate, and then false positive appears.

Notes:

For skin tissues, remove fat layer and bottom sediment after centrifugation. Take the sample of middle layer for the detection.

C. Cell Sample

1. Cell Culture Supernatant

1) Adherent or suspension cell density is required to reach 60-90% using 96/24/6 well plate;

2) Adherent or suspension cell density is required to reach 60-90% using 6-10mm petrie dish;

3) Suspension cell: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant;

4) Adherent cell: Collect supernatant directly; centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant;

5) Perform the assay immediately or sub-pack for cryopreservation at -80℃.

2. Cell Lysate

(1) Suspension Cell

1.1. Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell;

1.2. Add pre-cooling PBS into collected cell and gently mix; Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell;

1.3. Add 0.5-1ml RIPA lysis buffer (medium). RIPA lysis buffer requires for pH7.3. Components containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentration are not recommended due to the interfering with antigen-antibody reaction. Alternatively, use 50mM Tris+0.9% NaCL+0.1% SDS, pH7.3. Add some protease inhibitors(e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on the ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. If sticky DNA appears, ultrasound can disrupt DNA. (Another ultrasonic condition for processing sample on the ice: 3-5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working);

1.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into an EP tube.

1.5. In order to facilitate statistical analysis of data, it is necessary to use BCA kit to quantify total protein first, and generally adjust the total protein concentration to 1-3mg/ml. Use immediately for testing, or subpack at -80℃ frozen reserve.

(2) Adherent Cell

1.1. Absorb supernatant and add pre-cooling PBS to wash three times;

1.2. Add 0.5-1ml RIPA lysis buffer and some protease inhibitors(read requirements in suspension cell). Scrape adherent cell gently with a cell scraper;

1.3. Add the cell suspension into centrifugal tube. Lyse the cell on the ice for 30min-1h. Or disrupt the cell by ultrasound(read requirements in suspension cell);

1.4. At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm for 10 minutes. Then, the supernatant is added into EP tube.

1.5. In order to facilitate statistical analysis of data, it is necessary to use BCA kit to quantify total protein first, and generally adjust the total protein concentration to 1-3mg/ml. Use immediately for testing, or subpack at -80℃ frozen reserve.

Notes:

It's recommended to use ultrasound to disrupt during cell lysate preparation. Ultrasound can break the DNA efficiently. DNA fragments won't greatly interfere with the performance of the Elisa kit.

Selection of Cell Culture Supernatant and Cell Lysate

Select cell lysate or cell culture supernatant as the sample according to the position of target object;

If the target object is secreted (e.g. secreted membrane protein), cell culture supernatant can be used as the sample;

It's recommended to select cell lysate for intracellular target object. Some intracellular proteins may leak into the medium by secretion or apoptosis. However, the supernatant is still detectable.

D. Sputum Sample

1) Weigh the viscous part in the sputum. Add 0.1% DTT(Dithiothreitol, responsible for dissolving mucus) at twice the volume of sputum. Repeat pipetting and then use a vortex mixer to oscillate for 15s. Oscillate with water bath at 37℃ for 5 min;

2) Add PBS buffer at twice the volume of the sputum. Continue to oscillate for 15-20min. Use 150ft wire mesh for filtration. Centrifuge at 1500 rpm for 10 minutes. Absorb the supernatant to perform the assay.

E. Saliva / Urine / Milk Sample

Use a sterile tube to collect the sample. Centrifuge for 2 minutes at 10,000 x g at 2-8℃. Or centrifuge at 2000-3000 rpm for 20 minutes; Collect the supernatant to perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃; Try to reduce free-thaw cycles.

F. Stool Sample

1) Collect the stool as dry as possible. It's difficult to process diluted stool and the detection accuracy decreases. The weight of collected stool should be above 50mg;

2) Wash the stool with PBS three times. Weigh the centrifuged stool after precipitation;

3) Add PBS buffer (The volume of added PBS buffer depends on the weight of processed stool. Usually, 9ml PBS buffer is appropriate for 1g stool. Pulverize it with ultrasound or smash it;

4) Centrifuge for 10 minutes at 5000×g. Collect the supernatant to perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃.

G. Semen Sample

1) Collect the semen into a sterile container. Place the semen sample in a water bath at room temperature or 37℃. With the help of fibrinolytic enzyme secreted by the prostate, the semen is liquefied and thin.

2) After the semen is completely liquefied, centrifuge it at 4000 rpm for 10 minutes; Perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃.

H. Cerebrospinal Fluid, Bronchoalveolar Lavage Fluid (BALF), Synovial Fluid, Ascites Sample

Collect the sample and centrifuge for 20 minutes at 1000×g. Then collect the supernatant. Perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃. Try to reduce free-thaw cycles.

I. Breast Milk Sample

Collect the sample and centrifuge at 12000 rpm at 2-8℃ for 30 minutes; Remove fat layer and bottom sediment after centrifugation. Take the sample of middle layer for the detection or sub-pack for cryopreservation at -20℃ or -80℃. Try to reduce free-thaw cycles.

J. Yolk Sample (Detect Bird IgY(Synonym: IgG)

1) The fresh yolk is diluted 10 times by the ultrapure water;

2) Use HCL to adjust pH around 5.0-5.2; Let stand 6h at 2-8℃; Centrifuge at 10000 rpm for 10 minutes; Collect the supernatant and adjust pH around 7.2-7.4; Perform the assay immediately or sub-pack for cryopreservation at -20℃ or -80℃.

K. Exosomes

1）First, use a commercial exosome extraction kit to extract exosome particles. If the exosome concentration obtained is relatively low, ultrafiltration tubes can be used to concentrate;

2）Add 0.1-0.5ml RIPA lysis buffer (medium). RIPA lysis buffer requires for pH7.3. Components containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentration are not recommended due to the interfering with antigen-antibody reaction. Alternatively, use 50mM Tris+0.9% NaCL+0.1% SDS, pH7.3. Add some protease inhibitors(e.g. PMSF, working concentration: 1mmol/L). Lyse the sample on the ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. (Another ultrasonic condition for processing sample on the ice: 3-5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working);

3）At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm for 10 minutes. Then, the supernatant is added into EP tube.

4） In order to facilitate statistical analysis of data, it is necessary to use BCA kit to quantify total protein first, and generally adjust the total protein concentration to 1-3mg/ml. Use immediately for testing, or subpack at -80℃ frozen reserve.