ELISA Experimental Tips: 3 Types of Sample Collection

Since ELISA kits can only detect soluble proteins, the samples must be clear, transparent, and centrifuged to remove precipitates or suspended material. To ensure the accuracy of the assay, samples stored at -20℃ or -80℃ should be tested within 1-6 months, and samples stored at 4℃ should be tested within 1 week. In addition, the samples should not contain NaN3 which will inhibit HRP activity, as this will cause false-negative results.

Samples that can be used for ELISA experiments generally include serum, plasma, urine, cell culture supernatants, and tissue homogenates. Pretreatment methods vary for different sample types. Proper sample pretreatment is the first step to ensure the correctness and accuracy of ELISA experiments. Here, we will introduce several processing methods for different sample types.

Liquid Samples

Principles of liquid sample handling: all liquid samples are collected in sterile tubes, and centrifuged for about 20 minutes (2000-3000 rpm) at 2-8°C.

1. Serum

Serum is the most commonly used sample for ELISA experiments and its pretreatment is very simple. Blood samples are collected using pyrogen-free and endotoxin-free tubes or centrifuge tubes, and the serum is separated by leaving the tubes or centrifuge tubes at room temperature for natural coagulation (ranging from 10 minutes to 2 hours depending on the room temperature environment) or overnight at 4°C. It is recommended to tilt the tube or centrifuge tube to enlarge the cross-section of the liquid surface so that the serum can be separated to a greater extent. Centrifuge the serum for about 5 minutes (5000-6000 rpm) at 2-8°C, collect the supernatant carefully, and perform the assay immediately. It is recommended to store the collected serum in separate packages at -20°C or -80°C, avoiding repeated freezing and thawing, and centrifuge the serum again if precipitation occurs during the storage process.

Precautions: Haemolysis should be avoided during the collection of blood samples, as red blood cells release substances with peroxidase activity when lysed, in which case a non-specific color reaction will occur in the ELISA assay, leading to inaccurate results and false-positive results. Additionally, samples should be protected from

bacterial contamination, which may contain endogenous HRP, also leading to inaccurate results.

2. Plasma

EDTA, heparin sodium, or sodium citrate, should be selected as anticoagulants according to the requirements of the specimen, blood samples should be collected using blood collection tubes or centrifuge tubes containing anticoagulants, within 30 minutes of collection, after mixing for 10-20 minutes, centrifugation for about 5 minutes (5000-6000 rpm) at 2-8°C, and the supernatant (plasma) should be collected carefully, and it is recommended to -20°C or -80°C for the collected serum is stored in separate packages to avoid repeated freezing and thawing. Samples should be protected from hemolysis or hyperlipidemia. If precipitate forms during storage, it should be centrifuged again before use.

Precautions: Please read the ELISA kit instructions carefully before testing to check whether the kit has special requirements for anticoagulants.

3. Urine

Collect in a sterile tube and centrifuge for about 5 minutes (5000-6000 rpm) at 2-8°C. Collect the supernatant carefully and centrifuge again if any precipitate forms during storage.

4. Cell culture supernatant

For detection of secretory components, collect in sterile tubes. The cell culture supernatant is drawn into a centrifuge tube and centrifuged at 2-8°C for about 5 minutes (5000-6000 rpm) to remove cellular debris and impurities, and the supernatant is collected and set aside; samples are stored at -20°C or -80°C to avoid repeated freezing and thawing, and should be centrifuged again prior to use if a precipitate forms during storage.

5. Cerebrospinal fluid (CSF)

Collect in a sterile tube and centrifuge for about 5 minutes (5000-6000 rpm) at 2-8°C. Collect the supernatant carefully and centrifuge again before use if any precipitate forms during storage.

Methods of collecting cerebrospinal fluid from rats: To collect cerebrospinal fluid, the skin on the back of the rat's neck was wiped with wet gauze, and the dorsal hairs were clipped to expose the skin. A transverse incision (about 1.5cm) was made at the line connecting the two ears, and a 2cm subcutaneous cut was made at its midpoint towards the caudal side, separating the skin on both sides and expanding the field of

view. The skull of the rat was cut to expand the field of view, and the severed end was pulled to the caudal side in order. Note that, if there is bleeding, use dry gauze to press to stop the bleeding and keep the surgical opening clear. When approaching the posterior cervical ligamentum flavum, the overlying muscles were carefully separated with a 7-gauge syringe needle to expose the atlanto-occipital membrane. With a 1 ml syringe (needle with hemostatic forceps so that the tip of the needle makes an obtuse angle of 150 degrees with the body of the needle), the needle beveled upwards, the tip of the needle pierced into the subarachnoid space nearly horizontally fixed the needle, and the cerebrospinal fluid was slowly withdrawn, and the general volume of the collection was 100-200 microlitres.

6. Saliva

Collect in sterile tubes and centrifuge for about 5 minutes (5000-6000 rpm) at 2-8°C. Collect the supernatant carefully and centrifuge again before use if any precipitate forms during storage.

Solid Samples

Principles of solid sample handling: weigh 1g of solid sample and dissolve it with 9ml of suitable buffer. Secreted proteins can be tested by centrifuging the supernatant directly, while intracellular proteins should be collected from the cells first, then broken by suitable methods and centrifuged to obtain the supernatant for testing.

1. Specimen of Tissue

After cutting the specimen, weigh 1g of tissue, add 9ml of PBS of about pH 7.2-7.4, and homogenize the specimen well by hand or with a homogenizer. Centrifuge the specimen for about 5 minutes (5000-6000 rpm) at 2-8°C and collect the supernatant carefully. Dispense 1 portion for testing and freeze the rest for later use. If precipitate forms during storage, centrifuge again before use.

2. Intracellular Protein Sample

Many of the proteins to be tested are not secreted proteins, but proteins that are present inside the cells. In this case, the cells are collected, washed, then broken, and centrifuged for supernatants.

2-1. Cultured cells

Animal cells: Dilute the cell suspension with PH7.2-7.4 PBS to make the cell concentration reach about 1 million/ml. By repeated freezing and thawing (if

repeated freezing and thawing, the crushing effect is not good, then ultrasonic crushing is used), to make cell destruction and release intracellular components. Centrifugation for about 5 minutes (5000-6000 rpm) at 2-8℃, carefully collect the supernatant, and centrifugation should be done again before use if there is any precipitate formation during the preservation process.

Plant cells: Dilute the cell suspension with PBS of PH7.2-7.4 to reach a cell concentration of about 1 million/ml, place it on an ice box, and use an ultrasonic crusher with a setting of crushing for 2s and cooling for 30s to sufficiently crush the cells to disrupt and release intracellular components. centrifuge the cells for about 5 minutes (5000-6000 rpm) at 2-8°C, and carefully collect the supernatant. If a precipitate is formed during storage, centrifuge again before use.

2-2. Cells of tissues

After cutting the specimen, weigh 1g of tissue, add 9ml of PBS around pH 7.2-7.4, and homogenize the specimen well by hand or with a homogenizer. Centrifuge the specimen for about 5 minutes (5000-6000 rpm) at 2-8°C, remove the supernatant, and then carefully wash the precipitated cells 3 times with PBS around pH 7.2-7.4. The cells were then broken using the cell-breaking method described above.

Method of repeated freezing and thawing of cells

1) Aspirate the medium from the culture plate, digest the cells with trypsin, and then add the appropriate amount of medium to rinse the cells from the plate. This step can be omitted for suspension cells.

2) Collect the cell suspension into a centrifuge tube and centrifuge it at 1000×g for 10 minutes, then aspirate the medium and wash the cells with pre-cooled PBS 3 times.

3) Resuspend the cells by adding an appropriate amount of pre-cooled PBS (protease inhibitors are recommended to be added immediately before use). In a 6-well culture plate, each well requires 150-250 μL of PBS to resuspend the cells.

4) Allow the samples to freeze and thaw repeatedly at -20°C or -80°C conditions and at room temperature, repeating the freezing and thawing process several times until the cells are completely lysed. Cells can also be lysed by sonication of the suspension using an ultrasonic cell breaker.

5) Centrifuge at 10,000 x g for 10 minutes at 4°C to remove cell debris, collect the supernatant, and store at -20°C or -80°C to avoid repeated freeze-thawing.

3. Soil

Weigh 1g of soil, add 9ml of PBS of about pH 7.2-7.4, and mix the specimen well by hand. Centrifuge the sample for about 5 minutes (5000-6000 rpm) at 2-8°C and collect the supernatant carefully. Dispense one portion for testing and freeze the rest for later use. Any precipitate formation during storage should be centrifuged again. If you are measuring secreted proteins, take the supernatant directly for testing; to test intracellular proteins, break up the cells.

Plant Samples

1. Specimen Collection and Preservation

Take 0.1g (within ±3%) of fresh plant tissue sample and grind it thoroughly in liquid nitrogen; add 1ml of the extract (80% methanol) and leave it at -20℃ overnight; centrifuge it at 8000rpm at 4℃ for 1 hour and take the supernatant.

1) The supernatant was passed through a C-18 solid phase extraction column. The specific steps are: 80% methanol (1ml) equilibrium column → on the sample → collect the sample → remove the sample and wash the column with 100% methanol (5ml) → 100% ether (5ml) → 100% methanol (5ml) → cycle. After passing through the column, the sample is dried under vacuum or blown dry under nitrogen, and stored for spare use.

2) Add pH7.4 PBS buffer (1ml fixed volume) before sampling. After mixing, leave it at room temperature for 30 minutes, then centrifuge at 4℃ (8000rpm, 15 minutes), take the supernatant and store it temporarily at 4℃ for use.

2. Determination of Relevant Enzymes or Proteins in Plant Specimens

Fresh plant tissues should be ground thoroughly in liquid nitrogen; add 9 times the volume of the extract (pH 7.4 PBS buffer), centrifuge at 8000 rpm for 30 minutes at 4℃, take the supernatant, and store it temporarily at 4℃ for use.

We list the general sample treatment methods, which cannot cover all kinds of samples. For some special samples, it is recommended that experimenters refer to the published literature to design their own reasonable sample treatment methods.