Experiment Tips: Proper Handling of ELISA Assays

ELISA assay is now usually hand-operated with microtiter strips as the solid-phase assay mode, the assay operation is very simple and generally involves the collection and preservation of samples, reagent preparation, spiking, incubation, plate washing, color development, measure OD value, and the results interpretation. Inappropriate steps will affect the results of the assay, and in particular, the steps of the spiked samples, incubation, and washing the plate.

I. Samples Collection and Preservation

The most commonly used samples for ELISA are serum (plasma), and sometimes saliva, cerebrospinal fluid, urine, feces, and other samples are also used for specific testing purposes. Currently, serum samples are used for the determination of markers such as antigens and antibodies to infectious pathogens, tumor markers, hormones, special proteins, cytokines, and therapeutic drugs. For the collection of serum samples for hormone and therapeutic drug assays, it is important to note that the time of collection or body position may affect the results of the assay. For example, cortisone has a peak between 4 and 6 a.m.: growth hormone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are all released in a paroxysmal manner. Therefore necessary to take several blood samples at closely spaced intervals when measuring these hormones, with their mid-range values taken as the assay values. Another example is the marked increase in serum renin activity that occurs when changing from the prone to the standing position. In the case of therapeutic drug testing, blood should be drawn at the optimal time after drug administration based on pharmacokinetics. The collection of serum samples for the detection of antigens and antibodies to infectious pathogens, tumor markers, and specialized proteins has no temporal or positional implications, except for the following aspects to be considered in terms of handling and preservation:

(1) Take care to avoid severe hemolysis. Haemoglobin contains hematoxylin moiety, which has peroxide-like activity, therefore, in the ELISA assay with HRP as the labeling enzyme, if the concentration of hemoglobin in the serum sample is high, it will be easily adsorbed to the solid phase during the incubation, thus reacting with the HRP substrate added later to develop the color.

(2) Take care to avoid bacterial contamination in the collection of samples and serum isolation. Firstly, in the growth of bacteria, some of the enzymes secreted by them may have a decomposition effect on proteins such as antigens and antibodies; secondly, the endogenous enzymes of some bacteria, such as the β-galactosidase of Escherichia coli, may themselves cause non-specific interference with the assay methods labeled with the corresponding enzymes.

(3) Serum samples can be stored at 2-8°C for 1 week if separated by aseptic manipulation. Long-term storage of samples below -70°C.

(4) Avoid repeated freezing and thawing of frozen serum specimens due to power outages, etc. Repeated freezing and thawing of the sample will damage the protein

and other molecules, thus causing false-negative results. In addition, attention to the mixing of the frozen and thawed samples, which should not violent oscillations, and repeated inversion and mixing should be sufficient.
(5) If turbidity or flocculation due to bacterial contamination occurs while the sample is being stored, the sample should be centrifuged and precipitated and the supernatant taken for testing.

II. Reagent Preparation

Before starting the experiment, leave the kit at room temperature for more than 20 minutes to equilibrate the kit with room temperature before using it. The purpose is mainly to enable the temperature inside the reaction microwells to reach the required height faster to meet the assay requirements in the later incubation step. Secondly, the wash buffer must be prepared by diluting the supplied concentrate when using, so the distilled or deionized water used for dilution should be of good quality.

III. Addition of Serum Samples and Reaction Reagent Samples

Serum samples should be added using a microfuge. The key points to note are: do not add samples too quickly, avoid adding samples to the top of the well wall, and do not spatter or create bubbles. Adding the sample too quickly will not guarantee the accuracy and homogeneity of the micro-sample. Adding to the non-coated area in the upper part of the pore wall tends to lead to non-specific adsorption. Spillage can contaminate neighboring pores. The appearance of air bubbles is a difference in the interface of the reaction solution. The reagents are added from the drop bottle, in addition, pay attention to the angle of the drop, the speed of the drop is also very important. If the drop is too fast, it is easy to repeat the drop or add between the two wells, which will appear in the non-encapsulated area of the well's non-specific adsorption, resulting in non-specific color development. Therefore, sometimes a sample tested with the same kit may lead to inconsistent results, often due to the above spiking and reagent errors.