ELISA Protocol

(For scientific research purposes only, not for clinical diagnosis!)

Collection and Preservation of Samples

1. Animal and plant tissue samples (dry ice transport)

Accurately weigh the weight of animal tissue and add 9 times of the volume of homogenizing medium (0.86% or 0.9% normal saline is recommended) according to the proportion of weight (mg): Volume (UL): 1:9. Under the condition of ice water bath, mechanical homogenization is prepared into 10% homogenizing solution, 2500-3000 RPM, centrifugation for 10 minutes, and take the supernatant for determination.

2. Serum samples (dry ice transport)

The blood sample should be placed at room temperature for 2 hours or 4 ℃ overnight, then turned at 2-8 ℃ 3000 / centrifuged for 15 minutes, and the supernatant can be detected immediately; or it should be repackaged and stored at - 20 ℃ or - 80 ℃, but repeated freezing and thawing should be avoided. The thawed sample should be centrifuged again and tested.

3. Plasma sample (dry ice transport)

Heparin can be used as anticoagulant. Within 30 minutes after collection, the sample can be immediately detected at 2-8 ℃ for 3000 revolutions / centrifuged for 15 minutes; or it can be separately packed and stored at - 20 ℃ or - 80 ℃, but repeated freezing and thawing should be avoided. The thawed sample should be centrifuged again and tested.

4. Cell sample

Adherent cells: after washing the cells twice with PBS, scrape the cells carefully with cell scrape, and centrifugate the culture medium at 3000 rpm for 10min. Discard the supernatant and leave the cells to precipitate and transport with drikold.

Suspension cells: the culture medium was centrifuged for 10 minutes at 3000 rpm. Discard the supernatant and leave the cells to precipitate and transport with drikold.

Cell supernatant: the supernatant of the sample was centrifugated at 2-8 ℃, 3000 RPM for 15 minutes, and it was immediately used for the experiment, or stored at - 20 ℃ or - 80 ℃ after separation. Avoid repeated freezing and thawing.

Assay Procedure

According to the instructions of the kit, the operation of different indicators has difference.

The details are subject to the instructions of the ordered Kit (see the attachment). The basic steps are as follows:

1. **Coating:** dilute the antibody with carbonate coating buffer to 1-10 μg /ml protein content. Add 100 μl to each well of polystyrene enzyme plate, and stay overnight at 4 ℃. The next day, discard the solution and wash it with washing buffer for 3 times, each time for 3 minutes. (the antibody has been packed in the general commercial kit, this step can be omitted)
2. **Blocking**: add 200ul of blocking buffer into each hole and incubate at 37 ℃ for 1-2 H.
3. **Washing**: carefully remove the blocking buffer and put it into the washing machine for 3-5 times. You can also wash the board manually: discard the liquid, add 300ul of wash buffer into each well, soak for 1-2min, pat dry on the absorbent paper, and repeat for 3-5 times. (the first three steps can be omitted)
4. **Sample addition**: add 100 μ l of the sample to be tested to the coated reaction pore. (at the same time, blank well and standard well with multiple dilutions can be made. If possible, negative control and positive control can be added as quality control points).
5. **Warm Incubation**: seal the plate with plate sealer and incubate at 37 ℃ for 1-2 H.
6. **Washing**: synchronous step 3.
7. **Add antibody**: add 100 μ l diluted biotinylated antibody working solution to each well.
8. **Warm Incubation**: seal the plate with sealing membrane and incubate at 37 ℃ for 1 h.
9. **Washing**: synchronous step 3.
10. **Adding enzyme conjugates**: add 100 μ l diluted enzyme conjugates working solution to each well.
11. **Warm Incubation**: use the plate sealer to seal the plate and then incubate at 37 ℃ in dark for 30 min.
12. **Washing**: synchronous step 3.
13. **Add the chromogenic substrate**: add 100 μl of TMB substrate solution into each well, react in the dark at 37 ℃ for 10-30min, until there is an obvious color gradient in the times diluted standard pore.
14. **Stop reaction**: add 2 m sulfuric acid 100 μ l into each reaction well, and the color changes from blue to yellow.
15. **Results**: within 10 minutes, on the enzyme labeling instrument, at 450 nm, the O.D. of each well was measured after the blank control pore was zeroed.

Calculation of Results

The standard curve was made according to the concentration and O.D. of the standard, and then the sample concentration was calculated according to the standard curve equation. The results are sent as ECXEL.