ELISA Protocols

Sandwich ELISA

Additional material required

- 37°C±0.5°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Procedure

1. Determine the number of strips required for the assay. Insert the strips in the frames for use.
2. Add 50ul **standard** to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
3. Add 40ul sample to sample wells and then add **biotinylated antibody** to sample wells, then add 50ul **streptavidin-HRP** to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
4. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml **wash buffer** for 30 seconds to 1 minute for each wash.

1. Tips: For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Add 50ul **substrate solution A** to each well and then add 50ul **substrate solution B** to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
6. Add 50ul **stop solution** to each well, the blue color will change into yellow immediately.
7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Competitive ELISA

Biotinylated Antigen Preparation: Briefly centrifuge **the biotinylated antigen** vial then add 1ml **biotinylated antigen diluent** to mix well. And then pipette all this solution back into the **biotinylated antigen diluent** vial to mix well and generate a 6ml **stock solution**. Allow to sit for 10 minutes with gentle agitation prior to making dilutions.

Avidin-HRP Concentrate: Briefly low- speed centrifuge the **avidin-HRP Concentrates solution** and then pipette all **avidin-HRP** into the avidin **HRP Diluent** vial. Mix well to generate a 6ml stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions.

Procedure

1. Determine the number of strips required for the assay. Insert the strips in the frames for use.
2. **Blank well:** Only add **substrate solution A** , **substrate solution B** and **stop solution** as blank control.
3. Add 50ul **diluted standard** to standard well, add 50ul sample to the sample well, and add 50ul **biotinylated antigen** to each well. Mix well. Cover the plate with a sealer and incubate for 60 minutes at 37°C. Tips: Sample recommended dilution: 2-5 times when necessary.
4. Remove the sealer and the liquid in the well, wash five times with 300ul **wash buffer** manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to complete remove liquid. Tips: For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate on absorbent material.
5. Add 50ul **avidin-HRP** to the standard well and sample well, cover the plate with a sealer and incubate for 60 minutes at 37°C.
6. Remove the sealer and wash as described above.
7. Add 50ul **substrate solution A** to each well and then add 50ul **substrate solution B** to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
8. Add 50ul **stop solution** to each well, the blue color will change into yellow immediately.
9. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Competitive ELISA

Procedure

1. Determine the number of strips required for the assay. Insert the strips in the frames for use.
2. Set a Blank well without any solution.
3. Add 50ul **negative control** to each of the negative control wells and 50ul **positive control** to each of the positive control wells. Add 40ul **sample diluent** and then add 10ul sample to the sample well, mix well.
4. Cover with a plate sealer, and incubate for 30 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with **wash buffer**. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.
6. Tips: For automated washing, aspirate all wells and wash 5 times with **wash buffer**, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
7. 6. Add 50ul **HRP** to each well (except blank well). Cover with a plate sealer, and incubate for 30 minutes at 37°C.
8. Remove the sealer and wash as 5 times with **wash buffer**.
9. Add 50ul **substrate solution A** to each well and then add 50ul **substrate solution B** to each well. Mix well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
10. Add 50ul **stop solution to** each well, the blue color will change into yellow immediately.
11. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 15 minutes after adding the stop solution.