Protocol for Avian(chicken) Chlamydiosis ELISA Antibody Detection Kit

Name：Avian(chicken) Chlamydiosis ELISA Antibody Detection Kit

【Included in the kit:】

1. 96well immunoplate coated with rMOMP protein(1plate)
2. Acetate plate sealer(APS), (2pieces)
3. Chicken chlamydia negative control serum(0.5mL×1vial)
4. Chicken chlamydia positive control serum(0.5mL×1vial)
5. Secondary antibody/anti-birds IgY(IgG)-Peroxidase(400×0.2mL×1vial)
6. Substrate solution A(10mL)
7. Substrate solution B(10mL)
8. 10×dilution/washing buffer concentrate(50mL)
9. Secondary antibody diluent 20mL)
10. Stop solution(12mL)
11. Protocol (1copy)。

【Assay procedure】

1. Thoroughly read this protocol before performing an assay. Please allow all kit components to return to room temperature before use(25-40minutes).
2. Dilute the 10×dilution buffer concentrate with 450mL distilled water. This will be the 1× dilution buffer solution and used to wash plate or reconstitute samples.
   Note: If crystal appear in 10×dilution buffer ,the buffer can be placed in a warm water bath for approximately 30 minutes or until no crystals are visible. Mix thoroughly before use.
3. Dilute the samples 100times with dilution buffer.
4. Add 100μL of positive control serum into wells A1 and A2
5. Add 100μL of negative control serum into wells A3 and A4
6. Add tested sample into the each well except the control serum wells.
7. Seal the plate with acetate plate sealer, incubate the immunoplate for 30 minutes at 37℃.
8. Centrifuge the IgY-HRP vial provided in this kit(3000-5000rpm,5seconds)and dilute it 400times with secondary antibody dilution buffer to make 1×IgY-HRP solution, vortex thoroughly.
9. Remove APS from the plate. Discard contents of the wells.
10. Wash each well with 250μL of 1×washing buffer, discard the buffer, invert and blot dry plate. Repeat 5times.

11. Add 100μL secondary antibody to each well.
12. Reseal the immunoplate with APS, incubate for 30minutes at 37℃.
13. Repeat step9and step10.
14. Mix the substrate solution A and substrate solution B at the equal volume to make work solution.
15. Add 100μL of substrate solution into each well.
16. Reseal the immunoplate with APS, incubate for 10 minutes at 37℃.
17. Remove APS from the plate. Add 100μL of stop solution into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uniform, gently tap the plate to ensure thorough mixing. Proceed to the next step within 20minutes.
18. Load the immunoplate onto a Microtiter Plate Reader read absorbance O.D.at 450 and 630.

【Result Interpretation】

Validation criteria

1. The mean OD_{450}(OD_{450}-OD_{630}) of the Negative Control(wells A3 and A4)must be ＜0.1
2. The mean OD_{450} (OD_{450}-OD_{630}) of the Positive Control(wells A1 and A2)must be ≥0.6
3. Not meeting these criteria should the assay result be discarded.

Calculation of results

S/P =（OD_{450} sample/OD_{450}positive）x100%。

Determination of results

S/P＜20%　negative; S/P≥20% positive

【Application】*For research only.*

【Package】96-well/plate

【Storage】 Store at 2～8℃

【Expiration】6 months。

Additional Recommended Procedural Notes

- Reagents of different lot numbers should not be mixed.
- Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
- When handling the plate, avoid touching the bottom.
- Unused microplate strips should be placed back in the sealing bag.

- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times before loading.
- It is highly recommended that solutions be used as soon as possible after rehydration.
- Modification of the existing protocol (i.e. pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the assay results.