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User's Manual

Monkey Virus Multiple Combination 2 (BV SRV STLV-1 SIV) Antibody Detection Dot-ELISA Kit

REF **DEIA-JY2502**

Σ **100T/500T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Monkey Virus Dot Immunoassay Antigen Detection Kit is used to detect virus-specific IgG antibodies in experimental monkey serum.

Principles of Testing

The Dot-ELISA kit is based on the basic principle of dot immunoassay, using nitrocellulose membrane as the solid-phase carrier. The virus antigens and virus cultured cells used as reference are arranged in a matrix on the membrane. The antigens in each group are arranged in the following sequence: Vero cells, BV, SRV, STLV-1 and SIV. During the test, filter paper strips adsorbed with the serum to be tested are placed on the antigen spots. The specific binding between the relevant virus antibodies in the sample and the corresponding antigens on the membrane occurs. Subsequently, enzyme-conjugated secondary antibodies are added to form antigen-antibody-enzyme complexes. Finally, the corresponding substrate of the enzyme is added to induce an enzymatic reaction, causing the corresponding antigen spots to exhibit visible blue dots to achieve the qualitative detection of the relevant virus antibodies. If the membrane is imaged using a scanner or digital camera, the results can be relatively quantitatively analyzed. This method is simple to operate, has a short process, high throughput, does not require special equipment, and provides reliable results. It is suitable for use in large-scale routine screening in monkey facilities to improve animal quality.

Reagents And Materials Provided

1. 20× Wash Buffer
2. Sample Dilutant
3. Chromogen
4. AP Conjugate
5. Control Cut-off
6. Positive Control PC
7. Negative Control NC
8. DIA Antigens Membrane
9. Filter Paper Strips
10. Filter Paper Pad

Materials Required But Not Supplied

1. Forceps: Non-toothed pointed and flat-head stainless steel forceps;
2. Ultra-pure deionized water or distilled water;
3. Small test tubes for diluting serum (12×55 mm);
4. 50 mL centrifuge tubes, capacity bottles of 250 mL and 2000 mL for high-pressure sterilization;
5. A constant temperature incubator or water bath;

6. A shaker;
7. A vortex mixer;
8. Adjustable micropipettes of 20, 100, and 1000 µL with corresponding tips;
9. A repeater pipette with corresponding tips (5 or 10 mL);
10. 50-100 mL serological pipette (for 2000 mL reagent bottles).

Storage

2-8°C

Specimen Collection And Preparation

1. Use a vacutainer without anticoagulant to draw approximately 1.5 ml of venous blood. Be cautious not to draw too quickly or shake vigorously to avoid hemolysis of the sample.
2. Allow the blood to stand for half an hour after collection to allow it to clot before centrifugation. Separate the serum by centrifuging at 3000 rpm for 10-15 minutes.
3. Transfer the serum into a 1.5-2.5 ml cryotube with a sealing ring. Do not reuse the pipette tip during aspiration to prevent cross-contamination.
4. Serum samples can be temporarily stored at 4°C; if storage exceeds five days, it should be stored at -25°C.

Reagent Preparation

1. Pour 100 mL of phosphate buffer reagent into a 2000 mL reagent bottle, add 1900 mL of deionized water to prepare 1x wash solution (pH 7.2-7.4).
2. Add 5 g of sample dilution reagent to 100 mL of wash solution to prepare a 5% sample dilution solution, or prepare according to the sample quantity, with 100 mL needed for every 100 samples. This working solution should not be stored at 4°C for more than 2 days.
3. Remove the color developer from the refrigerator half an hour before staining and allow it to equilibrate to room temperature.

Assay Procedure

1. For **serum dilution** , label small test tubes with corresponding numbers and arrange them in sequence on the test tube rack.

● Use a repeat pipette to add 400 µl of sample dilution solution to each dilution tube. Then, use a 100 µl micropipette to add 100 µl of the corresponding serum to be tested, as well as the critical value control and positive control sera provided by the kit.

2. **Preparing Antigen Membrane:**

● Wear gloves, remove the antigen membrane, label with numbers, and place it in a 10×10 cm plastic petri dish (1-2 membranes per dish).

● Add 15 ml of sample dilution solution, cover, and let it shake gently on a shaker for 30 minutes at room temperature.

● After incubation, quickly wash the membrane three times with wash solution for one minute each time. Then, place a piece of filter paper under the antigen membrane, pour an adequate amount of wash solution to ensure the membrane and filter paper are thoroughly moistened, and remove excess liquid to prevent the membrane from becoming dry.

3. **Affixing the Membrane:**

● Firstly, thoroughly mix the diluted serum on a shaker. Using fine-tip tweezers, lightly grip one end of the provided small filter paper strip, dip the other end into the diluted sample, allow the paper to absorb the liquid fully, remove excess liquid, affix it carefully onto the corresponding location on the antigen membrane matrix, and wipe the tweezers dry with absorbent paper before affixing the next sample.

● Each membrane has 3 columns and 9 rows, totaling 27 groups of antigen spots, for testing 25 samples and 2 controls. Affix the leftmost column first from the top to the bottom, then proceed from left to right for the middle and right columns. Lastly, affix the critical value control and positive control in the last two groups of antigen spots.

● During affixing, avoid contact or close proximity of filter paper strips from different samples to prevent cross-contamination, and record the affixing order in detail.

4. **Incubation:**

● After affixing, cover the petri dish and place it in a water bath or constant temperature chamber at 37°C for 30 minutes. Ensure no water enters the petri dish during the water bath, and maintain humidity when using a constant temperature chamber to prevent the antigen membrane from drying out.

5. **Washing:**

● After incubation, remove the petri dish and quickly rinse off the filter paper covering the membrane with pre-cooled wash solution, discard the filter paper. Then, wash the membrane three times for 5 minutes each, with the petri dish placed on a shaker at medium speed during the washing process.

6. **Enzyme-Labeled Secondary Antibody:**

● Dilute the provided enzyme-labeled secondary antibody in wash solution at a 1:1000 ratio (take 15 µl of antibody per membrane, add to 15 ml of wash solution), pour the mixture into a petri dish to cover the entire membrane, and incubate at room temperature for 30 minutes.

● When reacting multiple membranes simultaneously, place two membranes back to back in one petri dish, add 25 ml of the secondary antibody solution, and shake gently at low speed on the shaker for 30 minutes.

7. **Washing:**

● Discard the secondary antibody solution, wash the antigen membrane with wash solution three times for 5 minutes each, with the petri dish placed on the shaker at medium speed. After the final wash, remove excess liquid as much as possible.

8. **Color Development:**

● Add 15 ml of color developer to each dish, gently shake the petri dish, and carefully observe the membranes. When the critical value control reaches a deep "+" while the background is still

light (approximately 2-5 minutes, subject to environmental temperature changes), terminate the reaction.

9. **Termination:**

● When terminating, quickly rinse the membrane five times with tap water, allow it to air dry, and then read the results.

Interpretation Of Results

Positive (P): If the reference cell spot is colorless or very light in color, but the virus antigen spot color reaches the depth of weak positive control or deeper, equivalent to "+~-+++" depth as shown in the diagram, then it is considered positive.

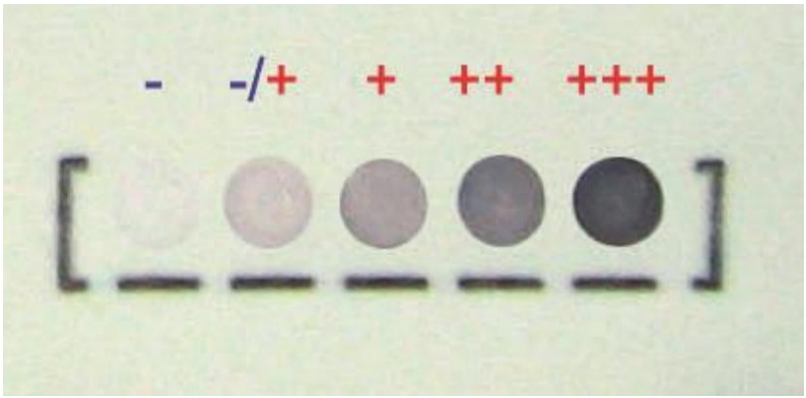
Suspected (I): If the virus antigen spot is darker than the corresponding reference cell spot but lighter than the weak positive control, equivalent to "+/-" depth as shown in the diagram, then it is considered suspected.

Negative (N): If the virus antigen spot is colorless, or very light in color and close to the corresponding reference cell spot, then it is considered negative.

For animals suspected of B virus infection, it is recommended to retest with a new blood sample two weeks later. If still inconclusive, it is advised to send both blood samples to laboratory for comprehensive confirmation using DIA, ELISA, and Western blot methods.

For animals with suspected or positive STLV, it is recommended to send whole blood to laboratory for further confirmation using Western blot or PCR.

For animals with suspected or negative Measles, it is recommended to consider re-vaccination.



Precautions

1. The reagent kit should be transported at room temperature and stored at 2-8°C.
2. It is necessary to change the pipette tip for each sample to prevent cross-contamination.
3. Gloves should be worn at all times during the operation to avoid direct contact with the antigen membrane.
4. Throughout the experiment, the antigen membrane should be kept moist and not allowed to dry out.
5. The room temperature should be maintained at 22-25°C.