Dog Rabies virus Antibody IgG (RABV) ELISA Kit

Catalog No.: abx055582

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C. Once opened, use within 2 weeks.

Application: For qualitative detection of RABV in Dog Serum.

Introduction: Rabies virus is a neurotropic virus that causes rabies in humans and animals. Rabies transmission can occur through the saliva of animals and less commonly through contact with human saliva. Rabies virus, like many rhabdoviruses, has an extremely wide host range. In the wild it has been found infecting many mammalian species, while in the laboratory it has been found that birds can be infected, as well as cell cultures from mammals, birds, reptiles and insects.

Principle of the Assay

This kit is based on a qualitative enzyme-linked immuno-sorbent assay technology. RABV antigen is precoated onto 96-well plates. Samples are added to the appropriate wells. Any antibodies present that are specific for the antigen present will bind to the precoated antigen. Horseradish Peroxidase (HRP) conjugated antibody is added and the plate is incubated. A substrate solution is added to the wells and the colour intensity develops in proportion to the amount of RABV antibody in the sample. The colour development is stopped and the intensity of the colour is measured.

Kit components

1. One pre-coated 96 well plate
2. Positive Control: 0.8 ml
3. Negative Control: 0.8 ml
4. Wash buffer (25X): 20 ml
5. Sample diluent: 2 x 20 ml
6. HRP Conjugate (100X): 120 µl
7. HRP Conjugate Diluent: 20 ml
8. TMB Substrate: 10 ml
9. Stop Solution: 10 ml
10. Adhesive Strip (for 96 wells): 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm, correction wavelength set at 540 nm or 570 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter paper for blotting the microtiter plate
9. 100 ml and 500 ml volume graduated cylinders
10. Deionized or distilled water

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately at 1:5 dilution (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the blood at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.

Note:

» Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

» Serum samples require approximately a 10 fold dilution.

2. Wash buffer

If crystals have formed in the concentrated wash buffer, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 20 ml of concentrated wash buffer into 480 ml of distilled water).

3. HRP Conjugate

Centrifuge the vial before opening. HRP Conjugate requires a 100-fold dilution. (i.e. add 10 µl of HRP Conjugate into 990 µl of HRP Conjugate Diluent).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature for 30 minutes prior to use.

1. Prepare all reagents and samples as directed in Section A.
2. Determine the number of wells to be used. Any strips that are not being used should be kept dry and stored at 4°C.
3. Set up two blank wells without any solution.
4. Set up two Negative Control wells with 100 µl of Negative Control per well.
5. Set up two Positive Control wells with 100 µl of Positive Control per well.
6. Set up the sample wells with 100 µl of diluted sample per well.
7. Cover the plate with the adhesive strip provided. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes.
8. Remove the cover, and wash the plate 3 times. Fill each well with wash buffer (200 µl) and soak for 2 minutes. Discard the contents and repeat two more times. After the last wash, blot the plate on an absorbent material.
9. Add 100 µl of diluted HRP conjugate to each well (except the blank well). Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes.
10. Repeat the wash process (Step 8) 5 times.
11. Add 90 µl of TMB Substrate to each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 20 minutes in dark conditions.

12. Add 50 µl of Stop Solution into each well. There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
13. By taking the blank well as zero, determine the the optical density of each well within 10 minutes of adding the Stop Solution, using a microplate reader set to 450 nm.

C. Analysis

1. Negative Result: (sample OD value) < (2.1 x Average negative control OD value).
2. Positive Result: (sample OD value) ≥ (2.1 x Average negative control OD value).

D. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. It is recommended to assay all standards, controls and sample in duplicate.
3. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
4. Ensure plates are properly sealed or covered during incubation steps.
5. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
6. To avoid cross contamination do not reuse pipette tips and tubes.
7. Do not use components from a different kit or expired ones.
8. The stop solution provided with this kit is an acidic solution. Wear eye, hand, face protection and protective clothing when using this material.
9. Upon addition of the Stop Solution, the colour of the wells should change from blue to yellow. Wells that are green in colour indicate that the Stop Solution has not been mixed thoroughly in the wells.