Dog CRV / Canine Rabies Virus IgM antibody ELISA Kit

Enzyme Immunoassay for the screening of IgM antibody to Canine Rabies Virus in dog's serum or plasma.

Catalog number: ARG80999

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

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PRINCIPLE OF THE ASSAY

This is an Enzyme immunoassay for the identification of IgM antibody to Rabies Virus in dog's serum or plasma.

Purified inactivated Canine Rabies Virus has been pre-coated onto a microtiter plate. Controls or samples are pipetted into the wells and any antibody present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti-dog IgM antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of antibody bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Rabies Virus-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Positive Control	1 vial (Ready-to-use)	-20 °C
Negative control	1 vial (lyophilized)	4°C (store at -20 °C after reconstitution)
HRP-Antibody conjugate	13 ml (Ready-to-use)	4°C
ELISA buffer	18 ml (Ready-to-use)	4°C
200X Wash buffer	20 ml	4°C
Plastic cover seal	1	RT

Substrate A	8 ml	4°C
Substrate B	8 ml	4°C
STOP solution	8 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Controls should be stored at-20 °C in aliquots after reconstitution.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 200X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried

out with care to guarantee reproducible inter- and intra-assay results. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Samples should preferable be adsorbed with protein A or anti-dog IgG magnetic beads, to adsorb IgG antibodies in the samples which otherwise might block the large IgM and course competition.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- **Negative Control**: Briefly centrifuge the tube. Reconstitute negative control with 1 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use (stock solution). Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE (QUALITATIVE)

All materials should be equilibrated to room temperature (RT) 15 min before use (Place the reagents back at 4 °C immediately after use). Samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Negative Control: (for duplicates) Dilute stock solution 1:50 in ELISA buffer to make working solution. Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL for each well in the coated plate.
4. Positive Control: (for duplicates) Make a 1:2 dilution of the positive control in ELISA buffer starting with 1:2, 1:4, and 1:8 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL for each well in the coated plate.
5. Serum or Plasma: (for duplicates) Dilute serum or plasma samples 1:50 in ELISA buffer. Make sure to make minimal 250 µL of every dilution to be

able to transfer 100 µL for each well in the coated plate.

6. Take 2 wells as substrate controls, add only 100 µL ELISA buffer to these well.
7. Add 100 µl of diluted samples, positive and negative controls into respective wells.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. Prepare immediately before use.
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-15 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately (620 nm as optional reference wave length) and use the substrate controls as blank.

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3. Negative Control: (for duplicates) Make a 3-step dilution of the negative control in ELISA buffer starting with 1:30, 1:90, 1:270, and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL for each well in the coated plate.
4. Positive Control: (for duplicates) Make a 3-step dilution of the positive control in ELISA buffer starting with 1:3, 1:9, 1:27, and 1:81 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL for each well in the coated plate.
5. Serum or Plasma: (for duplicates) Make 3-step dilutions of each sample in ELISA buffer starting with 1:30, 1:90, 1:270, and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL for each well in the coated plate.
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VALIDATION OF THE TEST

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.700 OD units (at 450 nm)
- The mean value (MV) of the measured OD value for the Negative Control (NC) must be ≤ 0.350 OD units (at 450 nm) and give an endpoint titer of ≤ 30.

Qualitative: (positive/ negative)

A sample is scored as positive when the OD is higher than the OD of the negative control plus 0.200.

Quantitative: (end point titer)

The end-point titer of the sample is the dilution which gives an extinction just above the OD of the negative control plus 0.150. I

The RIFFIT test is still seen as the standard but final correlation with ELISA depends on the laboratory performing the RIFFIT test.

Small lab to lab variation in RIFFIT test will always been seen due to the nature of biological material (cells and virus)