Dog CDV / Canine Distemper Virus IgM antibodies ELISA Kit

Enzyme Immunoassay for the screening of IgM antibodies to CDV in serum or plasma.

Catalog number: ARG80988

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

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PRINCIPLE OF THE ASSAY

This is an Enzyme immunoassay for the screening of IgM antibodies to CDV in dog's serum or plasma.

Purified Canine Distemper Virus (CDV) protein has been pre-coated onto a microtiter plate. Controls, standards or samples are pipetted into the wells and any Ab 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 Ab 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. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
CDV-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Positive Control	1 ml	4°C.
Negative control	1 ml	4°C.

HRP-Antibody conjugate	2 X 6 ml (Ready-to-use)	4°C
ELISA buffer	3 X 6 ml (Ready-to-use)	4°C
200X Wash buffer	60 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.
- 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- We advise that you preincubate all your sera with a solution of α-IgG or protein-G in order to eliminate any IgG that might prevent IgM from reacting with the antigen on the microtiter plate.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before 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.
2. Make 3 steps dilutions of each sample with ELISA buffer (1:30; 1:90; 1:270; 1:810) in another round bottomed microtiter plate (not provided).
3. Make the following dilutions of the positive (1:5, 1:15, 1:45, 1:135) and negative controls (1:30; 1:90; 1:270 and 1:810) in ELISA Buffer.
4. Add 100 µl of diluted samples, positive and negative controls into respective wells.
5. Seal and incubate for 60 minutes at 37°C.
6. 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.
7. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
8. Wash as according to step 6.
9. Mix equal part of substrate A and substrate B. Prepare immediately before use.

10. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark.
11. Add 50 µl of Stop Solution to each well.
12. Read the OD with a microplate reader at 450 nm immediately.

VALIDATION OF THE TEST

The negative control should give an OD < 0.200.

The end point titer of the positive control should be approximately 1:150 according to the instructions for interpretation of test results.

INTERPRETATION OF TEST RESULTS

The titer of the sample is the dilution which gives an extinction above 0.200 OD units (450 nm).

The test is valid if the first two dilutions of the positive control are above 0.200 (OD 450 nm).