Dog CDV / Canine Distemper virus IgG antibody ELISA Kit

Enzyme Immunoassay for the screening of IgG antibody to Canine Distemper virus (CDV) in dog's serum or plasma.

Catalog number: ARG80987

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

For diagnosis of Canine Distemper Virus (CDV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. Antibodies induced through infection or vaccination are caught by the virus, which is attached to the solid phase by use of monoclonal antibodies. IgG antibody titers above dilutions of 1:250 are considered protected. After reaching peak values within two or three weeks, antibody titers fall back to a threshold level at which they persist. Re-exposure results in an anamnestic response.

PRINCIPLE OF THE ASSAY

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

Purified Canine Distemper Virus (CDV) protein has been pre-coated onto a microtiter plate by CDV specific monoclonal antibody. 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 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 vial (lyophilized)	4°C (store at -20 °C after reconstitution)
Negative control	1 vial (lyophilized)	4°C (store at -20 °C after reconstitution)
HRP-Antibody conjugate	12 ml (Ready-to-use)	4°C
ELISA buffer	18 ml (Ready-to-use)	4°C
200X Wash buffer	20 ml	4°C
Substrate A	8 ml	4°C
Substrate B	8 ml	4°C
STOP solution	8 ml (Ready-to-use)	4°C
Plastic cover seal	1	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm and a 620 nm as the reference wavelength.
- Round bottomed microtiter plate
- 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.
- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Store the kit at 4°C at all times. An open packet should be used within 10 days.
- Do not use components past the expiry date and do not mix components from different serial lots. An open packet should be used within 10 days.
- Positive and negative controls should be stored at-20 °C in aliquots after reconstitution. Avoid repeated freezing and thawing as this increases non-specific reactivity.
- Buffer, controls, samples and antibody need to be shaken gently before use, in order to dissolve/mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or resuspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.

- If crystals are observed in the 200X Wash buffer, warm to RT or 37°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.
- Wash step: 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.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

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. Store the diluted 1X Wash buffer at 4°C.
- Negative Control: Briefly centrifuge the tube. Reconstitute negative control with 1 ml of distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use (stock solution). Avoid repeated freeze-thaw cycles.
- Positive Control: Briefly centrifuge the tube. Reconstitute positive control with 0.5 ml of distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE (QUALITATIVE)

All materials should be equilibrated to room temperature (RT, around 21°C) 15 min before use (without exposing them to direct sunlight or any heat sources and 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. **Dilute Negative Control**: (for duplicates) Dilute stock solution **1:50** in ELISA buffer to make working solution. Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.
4. **Dilute Positive Control**: (for duplicates) Dilute stock solution **1:50** in ELISA buffer to make working solution. Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.
5. **Dilute Serum or Plasma**: (for duplicates) Dilute serum or plasma samples **1:150** in ELISA buffer. Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.

   Dilution Note (For duplicate):

   a) For 1:10 dilution: add 5 µL of samples into 45 µL of ELISA buffer, mix well.

   b) For 1:150 dilution: add 16 µL of diluted samples from **a)** into 224 µL of ELISA buffer, mix well.

   c) For 1:50 dilution: add 5 µL of controls into 245 µL of ELISA buffer, mix well.
6. Take 2 wells as substrate controls, add only **100 µL** of **ELISA buffer** to the wells.
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** of **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 **10-20 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. It is recommended read the absorbance within 10 min after adding STOP solution.

ASSAY PROCEDURE (QUANTITATIVE)

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, 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. **Dilute Negative Control**: (for duplicates) Make a 3-step dilution of the negative control in ELISA buffer starting with **1:50, 1:150, 1:450, 1:1350** in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.
4. **Dilute Positive Control**: (for duplicates) Make a 3-step dilution of the positive control in ELISA buffer starting with **1:50, 1:150, 1:450, 1:1350** in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.
5. **Dilute Serum or Plasma**: (for duplicates) Make 3-step dilutions of each sample in ELISA buffer starting with **1:50, 1:150, 1:450, 1:1350** in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 240 µL of every dilution to be able to transfer 100 µL for each well to the coated plate.

   Dilution Note (For duplicate):

   a) For **1:5** dilution: add 20 µL of samples/controls into 80 µL of ELISA buffer, mix well.

   b) For **1:50** dilution: add 40 µL of diluted samples/controls from **a)** into 360 µL of ELISA buffer, mix well.

   c) For **1:150** dilution: add 120 µL of diluted samples/controls from **b)** into

240 µL of ELISA buffer, mix well.

d) For **1:450** dilution: add 120 µL of diluted samples/controls from **c)** into 240 µL of ELISA buffer, mix well.

e) For **1:1350** dilution: add 120 µL of diluted samples/controls from **d)** into 240 µL of ELISA buffer, mix well.

6. Take 2 wells as substrate controls, add only **100 µL** of **ELISA buffer** to the wells.
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** of **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 **10-20 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. It is recommended read the absorbance within 10 min after adding STOP solution.

VALIDATION OF THE TEST

Qualitative:

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD_{450} value for the 1:50 diluted Positive Control (PC) must be ≥ 0.850
- The MV of the measured OD_{450} value for the 1:50 diluted Negative Control (NC) must be ≤ 0.400

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 90. The negative control should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 30.

INTERPRETATION OF TEST RESULTS

Qualitative: Positive – Negative

The results are valid if the following criteria are met:

A sample with the S/P ratio < 0.25 is negative

(Specific antibodies to Distemper Virus could not be detected)

A sample with the S/P ratio ≥ 0.25 is positive

(Specific antibodies to Distemper Virus were detected)

Quantitative: End point titer

The ELISA titer can be calculated by constructing a curve and using a cut-off line (dilution 1:50 → 1:150→ 1:450→ 1:1350→ 1:4050→ 1:12150, etc., total 8 dilutions of 3 steps) OD on Y-axis and titer on X-axis.

ELISA titers can be calculated using as cut-off 2.5 times OD value of negative control at 1:50.