Dog CPV / Canine Parvo Virus IgG antibody ELISA Kit

Enzyme Immunoassay for the screening of IgG antibody to Canine Parvo Virus (CPV) in dog's serum or plasma.

Catalog number: ARG80990

Package: 96 wells

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

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INTRODUCTION

For diagnosis of Canine Parvo Virus (CPV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. The virus that is attached to the solid phase by use of monoclonal antibodies catches antibodies induced through infection or vaccination.

IgG antibody titers above a dilution of 1:810 are considered protected.

PRINCIPLE OF THE ASSAY

This is an Enzyme immunoassay for the screening of IgG antibody to Canine Parvo Virus (CPV) in dog's serum or plasma.

A monoclonal antibody to Canine Parvo Virus has been pre-coated onto a microtiter plate. Purified inactivated Canine Parvo Virus would be captured onto the microtiter plate by the CPV monoclonal antibody. Controls or samples are pipetted into the wells and any CPV antibody present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti-dog IgG 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
CPV antibody coated Microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Inactivated Canine Parvo Virus antigen	11 ml (Ready-to-use)	4°C
Positive Control	1 vial (lyophilized)	4°C (aliquot and store at -20 °C after reconstitution)
Negative control	1 vial (lyophilized)	4°C (aliquot and 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.
- Store the kit at 4°C at all times. An open packet should be used within 10 days.
- Place the reagents back at 4°C - 8°C immediately after use.
- 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.

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.
- 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 as this increases non-specific reactivity.
- Positive Control: Briefly centrifuge the tube. Reconstitute positive control with 0.5 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use. Avoid repeated freeze-thaw cycles as this increases non-specific reactivity.

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. Add **100 µL** of **inactivated Canine Parvo Virus antigen** to all wells to be used and incubate for **75 min at 37°C.**
4. **Dilute Negative Control**: (for duplicates) Dilute stock solution 1:100 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.
5. **Dilute Positive Control**: (for duplicates) Dilute stock solution 1:250 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.

6. **Dilute Serum or Plasma**: (for duplicates) Dilute serum or plasma samples 1:250 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.

   Dilution Note (For duplicate):

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

   b) For 1:250 dilution: add 10 µL of diluted samples/controls from **a)** into 240 µL of ELISA buffer, mix well.

   c) For 1:100 dilution: add 20 µL of diluted samples/controls from **a)** into 180 µL of ELISA buffer, mix well.

7. After antigen buffer incubation. 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.
8. Take 2 wells as **substrate controls**, add only **100 µL** of **ELISA buffer** to the wells.
9. Add **100 µL** of **all diluted samples, positive and negative controls** into respective wells.
10. Seal and incubate for **60 minutes at 37°C**.
11. **Wash** as according to **step 7**.
12. Add **100 µL** of **HRP-antibody conjugate solution** into each well. Cover the

plate and incubate for **60 minutes at 37°C.**

13. **Wash** as according to **step 7**.
14. **Mix equal part of substrate A and substrate B** with gentle shaking. Prepare immediately before use.
15. Add **100 µL** of **mixed substrate reagent** to each well. Cover the plate and incubate for **10-20 minutes at room temperature** in dark. Make sure the negative control does not become too dark.
16. Add **50 µL** of **Stop Solution** to each well, mix well.
17. **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 to read the absorbance within 10 min after adding STOP solution.

ASSAY PROCEDURE (QUANTITATIVE)

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. Add **100 µL** of **inactivated Canine Parvo Virus antigen** to all wells to be used and incubate for **75 min at 37°C.**
4. **Dilute Negative Control**: (for duplicates) Make a 3-step dilution of the negative control in ELISA buffer starting with **1:100, 1:300, 1:900, and 1:270**0 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. **Dilute Positive Control**: (for duplicates) Make a 3-step dilution of the positive control in ELISA buffer starting with **1:100, 1:300, 1:900, and**

1:2700 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.

6. Dilute Serum or Plasma: (for duplicates) Make 3-step dilutions of each sample in ELISA buffer starting with 1:100, 1:300, 1:900, and 1:2700 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.

   Dilution Note (For duplicate):

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

   b) For 1:100 dilution: add 30 µL of diluted samples/controls from a) into 270 µL of ELISA buffer, mix well.

   c) For 1:300 dilution: add 100 µL of diluted samples/controls from b) into 200 µL of ELISA buffer, mix well.

   d) For 1:900 dilution: add 100 µL of diluted samples/controls from c) into 200 µL of ELISA buffer, mix well.

   e) For 1:2700 dilution: add 100 µL of diluted samples/controls from d) into 200 µL of ELISA buffer, mix well.

7. After antigen buffer incubation. 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.

8. Take 2 wells as **substrate controls**, add only **100 µL** of **ELISA buffer** to the wells.
9. Add **100 µL** of all **diluted samples, positive and negative controls (1:100, 1:300, 1:900, and 1:2700 diluted)** into respective wells.
10. Seal and incubate for **60 minutes at 37°C**.
11. **Wash** as according to **step 7**.
12. Add **100 µL** of **HRP-antibody conjugate solution** into each well. Cover the plate and incubate for **60 minutes at 37°C.**
13. **Wash** as according to **step 7**.
14. **Mix equal part of substrate A and substrate B** with gentle shaking. Prepare immediately before use.
15. Add **100 µL** of **mixed substrate reagent** to each well. Cover the plate and incubate for **10-20 minutes at room temperature** in dark. Make sure the negative control does not become too dark.
16. Add **50 µL** of **Stop Solution** to each well, mix well.
17. **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 to 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 value for the 1:100 diluted Positive Control (PC) must be ≥ 1.000
- The mean value (MV) of the measured OD value for the 1:250 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 1:100 diluted positive control should be ≥ 1.000 OD units (450 nm). The 1:100 diluted negative control should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 50.

INTERPRETATION OF TEST RESULTS

Qualitative: Positive – Negative

The results are valid if the following criteria are met:

A sample with the S/P ratio < 0.22 is negative

(Specific antibodies to Canine Parvo Virus could not be detected)

A sample with the S/P ratio ≥ 0.22 is positive

(Specific antibodies to Canine Parvo Virus were detected)

Quantitative: End point titer

The ELISA titer can be calculated by constructing a curve and using a cut-off line (dilution 1:100 → 1:300→ 1:900→ 1:2700→ 8100→ 24300, 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 1:100.