Dog Parvovirus (PV IgG) ELISA Kit

Catalog No.: abx159778

Size: 96 tests

Specificity

This assay has high sensitivity and excellent specificity for detection of dog parvovirus (PV) antibody (IgG). No significant cross-reactivity or interference between dog parvovirus (PV) antibody (IgG) and analogues was observed.

Precision:

CV (%) = (Standard Deviation / mean) × 100

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

Storage:

Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit May be stored for up to one month at 2 - 8° C.

Application: For the qualitative determination of dog parvovirus (PV) antibody (IgG) concentrations in serum.

Principle of the Assay

The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells with anti-dog IgG conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of dog parvovirus (PV) antibody (IgG) bound in the initial step. The color development is stopped and the intensity of the color is measured. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of PV IgG can be calculated.

Kit components

1. One pre-coated 96 well plate (12 × 8 well strips)
2. Negative Control: 0.6 ml
3. Positive Control: 0.6 ml
4. Sample diluent buffer: 25 ml
5. HRP-conjugate 11 ml
6. Wash buffer (20X): 25 ml
7. TMB substrate: 12 ml
8. Stop solution: 6 ml
9. Plate sealer: 4

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader capable of measuring absorbance at 450/630 nm.
3. High-precision pipette and sterile disposable pipette tips
4. Squirt bottle or automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare samples
7. Distilled water
8. Absorbent filter papers
9. 100 ml and 500 ml graduated cylinders

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4 °C or at room temperature for up to 2 hours. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20 °C or -80 °C.

2. Wash buffer

Dilute 25 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x). If crystals have formed, bring to room temperature and mix gently until the crystals have completely dissolved.

Note:

- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
- NaN_3 cannot be used as test sample preservative, since it inhibits HRP.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30min.

B. Assay Procedure

Centrifuge the sample again after thawing before the assay.

Prepare all reagents, and samples as directed in the previous sections.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of sample diluent buffer into the control (zero) well.
4. Add 100 µl of sample into test sample wells.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37 °C for 30 minutes.
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 10 seconds. The complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of HRP-conjugate to each well (not to Blank!). Cover the microtiter plate with the adhesive strip. Incubate for 30 minutes at 37°C.
8. Repeat the aspiration/wash process for three times as in step 5.

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9. Add 100 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 10 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
10. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. The Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling these solutions. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Calculation 0f Results

For calculation the valence of dog parvovirus (PV) antibody (IgG), compare the sample well with control. The average OD value of the Negative Control must less than 0.1, and the average OD value of the Positive Control must be no less than 0.6. If this is false, repeat the test.

S/P=(Sample $OD_{450/630}$-$NC\bar{x}$)/($PC\bar{x}$-$NC\bar{x}$)

$NC\bar{x}$: the average $OD_{450/630}$ value of Negative Control

$PC\bar{x}$: the average $OD_{450/630}$ value of Positive Control

- While S/P≥0.2: Positive
- While S/P＜0.2: Negative

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