IVD TECHNOLOGIES

Parvovirus Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN CANINE SERUM OR PLASMA

Product Number: CPV-1000

CANINE PARVO

INTENDED USE

The Parvovirus Antibody Test Kit is used for the detection of Parvovirus IgG antibody in canine plasma and serum.

ANALYTICAL PRINCIPLE

Parvirus IgG-specific antibody in diluted samples is allowed to bind to microwell-bound Parvovirus antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Parvovirus IgG antibody-antigen complex. Unbound HRP-conjugate is washed away and TMB is allowed to react with bound HRP-conjugate. The reaction is stopped and the microwell is read. The intensity of the color produced in the HRP-TMB reaction is proportional to the amount of IgG-specific antibody in the sample.

SPECIMEN REQUIREMENTS

Serum and plasma samples are acceptable. Avoid repetitive freezing and thawing of samples.

REAGENTS

Precautions & Safety Notes

- WEAR LATEX GLOVES, FACE SHIELDS AND A LAB COAT WHEN HANDLING SPECIMENS AND OTHER HAZARDOUS REAGENTS
- FOR *IN VITRO* USE, POTENTIAL BIOHAZARDOUS MATERIAL. HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- The Sample Diluent, Positive Control, Negative Control, and Wash Buffer contain 0.05% ProClin 300. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	Parvovirus Antibody Test Kit	**CPV-1000**	**96 Test**
SD	Sample Diluent [Contains: PBS, BSA, 0.05% ProClin 300]	**SDFEA-1001**	**35 ml**
A	Negative Control [Contains: Canine Serum Negative for Parvovirus IgG, 0.05% ProClin 300] See QC Certificate for value	**CPV - 1001**	**70 µl**
B	Positive Control [Contains: Canine Serum Positive for Parvovirus IgG, 0.05% ProClin 300] See QC Certificate for value	**CPV - 1002**	**70 µl**
C	Microwell Plate [Contains: Parvovirus Antigen]	**CPV - 1003**	**96 Well Plate**
D	HRP Conjugate [Contains: HRP Conjugate]	**CPV - 1004**	**10 ml**
WB	20X Wash Buffer Concentrate [Contains: Tris Buffer, 0.01% Tween-20, NaCl, 0.05% ProClin 300]	**WBFEA-1001**	**50 ml**
TS	TMB Solution [Contains TMB] Keep away from light	**TMBS-1001**	**10 ml**
SS	Stop Solution [Contains: 1N Sulfuric Acid]	**SSFEA-1001**	**10 ml**

MATERIALS

The following materials are needed but not supplied.

- Variable Pipettors and Tips
- Stir Bar & Stirrer
- 12x75 mm disposable borosilicate glass culture tubes
- Test Tube Rack, polypropylene
- Vortexer
- Microwell Plate Film Sealer
- ELISA Plate Washer
- ELISA Plate Reader
- Refrigerator (for kit storage)

PROCEDURE PRECAUTIONS

- Bring reagents to room temperature before use.
- Use clean instruments & equipments.
- Unused microwells should be sealed tightly using laboratory tape in the foil pouch until further use.
- Handle microwells with care.
- Minimize air bubbles in microwells.
- Microwells should not contain liquid after the washing steps. To ensure that liquids are removed from microwells after washing steps, microwells can be tapped against clean paper towels.

REAGENT PREPARATION

1X Wash Buffer Preparation

1. Add 1 unit of volume of 20X Wash Buffer Concentrate (**WB**) to 19 units of volume of DI water. For example, add **50 ml** 20X Wash Buffer Concentrate (**WB**) to **950 ml** of DI water.
2. Mix the Wash Buffer Preparation well.

PROCEDURE

1. Dilute controls (**A**, **B**) and samples **1:25** by adding **10 µl** of sample to **240 µl** of Sample Diluent (**SD**) in a glass tube.
2. Vortex the tubes.
3. Dilute samples for a final dilution of **1:100** by adding **40 µl** of the **1:25 diluted samples** from step 1 to **120 µl** of Sample Diluent (**SD**) into a separate glass tube.
4. Vortex the tubes.
5. Dispense **100 µl** of **diluted Negative Control**, **Positive Control**, and the **diluted samples** into designated wells of the Microwell Plate (**C**). The 1:25 and 1:100 dilutions of samples should both be assayed – each being dispensed into separate wells.
6. Cover the wells.
7. Incubate the wells for **30 minutes** at **room temperature**.
8. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**.
9. Dispense **100 µl** of HRP Conjugate (**D**) to each well.
10. Cover the wells.
11. Incubate the wells for **30 minutes** at **room temperature**.
12. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**.
13. Dispense **100 µl** of TMB Solution (**TS**) to each well.
14. Cover the wells.
15. Incubate the wells for **15 minutes** at **room temperature** and **protect from light**.
16. Dispense **100 µl** of Stop Solution (**SS**) to each well.
17. Read the wells in a microwell reader at **450 nm**.

PROCEDURAL NOTES

•Evaluation of samples should be based on the Sample to Negative Ratio.

EXPECTED VALUES

A total of 93 dogs were evaluated for the Parvovirus antibody using IVD Technologies' Parvovirus Antibody Test Kit and an immunofluorescense assay. Twenty-four (24) dogs were evaluated before and three months after they received a Parvovirus vaccination. The optical density (OD) before vaccination was 0.144 – 0.432. After vaccination, the OD was 0.500 – >3. Sixty-nine (69) dogs were evaluated at least three months after receiving a Parvovirus vaccination. The OD for these dogs was 0.362 – >3. The sample to negative (SN) ratios were based on these data.

Sample to Negative Ratio Calculation

Sample to Negative Ratio
= O.D. Sample / O.D. Negative Control

- The Sample to Negative Ratio is equal to the O.D. of the Sample divided by the O.D. of the Negative Control.

Sample to Negative Ratio Interpretation

At 1:25 if the Sample to Negative Ratio (S/N Ratio) is > 1 then the patient is positive for Parvovirus antibody. If the S/N Ratio is < 1 then the patient is negative for Parvovirus antibody.

At 1:100 if the S/N Ratio is > 1 then the patient has protective immunity. If the S/N Ratio is < 1 then the patient has low immunity.

Sample to Negative Ratio Table

S/N Ratio at 1:25 dilution	
< 1.0	Negative
> 1.0	Positive
S/N Ratio at 1:100 dilution	
< 1.0	Low Immunity
> 1.0	Protective Immunity

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