IVD TECHNOLOGIES

Distemper Virus Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN CANINE SERUM OR PLASMA

Product Number: CDVG-1000

CANINE DISTEMPER

INTENDED USE

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

ANALYTICAL PRINCIPLE

Distemper virus IgG-specific antibody in diluted samples is allowed to bind to microwell-bound Distemper virus antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Distemper virus 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.
- 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
	Distemper Virus Antibody Test Kit	**CDVG-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 Distemper IgG, 0.05% ProClin 300] See QC Certificate for value	**CDVG-1001**	**70 µl**
B	Positive Control [Contains: Canine Serum Positive for Distemper IgG, 0.05% ProClin 300] See QC Certificate for value	**CDVG-1002**	**70 µl**
C	Microwell Plate [Contains: Distemper Antigen]	**CDVG-1003**	**96 Well Plate**
D	HRP Conjugate [Contains: HRP Conjugate]	**CDVG-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 all reagents to room temperature before use.
- Use clean instruments & equipments.
- Be careful not to splash contents in the microwells.
- Minimize air bubbles in microwells.
- Minimize touching the bottom of the wells with pipette tips.
- Microwells should not contain liquid after each wash.

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 1X 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 **1:50** by adding **80 µl** of the **1:25 diluted samples** from step 1 to **80 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 1:50 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

Reference Range

A total of 119 dog samples were assayed; 94 and 25 samples were found to be normal and positive, respectively. The OD of the normal and positive samples ranged from 0.058 to 0.256 and 0.356 to > 3, respectively. An OD cut-off of 0.271 was determined based on ROC and sensitivity and specificity analysis.

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 Distemper antibody. If the S/N Ratio is < 1 then the patient is negative for Distemper antibody.

At 1:50 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 1:50 dilution	
< 1.0	Low Immunity
> 1.0	Protective Immunity

CORRELATION ANALYSIS

A total of 140 dog samples were assayed for Distemper virus IgG antibody using IVD Technologies' Distemper virus Antibody Test Kit and a reference laboratory. Both laboratories found 114 samples to be normal and 26 samples to be positive. ELISA and IFA methods were used by the reference laboratory to assay the samples. The correlation analysis shows an overall agreement of 100%.

CORRELATION ANALYSIS TABLE

		Reference Laboratory	
		Positive	Negative
IVD Tech.	Positive	26	
	Negative		94
	Total	**26**	**94**

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