IVD TECHNOLOGIES

Feline Coronavirus (FCoV) Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN FELINE SERUM OR PLASMA

Product Number: FIPV-1000

FIP FCoV IgG

INTENDED USE

The Feline Coronavirus Antibody Test Kit is used for the detection of Feline Coronavirus IgG antibody in feline plasma or serum.

ANALYTICAL PRINCIPLE

Diluted samples with Feline Coronavirus specific antibody is allowed to bind to microwell-bound FCoV antigen forming a FCoV antibody-antigen complex. After washing unbound materials, Goat anti-cat IgG-HRP is allowed to bind to the 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 reagents contain 0.05% ProClin 150. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	FCoV Antibody Test Kit	**FIPV-1000**	**96 Test**
SD	Sample Diluent [Contains: PBS, BSA, 0.05% ProClin 150]	**SDFEA-1001**	**35 ml**
A	Negative Control [Contains: Normal Feline Serum, 0.05% ProClin 150] Ready to Use	**FIPV-1001**	**1 ml**
B	Positive Control [Contains: Feline Serum Positive for FCoV Antibody, 0.05% ProClin 150] Ready to Use	**FIPV-1002**	**1 ml**
C	FCoV Microwell Plate [Contains: FCoV Antigen]	**FIPV-1003**	**96 Well Plate**
D	HRP Conjugate [Contains: anti-Cat IgG-HRP]	**FIPV-1004**	**10 ml**
WB	20X Wash Buffer Concentrate [Contains: Tris Buffer, 1% Tween-20, NaCl, 0.05% ProClin 150]	**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 in the foil pouch until further use.
- Ensure that the bottom surface of the microwells are clean before reading by wiping with low lint non abrasive paper towels.
- 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 samples by **1:25** by adding **10 µl** of sample to **240 µl** of Sample Diluent (**SD**) in a glass tube.
2. Vortex the tubes.
3. Dispense **100 µl** of the diluted samples, Negative Control (**A**), and Positive Control (**B**) into designated wells of the Microwell Plate (**C**).
4. Cover the wells.
5. Incubate the wells for **30 minutes** at **room temperature**.
6. Wash each well **3 times** with **300 µl** of the **1X Wash Buffer Preparation**. Tap microwells against clean paper towels to ensure no liquid remains.
7. Dispense **100 µl** of HRP Conjugate (**D**) to each well.
8. Cover the wells.
9. Incubate for **30 minutes** at **room temperature**.
10. Wash each well **3 times** with **300 µL** of the **1X Wash Buffer Preparation**. Tap microwells against clean paper towels to ensure no liquid remains.
11. Dispense **100 µl** of TMB Solution (**TS**) to each well.
12. Cover the wells.
13. Incubate the wells for **15 minutes** at **room temperature** and **protect from light**.
14. Dispense **100 µl** of Stop Solution (**SS**) to each well.
15. Read 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 154 cat samples were assayed; 105 and 49 samples were found to be negative and positive, respectively. An O.D. Cutoff of 0.273 was determined based on ROC and sensitivity and specificity analysis.

Sample to Negative Ratio Calculation

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

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

Sample to Negative Ratio Table

< 0.900	Negative
0.900 – 1.20	Borderline
> 1.20	Positive

CLINICAL CORRELATION ANALYSIS
Sample to Negative Ratio

	FCoV		
INDEX	POS	NEG	Total
Positive test ≥ 1.2	48	1	49
Negative test < 1.2	1	104	105
Total	49	105	154

METHOD CORRELATION ANALYSIS

A total of 183 cat samples were assayed by IVD Technologies' FCoV Antibody ELISA Test Kit and IFA. By ELISA, 57 samples were reported as positive with an OD of >0.40 and sample to negative ratio of >1.20. The titer for these samples ranged of 1:32 to 1:4,096. By IFA, 53 samples were reported as positive with a titer range of 1:64 to 1:25,600. By ELISA, 126 samples were reported as negative with an OD of <0.40 and sample to negative ratio of <1.20. By IFA, 130 samples were reported as negative with a titer range of <1:64. Five (5) samples were found to be positive by ELISA and negative by IFA. One (1) sample was reported negative by ELISA and positive by IFA. Kappa statistical analysis indicate a 0.967 agreement.

COMPARISON STUDY TABLES

	ELISA		
IFA	POS	NEG	Total
POS	52	1	53
NEG	5	125	130
Total	57	126	183

Agreement		95% CI	
POS	0.912	0.807	to 0.971
NEG	0.992	0.957	to 1.000
Observed Overall	0.967	0.930	to 0.988

REPRODUCIBILITY

A total of 6 samples were assayed in duplicates over 3 assays using the IVD Technologies' FCoV Antibody Test Kit. Three (3) samples were found to be negative, 1 sample was borderline, and 2 samples were positive. The sample to negative ratio, mean, standard deviation, and coefficient of variation was calculated for each sample. The CV ranged from 1.7 to 8% indicating good reproducibility.

Assay	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Assay 1	0.16	0.32	0.74	1.02	1.60	2.90
	0.13	0.33	0.71	1.00	1.62	3.04
Assay 2	0.14	0.33	0.68	1.05	1.67	3.12
	0.13	0.32	0.70	1.02	1.61	3.16
Assay 3	0.14	0.33	0.72	1.00	1.61	2.76
	0.15	0.32	0.71	1.02	1.64	2.84
Mean	0.14	0.33	0.71	1.02	1.624	2.968
SD	0.01	0.01	0.02	0.02	0.028	0.161
% CV	8.04	1.77	2.75	1.71	1.704	5.428

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