IVD TECHNOLOGIES

Feline Immunodeficiency Virus Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN FELINE SERUM OR PLASMA

Product Information: FIVG-1000

FIV

INTENDED USE

The Feline Immunodeficiency Virus Antibody Test Kit is used for the detection of Feline Immunodeficiency Virus IgG antibody in feline plasma and serum.

ANALYTICAL PRINCIPLE

Feline Immunodeficiency Virus IgG-specific antibody in diluted samples is allowed to bind to microwell-bound Feline Immunodeficiency Virus antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Feline Immunodeficiency 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. Do not use hemolyzed samples. 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 0.1% ProClin 150. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	FIV Antibody Test Kit	**FIVG-1000**	**96 Test**
SD	Sample Diluent [Contains: PBS, BSA, 0.1% ProClin 150]	**SDFEA-1001**	**35 ml**
A	Negative Control [Contains: Normal Feline Serum, 0.1% ProClin 150] See QC Certificate for value	**FIVG-1001**	**100 µl**
B	Positive Control [Contains: Feline Serum Positive for FIV IgG, 0.1% ProClin 150] See QC Certificate for value	**FIVG-1002**	**100 µl**
C	Microwell Plate [Contains: FIV Antigen]	**FIVG-1003**	**96 Well Plate**
D	HRP Conjugate [Contains: HRP Conjugate]	**FIVG - 1004**	**10 ml**
WB	20X Wash Buffer Concentrate [Contains: Tris Buffer, 0.01% Tween-20, NaCl, 0.1% 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

Materials 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)
- Laboratory Tape

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.
- Run the Controls (**A, B**) in duplicates from one dilution preparation. Each dilution (10µl Control into 240 µl Sample Diluent) yields enough diluted Controls for a duplicate in a run.

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 the samples, and Controls (**A, B**) by **1:25** by adding **10 µl** of the samples and Controls to **240 µl** of the Sample Diluent (**SD**) in a glass tube.
2. Vortex the tubes.
3. Dispense **100 µl** of the diluted Controls and samples (from step 1) 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 **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to ensure that no liquid remains.
7. Dispense **100 µl** of HRP Conjugate (**D**) to each well.
8. Cover the wells.
9. Incubate the wells for **30 minutes** at **room temperature**.
10. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**. Tap the 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 the wells in a microwell reader at **450 nm**.

PROCEDURAL NOTES

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

CALCULATION OF RESULTS

• Calculate the Sample to Negative Ratio for the Positive Control and each of the samples by using the equation provided in this section, below.

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 over the O.D. of the Negative Control.

Sample to Negative Ratio Table

< 0.900	Negative
0.900 – 1.20	Borderline
> 1.20	Positive

VALIDITY OF ASSAY

An assay is valid if the following criteria are met:
1. The OD of the Negative Control should be >0.20 but <0.550.
2. The Sample to Negative Ratio (SN Ratio) of the Positive Control should be >1.2.

If the assay fails to meet these criteria, then the assay is not valid and the results may not be reliable.

CORRELATION

A total of 138 samples were compared using IVD Technologies' kit and a reference laboratory. IVD Technologies' kit found 38 positive samples out of the 39 positive samples that the reference laboratory reported as positive. IVD Technologies' kit as well as the reference laboratory found 99 samples to be negative. One (1) sample was found to be negative by IVD Technologies' kit but positive by the reference laboratory.

IVD Technologies	Reference Laboratory Pos.	Reference Laboratory Neg.	*Total*
Pos.	38	0	*38*
Neg.	1	99	*100*
Total	*39*	*99*	*138*

SENSITIVITY AND SPECIFICTY

Using IVD Technologies' kit, 38 samples were found to be positive, out of 39 true positive samples; and 99 samples were found to be negative, out of 99 true negative samples. Based on these results, the sensitivity of the kit is calculated to be 97.4% and the specificity of the kit is calculated to be 100%.

TP (Sensitivity)	97.4%
TN (Specificity)	100%

REPRODUCIBILITY

Intra-assay

Five sample were assayed 18 times using IVD Technologies' kit. The SD and %CV were calculated using the Serial to Negative Ratios of the samples.

Sample	Mean S/N Ratio	SD	%CV
Positive	3.122	0.259	8.304
Sample 1	0.265	0.026	9.790
Sample 2	0.720	0.035	4.929
Sample 3	2.494	0.135	5.404
Sample 4	5.547	0.438	7.890

Inter-assay

Three (3) samples were assayed in 8 replicates in the same assay. The Mean, SD, and %CV were calculated for the ODs and Serial to Negative Ratios of the samples.

	Neg	Sample A		Sample B		Sample C	
	OD	OD	S/N	OD	S/N	OD	S/N
Mean	0.355	0.332	0.933	0.510	1.435	1.279	3.603
SD	0.007	0.033	0.083	0.019	0.037	0.030	0.075
%CV	1.946	9.962	8.943	3.761	2.613	2.339	2.081

REFERENCES

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