IVD TECHNOLOGIES

Canine Thyroglobulin Auto-Antibody Test Kit

FOR DETECTION OF THYROGLOBULIN AUTO-ANTIBODY IN CANINE SERUM OR PLASMA

Product Number: CTGA-1000

CANINE TgAA

INTENDED USE

The Thyroglobulin Auto-Antibody Test Kit is used for detection of Thyroglobulin Auto-Antibody (TgAA) in canine plasma or serum.

ANALYTICAL PRINCIPLE

Canine Thyroglobulin IgG-specific antibody in diluted samples is allowed to bind to microwell-bound canine thyroglobulin antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Thyroglobulin 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 EDTA 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, Cutoff Calibrator, Wash Buffer, and HRP-Conjugate contain 0.1% ProClin 150. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	Canine Thyroglobulin Auto-Antibody Test Kit	**CTGA-1000**	**96 Test**
SD	Sample Diluent	**SDFEA-1003**	**2 x 50 ml**
A	Cutoff Calibrator See QC Certificate for value	**CTGA-1001**	**1.5 ml**
B	Positive Control See QC Certificate for value	**CTGA-1002**	**1.5 ml**
C	Microwell Plate	**CTGA-1003**	**96 Well Plate**
D	HRP Conjugate	**CTGA-1004**	**10 ml**
WB	20X Wash Buffer Concentrate	**WBFEA-1001**	**50 ml**
TS	TMB Solution Keep away from light	**TMBS-1001**	**10 ml**
SS	Stop Solution	**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

- 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 samples by **1:100** by adding **10 µl** of samples to **990 µl** of Sample Diluent (**SD**) in a glass tube.
2. Vortex the tubes.
3. Dispense **100 µl** of the diluted samples, ready to use Cutoff Calibrator (**A**), and ready to use Positive Control (**B**) 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 **15 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

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

EXPECTED VALUES

Normal Sample Study

One-hundred and sixty (160) normal dog samples were assayed with IVD Technologies' Canine Thyroglobulin Auto-Antibody Test Kit. The O.D. ranged from 0.07 to 0.500.

Sample to Negative Ratio Calculation

Sample to Negative Ratio
= O.D. Sample / O.D. Cutoff Calibrator

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

Sample to Negative Ratio Table

< 0.900	Negative
0.900 – 1.20	Borderline
> 1.20	Positive

CORRELATION ANALYSIS

A total of 180 canine samples were assayed for thyroglobulin antibody using IVD Technologies' Canine Thyroglobulin Auto-Antibody ELISA Test Kit and a Reference Laboratory's Canine Thyroglobulin Auto-Antobody ELISA Test Kit. Fifteen (15) samples were found to be positive by IVD Technologies, while 14 samples were found to be positive by the Reference Laboratory. One-hundred and sixty-five (165) samples were found to be negative by IVD Technologies, while 166 samples were found to be negative by the Reference Laboratory.

Correlation Analysis Table
Reference Laboratory
CTgAA ELISA

IVD Technologies CTgAA ELISA	POSITIVE	NEGATIVE	Total
POSITIVE	13	2	**15**
NEGATIVE	1	164	**165**
Total	**14**	**166**	**180**

Assay Sensitivity and Specificity

Based on the correlation table, IVD Technologies' Canine Thyroglobulin Auto-Antibody ELISA Kit has a sensitivity of 92.9% and a specificity of 98.8%.

Sensitivity	92.9%
Specificity	98.8%

REPRODUCIBILITY

Intra-Assay

Three (3) samples were assayed in eight (8) replicates in a single assay. The mean, standard deviation, and CVs are listed in the table below.

Sample ID	1	2	3
Replicates	8	8	8
Mean	0.25	0.12	1.87
SD	0.0250	0.0030	0.107
CV	**10**	**3.1**	**5.7**

Inter-Assay

Three (3) samples were tested in six (6) assays. The mean, standard deviation, and CVs are listed in the table below

Sample ID	A	B	C
Assays	6	6	6
Mean	0.1	0.34	1.09
SD	0.001	0	0.046
CV	**1.03**	**1.32**	**4.27**

REFERENCES

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