

**Toxoplasma gondii Antibody Test Kit**FOR DETECTION OF IgG ANTIBODY IN CANINE SERUM OR PLASMA

Product Number: CTEIGG-1000

**INTENDED USE**

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

**ANALYTICAL PRINCIPLE**

Toxoplasma IgG-specific antibody in diluted samples is allowed to bind to microwell-bound Toxoplasma antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Toxoplasma 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, Cut-Off 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
	<b>Toxoplasma gondii Antibody Test Kit</b>	<b>CTEIGG-1000</b>	<b>96 Test</b>
<b>SD</b>	Sample Diluent [Contains: PBS, BSA, Bovine Protein 0.1% ProClin 150]	<b>SDFEA-1001</b>	<b>35 ml</b>
<b>A</b>	Negative Control [Contains: Normal Canine Serum, 0.1% ProClin 150] See QC Certificate for value	<b>CTEIGG-1001</b>	<b>1 ml</b>
<b>B</b>	Positive Control [Contains: Canine Serum Positive for Toxoplasma IgG, 0.1% ProClin 150] See QC Certificate for value	<b>CTEIGG-1002</b>	<b>1 ml</b>
<b>C</b>	Toxo IgG Microwell Plate [Contains: Toxoplasma gondii Antigen]	<b>EFTIGG-1003</b>	<b>96 Well Plate</b>
<b>D</b>	HRP Conjugate [Contains: HRP Conjugate, 0.1% ProClin 150]	<b>CTEIGG-1004</b>	<b>10 ml</b>
<b>WB</b>	20X Wash Buffer Concentrate [Contains: Tris Buffer, 0.01% Tween-20, NaCl, 0.1% ProClin 150]	<b>WBFEA-1001</b>	<b>50 ml</b>
<b>TS</b>	TMB Solution [Contains TMB] Keep away from light	<b>TMBS-1001</b>	<b>10 ml</b>
<b>SS</b>	Stop Solution [Contains:	<b>SSFEA-</b>	<b>10 ml</b>

1N Sulfuric Acid]

1001

**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.

**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 **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 Negative Control (**A**), Positive Control (**B**), and diluted 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 (20°C - 23°C)**.
6. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to remove any liquid that 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 (20°C - 23°C)**.
10. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to remove any liquid that 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 (20°C - 23°C)** and **away 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**

**Reference Range**

A total of 94 dog samples were assayed using IVD Technologies' Canine Toxoplasma IgG ELISA Kit; 78 and 16 samples were found to be normal and positive, respectively. The OD of the normal and positive samples ranged from 0.06 to 0.360. An OD cut-off of 0.40 was determined based on ROC and sensitivity and specificity analysis. The cut-off for the Antibody Index Value is 1.20.

**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

**CORRELATION ANALYSIS**

A total of 94 dog samples were assayed for Toxoplasma IgG antibody using the IVD Technologies' Canine Toxoplasma IgG ELISA Kit and a reference laboratory. Both laboratories found 78 samples to be normal and 16 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			Total
		Positive	Borderline	Negative	
IVD Tech	Positive	16			16
	Borderline				0
	Negative			78	78
	Total	16	0	78	94

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