IVD TECHNOLOGIES

Toxoplasma gondii Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN FELINE SERUM OR PLASMA

Product Number: EFTIGG-1000

FELINE TOXO IgG

INTENDED USE

The Toxoplasma gondii Antibody Test Kit is used for the detection of Toxoplasma gondii IgG antibody in feline 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.
- 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
	Toxoplasma gondii Antibody Test Kit	**EFTIGG-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] See QC Certificate for value	**EFTIGG-1001**	**1 ml**
B	Positive Control [Contains: Feline Serum Positive for Toxoplasma IgG, 0.05% ProClin 150] See QC Certificate for value	**EFTIGG-1002**	**1 ml**
C	Microwell Plate [Contains: Toxoplasma gondii Antigen]	**EFTIGG-1003**	**96 Well Plate**
D	HRP Conjugate [Contains: Animal Proteins, HRP Conjugate]	**EFTIGG-1004**	**10 ml**
WB	20X Wash Buffer Concentrate [Contains: Tris Buffer, 0.05% ProClin 150, 1% Tween-20, NaCl]	**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.

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** (**20ºC - 23ºC**).
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** (**20ºC - 23ºC**).
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** (**20ºC - 23ºC**) 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 119 cat 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 1.459, respectively. An OD cutoff 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 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 140 cat samples were assayed for Toxoplasma IgG antibody using IVD Technologies' Toxoplasma gondii Antibody Test Kit and a reference laboratory. Both laboratories found 114 samples to be negative 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	Borderline	Negative	**Total**
IVD Tech	Positive	26			**26**
	Borderline				**0**
	Negative			114	**114**
	Total	**26**	**0**	**114**	**140**

REFERENCES

1. Aubert, D., Maine, G.T., Villena, I., Hunt, J.C., Howard, L., Sheu, M., Brojanac, S., Chovan, L.E., Nowlan, S.F., Pinon, J.M., 2000. Recombinant antigens to detect Toxoplasma gondii-specific immunoglobulin G and immunoglobulin M in human sera by enzyme immunoassay. J. Clin. Microbiol. 38, 1144–1150.
2. Boldbaataar, D., Xuan, X., Kimbita, E., Huang, X., Igarashi, I., Byambaa, B., Battsetseg, B., Battur, B., Battsetseg,G., Batsukh, Z., Nagasawa, H., Fujisaki, K., Mikami, T., 2001. Detection of antibodies to Hypoderma lineatum in cattle by Western blotting with recombinant hypodermin C antigen. Vet. Parasitol. 99, 151–158.
3. Burg, J.L., Perelman, D., Kasper, L.H.,Ware, P.L., Boothroyd, J.C., 1988. Molecular analysis of the gene encoding the major surface antigen of Toxoplasma gondii. J. Immunol. 141, 3584–3591.
4. Furuya, T., Kawaguchi, Y., Miyazawa, T., Fujikawa, Y., Tohya, Y., Azetaka, M., Takahashi, E., Mikami, T., 1990. Existence of feline immunodeficiency virus infection in Japanese cat population since 1968. Jpn. J. Vet. Sci. 52, 891–893.
5. Furuya, T., Hasegawa, A., Miyazawa, T., Miki, K., Mikami, T., 1992. Detection of anti-gag antibodies of feline immunodeficiency virus in cat sera by enzyme-linked immunosorbent assay. Arch. Virol. 124, 355–361.
6. Handman, E., Goding, J.W., Remington, J.S., 1980. Detection and characterization of membrane antigens of Toxoplasma gondii. J. Immunol. 124, 2578–2583.
7. Harning, D., Spenter, J., Metsis, A., Vuust, J., Petersen, E., 1996. Recombinant Toxoplasma gondii surface antigen 1 (P30) expressed in Escherichia coli is recognized by human Toxoplasma specific Immunoglobulin M (IgM) and IgG antibodies. Clin. Diagn. Lab. Immunol. 3, 355–357.
8. Iwata, H., Yamamoto, M., Hasegawa, A., Kurata, K., Inoue, T., 2000. Expression of interleukin-2 in Escherichia coli. J. Vet. Med. Sci. 62, 1101–1104.
9. Kasper, L.H., Bradley, M.S., Pfefferkorn, E.R., 1984. Identification of stage-specific sporozoite antigens of Toxoplasma gondii by monoclonal antibodies. J. Immunol. 132, 443.
10. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227, 680–685.

Manufacturer
Made in the USA

IVD Technologies
2002 S. Grand Avenue, Suite A
Santa Ana, CA 92705 USA
Tel: 1(714)549-5050
Fax: 1(714)549-5055
info@ivdtechnologies.com
www.ivdtechnologies.com