

Toxoplasma gondii (TOX) IgG antibody ELISA test kit

**INSTRUCTION MANUAL
FOR PRODUCT No: LT23003AYSL**



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MANUAL VERSION 1.01

PRINCIPLE

This kit use indirect ELISA method to test specific *Toxoplasma gondii* antibody in animal serum or plasma, it can be used as animal toxoplasmosis secondary inspection. If there is *Toxoplasma gondii* antibody in the sample, it will combine with coated *Toxoplasma* antigen on plate and SPA enzyme conjugate on the second step. The color reaction will reflect whether there is toxoplasma antibody in the sample.

MATERIALS SUPPLIED WITH THIS KIT

1	TOX-Ag coated plates	96T	7	Stop solution	15 ml
2	Enzyme conjugate	6 ml	8	Positive control	1 ml
3	10X concentrated Washing buffer	100 ml	9	Negative control	1 ml
4	Substrate A	15 ml	10	Adhesive Foil	3 pieces
5	Substrate B	15 ml	11	Instruction	1 piece
6	Sample diluent	100 ml	12	Serum diluent plate	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

- 1) Microplate Reader (wave length: 450/630 nm).
- 2) 37 °C incubator.
- 3) Micropipettes, adjustable.

SAMPLE REQUIREMENT

This kit is used only for dog, cat, rabbit and monkey serum and plasma. Collect

serum by regular method, the serum need to be clear, no hemolysis, no pollution. For short-term storage, the sample can store at 2-8°C, for long-term storage, at -20°C.

TEST PROCEDURE

1) Washing Solution preparation: Dilute 10X Washing solution with distilled water or deionized water at 1:10 (for example: take 100ml 10X Washing solution, add 900ml distilled water or deionized water, mix),mix it evenly to get washing solution.

2) Sample diluent and adding sample: Dilute serum with Sample diluent at 1:100, Add 100µl diluted serum sample into all sample wells; If using Serum diluent plate, the dilution method is like following:

Dilute at 1:100: Firstly add 50µl sample diluent into the coated sample wells. On Serum diluent plate, add 245µl Sample diluent, then add 5µl serum, use pipette to mix it evenly, then take 50µl in to coated sample wells for react. (Note: Serum diluent plate is disposable, cannot repeat use).

3) Add controls: Positive control and negative control do not need dilute, add directly. Set 2 blank controls, only add sample diluent, 100µl/well. Set 2 wells for negative control, only add 100µl negative control; 2 wells for positive control, only add 100µl positive control. Cover and **Incubate in dark at 37 °C for 30 minutes.**

4) Washing plate: Discard the liquid of the well, add washing solution into each well fully (250µl/well if using washing machine) for 3 times, incubate for 1 min each time, at last time flap to dry with the absorbent paper.

5) Adding Enzyme conjugate: Add 50µl Enzyme Conjugate into all wells (except blank well) and **incubate at 37 °C in dark for 30 minutes.** Discard the contents of

the wells and wash 3 times as described in step 3.

6) Adding substrates: according to quantity needed, Add equal volume of Substrate A and Substrate B, mix evenly. Add 100 μ l into all well, **incubate at 37 °C in dark for 10 minutes.**

7) Add 50 μ l Stop solution into all wells to stop reaction. Set zero at blank control, read the OD value at ELISA reader 450nm (630nm as a reference).

RESULTS INTERPRETATION

1) Cut-off value (COV)=Average OD value of negative control x 2.1 (calculate as 0.08 when the OD value of negative control is less than 0.08)

2) Validation judgement:

OD value of negative control \leq 0.15 (If $>$ 0.15, invalid);

OD value of positive control \geq 0.50 (If $<$ 0.50, invalid);

RESULTS

Sample OD value \geq COV, the result is Positive;

Sample OD value $<$ COV, the result is Negative;

LIMITATION

This kit is only for Screening, not basis for diagnosis..

NOTES

- 1) Microplate removed from the refrigerated environment should be balance to dry at room temperature, and seal the unused microplate with desiccant.
- 2) Before adding reagent, gently shake dropping bottle and mix even the reagent.
- 3) When incubation, must cover the plate with adhesive foil, do not repeat use the

adhesive foil.

- 4) Screw down the cap after use, don't mix caps between different bottles and don't mix components between different kits
- 5) The test kit and any samples should be regarded as the source of infection to properly handle. Stop solution is corrosive, be careful to use.

Specifications: 96 wells/kit. **Expiry date:** 12 months.

Storage: Storing at 2-8°C, in the dark.