VERSION 1.01

TOXOPLASMA GONDII ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Toxoplasma Gondii Antibody ELISA Kit For Pet

Catalogue Number. IP100199.

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.

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	TOX-Ag Coated plates	96 wells
2	Enzyme Conjugate	6 ml
3	10X Washing solution	100 ml
4	Substrate A	15 ml
5	Substrate B	15 ml
6	Sample diluent	100 ml
7	Stop solution	15 ml
8	Positive control	1 ml
9	Negative control	1 ml
10	Adhesive Foil	3 pieces
11	Instruction sheet	1 piece
12	Serum diluent plate	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample requirement

1) This kit is used for only dog, cat, rabbit and money etc. serum and plasma

2) To get the best test result, avoid using sample with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
3) The serum sample store at 2-8 ℃ for 3 days, if for long term, it should be kept at -20℃ or lower, avoid repeated freezing and thawing**.**

Operation procedures

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 50ul sample diluent into the coated sample wells. On Serum diluent plate, add 245ul Sample diluent, then add 5ul serum, use Pipette to mix it evenly, then take 50ul
Into coated sample wells for react. (Note: Serum diluent plate is disposable, can not repeat use.)
3) Add controls: Positive control and negative control do not need dilute, add directly. Set 2 blank controls, only add sample diluent,100ul/well. Set 2 wells for negative control, only add 100ul negative control; 2 wells for positive control, only add 100ul positive control. Cover and Incubate in dark at 37 ℃ for 30 minutes.
4) Washing plate: Discard the liquid of the well, add washing solution into each well fully(250ul/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 50ul Enzyme Conjugate into all wells (except blank well) and incubate at 37℃ 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 100ul into all well, incubate at 37℃ in dark for 10 minutes.
7) Add 50ul 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 ≤ 0.15 (If ＞ 0.15, invalid);
OD value of positive control ≥ 0.50 (If ＜ 0.50, invalid);

Results

Sample OD value ≥ COV, the result is Positive;
Sample OD value < COV, the result is Negative;
4) Limitation: This kit is only for Screening, not basis for diagnosis.

Notes

1.MicroWell plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 2~8 ℃.
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.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.