Toxoplasma gondii IgA ELISA Kit

Catalog Number KA1462

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

Toxoplasma gondii IgG ELISA Kit is for the detection of IgA antibodies to Toxoplasma gondii in human serum and plasma.

Principle of the Assay

Toxoplasma gondii IgA ELISA Kit is a solid-phase immunoanalytical test. The purified, homogeneous antigen is fixed to each well of the microtiterstrips. Specific antibodies present in the patient's sample are bound during the first incubation step. After removing unbound material by washing, the presence of the specific antibodies is detected using anti-human IgA conjugate during the second incubation. The unbound peroxidase conjugate is then removed and TMB substrate is added, resulting in the development of a blue colour. The enzyme reaction is terminated by addition of the stop solution. The intensity of the colour is proportional to the concentration of the antibodies in the sample.

Flow Chart

Step 1.	Prepare reagents and samples
	↓
Step 2.	Dispense 100 µL/well controls and samples
	↓
	Incubate 30 minutes at room temperature
	↓
	Wash 4 times (300 µL/well), aspirate
	↓
Step 3.	Dispense 100 µL/well of peroxidase conjugate
	↓
	Incubate 30 minutes at room temperature
	↓
	Wash 4 times (300 µL/well), aspirate
	↓
Step 4.	Dispense 100 µL/well of chromogenic substrate (TMB)
	↓
	Incubate 10 minutes at room temperature
	↓
Step 5.	Dispense 100 µL/well of Stop solution
	↓
Step 6.	Read the absorbance at 450 nm within 10 minutes

General Information

Materials Supplied

List of component

Component	Amount
ELISA break-away strips coated with the specific antigen	96 wells
Negative control r.t.u. [1)]	1.2 mL 1 vial
Positive control r.t.u.	1.2 mL 1 vial
Cut-off control r.t.u.	1.2 mL 1 vial
Peroxidase conjugate (anti-IgA/Px) r.t.u.	12 mL 1 vial
Wash buffer 25x concentrated	80 mL 1 vial
Dilution buffer r.t.u.	100 mL 1 vial
Chromogenic substrate TMB r.t.u.	13 mL 1 vial
Stop solution r.t.u.	15 mL 1 vial
Cover membrane	3
Bag with zipper + desiccant	1

[1)] r.t.u., ready to use

Storage Instruction

- ✓ Store the kit and the kit reagents at 2 to 8°C in a dry place and protected from the light, avoid from freezing.
- ✓ Use only intact vacuum-sealed strips. Store unused strips in the sealable pouch and keep the desiccant inside. These strips are then stable for 4 weeks.
- ✓ Unused diluted washing buffer is stable for 4 weeks when stored at 2°C to 8°C.
- ✓ Suitable specimens are serum or plasma (heparinized) samples obtained by standard techniques. The samples should not be heat-inactivated since non-specific results may occur. Store the unused undiluted tested samples in aliquots at -18°C to -28°C. Repeated freezing a thawing is not recommended. If you wish to store serum samples at 2°C to 8°C use them within one week.
- ✓ Do not store diluted samples, use them immediately.
- ✓ Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration.
- ✓ If you find damage at any part of the kit, please inform the manufacturer immediately.
- ✓ Expiration date is indicated at the ELISA kit label and at all reagent labels.

Materials Required but Not Supplied

Distilled or deionised water, test tubes for sample dilution, timer, micropipettes, multipipettes 10-1000 µl, graduated cylinder, ELISA washer or multichannel pipette, ELISA reader (450 nm/ reference wavelength

630/620 nm), paper towels, pipette tips.

Precautions for Use

- Safety Precautions

✓ All ingredients of this kit are intended for laboratory use only.

✓ Only qualified and well-trained employees should carry out the assay procedure.

✓ Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

✓ Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

✓ Autoclave all reusable materials that were in contact with human samples for 1 hour at 121℃, or disinfect with 3% chloramines for 30 minutes.

✓ Decontaminate liquid wastes with disinfection solution (Incidure, Incidine, chloramine). Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

✓ Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

✓ Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.

- Handling Precautions

✓ If user modifies the assay procedure mentioned in this Instruction manual then the user has to validate that method and be responsible for its use.

✓ Manufacturer guarantees performance of the entire ELISA kit. Washing solution 25x conc., Stop solution r.t.u. a dilution buffer r.t.u can be used in Toxoplasma gondii IgG and IgA ELISA Kit. The TMB solution r.t.u. is interchangeable only with the same lot on the bottle.

✓ Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents.

✓ Peroxidase conjugate and Sample diluent are conserved with 0.049% Thiomersal.

✓ Negative control and Calibrators are conserved with 0.095% sodium azide.

✓ Avoid contact of the TMB with oxidizing agents or metal surfaces.

✓ Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:

* Insufficient mixing of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique or spilling the rim of well with sample or Peroxidase conjugate
* Use of identical pipette tip for different solutions

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature.
- ✓ Prepare Wash buffer by diluting the Wash buffer concentrate (Wash 25x) 25 times with an appropriate volume of distilled or deionised water (e.g. 40 mL of the concentrated Wash buffer + 960 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 to 37℃ in a water bath. Diluted Wash buffer is stable for 4 weeks if stored at 2 to 8℃.
- ✓ Do not dilute the Controls, Peroxidase conjugate, Chromogenic substrate and Stop solution, they are ready to use.

Sample Preparation

- ✓ Vortex samples, Negative control, Positive control, Cut-off control and Peroxidase conjugate in order to ensure homogeneity and mix all solution well prior use.
- ✓ Dilute serum samples 1:100 in Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer).

Assay Procedure

1. Allow the vacuum-closed aluminium bag with strips to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided bag and seal it carefully with the desiccant kept inside.
2. Pipette 100 µL of Sample diluent, Controls and serum samples to the wells according to the pipetting scheme in Plate Layout: fill the first well with Dilution buffer (DIL) to determine the reaction background. Fill the next two wells with Cut-off control (CUTOFF). The next wells fill with Negative control (CONTROL-) and Positive control (CONTROL+). The remaining wells fill with diluted serum samples (S1...). It is satisfactory to apply one serum into one well (S1, S2, S3...). However, if you want to minimize a laboratory error, apply controls and samples as doublets. Cover the strips with the Cover membrane or cover.
3. Incubate 30 minutes (+/- 2 min.) at room temperature.
4. Aspirate the liquid from wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 300 µl/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
5. Add 100 µL Peroxidase conjugate r.t.u. into each well. Incubate 30 minutes (+/- 2 min) at room temperature.
6. Aspirate and wash four times with 300 µl/well of Wash buffer.

7. Dispense 100 µl of TMB substrate (TMB) into each well. Incubate 10 minutes (+/- 5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with opaque cover or keep them in the dark during the incubation with TMB.
8. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.
9. Read the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 630 (620) nm.

Data Analysis

Calculation of Results

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL well) from the absorbances of all other wells.

- Processing of results for Qualitative interpretation

✓ Compute the mean absorbance of the two wells of Cut-off (CUTOFF).

✓ Compute the Cut-off value. The Cut-off value is calculated from the absorbance of the CUTOFF and the absorbance of the Negative control.

Cut-off value = OD CUTOFF + OD CONTROL-

✓ Define the Cut-off range:

Cut-off range = Cut-off value +/- 10%

sample OD value < Cut-off value – 10% Negative Result

sample OD value > Cut-off value + 10% Positive Result

✓ The result is equivocal if a sample OD value ≥ Cut-off value – 10% and ≤ Cut-off value + 10 %.

The samples with equivocal results should be retested. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain new sample from the patient.

- Processing of results for Qualitative interpretation using Positivity index value

Determine the Positivity Index for each serum sample as follows:

✓ Define the Cut-off value (see the previous paragraph)

✓ Compute the Positivity Index according to the following formula:

$$\text{Sample Positivity Index} = \frac{\text{Sample OD value}}{\text{Cut-off value}}$$

✓ Determine the serum reactivity according to the following table:

Index value	Interpretation
< 0.9	Negative (-)
0.9 – 1.1	Equivocal (+/-)
> 1.1	Positive (+)

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain new sample from the patient.

Performance Characteristics

- Validity of the test

The test is valid if:

✓ The background of the reaction (the absorbance of the DIL well) is less than 0.100.

- ✓ The mean of Cut-off control (CUTOFF) absorbances is higher than 0.200.
- ✓ OD values of Negative control (CONTROL-) is less than 0.100.
- ✓ Index value of Positive control should be ≥ 1.5 (OD CONTROL+/Cut-off value ≥ 1.5).

- Precision and reproducibility of the test

  The intraassay variability (within the test) and the interassay variability (between tests) were performed with samples of variable absorbance values.

- Intraassay variability

  (n = number of parallels):

n	A	SD	CV
22	0.532	0.029	5.5 %
22	1.278	0.058	4.5 %

- Interassay variability

  (n = number of paralells):

n	A	SD	min – max	CV
10	0.529	0.042	0.401 – 0.658	7.9 %
10	1.301	0.097	1.009 – 1.595	7.5 %
10	1.985	0.133	1.583 – 2.387	6.7 %

- Interaction

  Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our experience they have no influence on results.

Resources

References

1. Gros, U. et al.: Immun. Insekt. 20, 151-154 (1992).
2. Janitschke, K.: Klin. Lab. 40, 1059-1064 (1994).

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL	S 4										
B	CUTOFF	S 5										
C	CUTOFF	S ...										
D	CONTROL -											
E	CONTROL +											
F	S 1											
G	S 2											
H	S 3											