

Human Toxoplasma IgG ELISA Kit (JN203018)

MATERIALS PROVIDED & STORAGE CONDITIONS

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

* Provided this is within the expiration date of the kit.

Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the TOX-IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified TOX-antigen. Samples are added to the ELISA Microtiter plate wells and the TOX antibody in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgG antibody is added to each well and react with the compound to form "TOX antigen-TOX antibody-HRP antibody" compound. The TMB substrate is added to initiate the color developing reaction. The presence of TOX-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

Other materials required but not supplied

Micro-plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37°C Incubator or water bath

Deionized water

Absorbent paper

Loading slot for Wash Buffer

Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the JN203018. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other JN203018 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store at 2-8°C. Avoid freeze.

Please store the opened plate at 2-8°C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum:** Use the conventional method to prepare serum. Fresh collected serum specimens should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not precipitated fully.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood lipids, cholerythrin, hemoglobin, rheumatoid factors positive samples and AFP positive samples may not affect the results. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, EB, HSV, RV and related diseases, will not affect the results.
3. Samples can be stored at 2-8°C for one week and stored at -20°C for more than a week. Avoid freeze-thaw cycles.
4. **Wash Buffer:** Dilute the **20×Concentrated Wash Buffer** for 20 times with distilled water.

Assay procedure

Bring all reagents to room temperature for 30 min. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** Number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control (empty), 3 wells for negative control and 1 well for positive control. **Samples need test in duplicate.**
2. **Add sample:** Add 100 µL **positive/negative control** to positive/negative control well. (Blank well is not necessary for dual-wavelength detection). Dilute the tested serum with **Sample Diluent** at 1:10 (add 100 µL of Sample Diluent to the reaction well, and then add 10 µL of serum sample), mix fully.
3. **Incubate:** Cover the plate sealer and mix thoroughly, incubate at 37°C for 30 min.
4. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time.
5. **HRP conjugate:** Add 100 µL of **HRP Conjugate** to each well except the blank control well.
6. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37°C.
7. **Wash:** Repeat step 4 for washing.
8. **Add substrate:** Add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 10 min at 37°C in shading light.
9. **Stop reaction:** Add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **OD Measurement:** Set the Micro-plate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

Reference value

Normally, Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08 . Positive control (PC) $A_{450} > 0.30$. Negative control (NC) $A_{450} < 0.08$.

Interpretation of the results

Use each test result independently. Determine the result according to the Cut Off value.

Calculate the Cut Off: Cut Off (C.O) = $0.10 + A$ value of average negative control (NC) (when A_{450} of average NC < 0.05 , calculate at 0.05; while A_{450} of average NC ≥ 0.05 , calculate at the actual value).

1. Positive result: A_{450} of Sample $\geq$ Cut Off.
2. Negative result: A_{450} of Sample $<$ Cut Off.
3. Negative result indicates no TOX-IgG antibody detected in samples, while positive result is just the opposite.
4. The positive result of TOX-IgG antibody is an important index for TOX infection.

Limitations of this test method

1. This test is only used as the qualitative detection of TOX-IgG antibodies in serum of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.