# Human Toxoplasma (TOX) IgG Avidity ELISA Kit

Catalog No: E-HD-E205

96T

| | |
|---|---|
| **Version Number:** | V1.1 |
| **Replace version:** | V1.1 |
| **Revision Date:** | 2026.02.04 |

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the TOX-IgG Avidity 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 control solution and the dissociation solution is added to each well. After the incubation reaction, the low-affinity Toxoplasma IgG antibodies were separated from the antigen under the action of the dissociation buffer. Free components are washed away. The HRP conjugate is added to each well and react with the compound to form "TOX antigen-TOX antibody-HRP antibody" compound, the substrate reagent is added to initiate the color developing reaction. After colorimetry on the microplate reader, the avidity index (AI) of the sample to be tested is calculated based on the ratio of the absorbance of the dissociation well to that of the parallel control well.

## Kit components

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

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

## 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℃ Incubator or water bath

Deionized or distilled 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. FOR RESEARCH USE ONLY. 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 tested sample should be kept fresh.
7. The results shall depend on the readings of the micro-plate Reader.
8. **Each reagent is optimized for use in the E-HD-E205. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E205 with different lot numbers.**
9. Do not use components from different batches of kit.

## Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, 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. Samples can be stored at 2-8℃ for one week and stored at -20℃ for more than a week. Avoid freeze-thaw cycles.
3. The specific-TOX-IgG in the sample: A450/630 ≥ 0.150.
4. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

## Assay procedure

Restore all reagents and samples to room temperature (25±2℃) before use. 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℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for Low/High Avidity Positive Control. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 100 μL of **Low/High Avidity Positive Control** respectively to **Control** wells.
   b) Add 100 μL of **Sample Diluent** to sample well , then add 10 μL of **Serum** sample.
3. **Incubate:** Gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with plate sealer. Incubate for 30 min at 37℃ in shading light.
4. **Wash:** Remove the plate sealer and aspirate the liquid of each well. The washing procedure for 1 time with **Wash Buffer** and immerse for 30-60 sec. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Add dissociation solution and control solution:**
   a) Add 100 μL of **Dissociation Solution** respectively to **Low Avidity Positive Control** wells.
   b) Add 100 μL of **Control Solution** respectively to **High Avidity Positive Control** wells.
6. **Incubate:** Cover the ELISA Microtiter plate with sealer. Incubate for 10 minutes at 37℃ in shading light.
7. **Wash:** 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. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
8. **HRP conjugate:** Add 100 μL of **HRP Conjugate** to each well.
9. **Incubate:** Cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37℃ in shading light.
10. **Wash:** Repeat step 7 for washing.
11. **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 plate sealer. Incubate for 15 min at 37℃ in shading light.
12. **Stop reaction:** Add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
13. **OD Measurement:** Set the Micro-plate Reader dual wavelength at 450 nm/630 nm to detect A value of each well. **Note: Read the results within 10 min.**

## Reference value

1. If the average OD value of High Avidity Positive Control ≥ 0.150, it indicates that the sample contains TOX-IgG and the avidity calculation can be performed. If average OD value of High Avidity Positive Control < 0.150, it means that the sample does not contain TOX-IgG or the content of TOX-IgG is not detectable, and the avidity calculation cannot be conducted.
2. Experimental validity determination: The Low/High Avidity Positive Control serve as internal controls for the reliability of the experiment, and must be performed in each experiment. It is required, Average OD value of Low Avidity Positive Control > 0.20, and the avidity index < 40%; that the Average OD value of High Avidity Positive Control > 1.50, and the avidity index > 60%.

## Interpretation of test results

Use each test result independently.

The avidity index of TOX-IgG (RAI) : (Average OD value of Low Avidity Positive Control / Average OD value of High Avidity Positive Control) × 100%.

1. Low avidity result: RAI of Sample < 40%.
2. High avidity result: RAI of Sample ≥ 60%.
3. Indeterminate result: 40% ≤ RAI of Sample < 60%.
4. Indeterminate samples should be re-tested after 2 weeks, or other methods can be used for confirmation.

## Limitations of test method

1. 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.