Human Aspergillus Thioredoxin Reductase (ATR) IgG ELISA Kit

Catalog No: E-HD-E215

96T

Version Number: V1.1

Replace version: V1.0

Revision Date: 2026.03.20

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.

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Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Aspergillus Thioredoxin Reductase (ATR) IgG in human serum and plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with genetic engineering ATR antigen. Samples are added to the ELISA Microtiter plate wells and the ATR-IgG 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 “antigen- antibody-HRP antibody” compound. The TMB substrate is added to initiate the color developing reaction. The presence of ATR-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	0.3 mL
Negative Control	0.3 mL
HRP Conjugate	10 mL
Sample Diluent	60 mL
20×Concentrated Wash Buffer	30 mL * 2
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 water

Absorbent paper

Notes

1. 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.
2. 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.
3. 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.
4. Concentrated washing liquid at low temperature condition is easy to crystallize, it should be adjusted to room temperature to dissolve completely before use.
5. Each well must be filled with liquid when washing to prevent residual free enzyme.
6. **Each reagent is optimized for use in the E-HD-E215. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E215 with different lot numbers.**
7. The results shall depend on the readings of the Micro-plate Reader.
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℃. 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.

Experimental preparation

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

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components at once!

Solution 1: **Wash Buffer**

The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

Sample preparation

The following are general guidelines for sample collection and storage only. Sodium azide shall not be used as a preservative during the collection and storage of all samples. If samples are not analyzed immediately, they should be aliquoted and stored frozen, with repeated freeze-thaw cycles avoided.

1. **Cell culture supernatant:** Remove precipitates by centrifugation. Analyze immediately or aliquot and store frozen at −20 °C.
2. **Serum:** Collect blood in clean tubes. Allow to clot at room temperature for 30 minutes, then centrifuge at 2000×g for 20 minutes and collect serum. Analyze immediately or aliquot and store frozen at −20 °C.

3. **Plasma:** Use heparin, citrate, or EDTA as anticoagulant. Centrifuge at 2000×g for 20 minutes at 2–8 °C within 30 minutes after blood collection. To eliminate platelet interference, further centrifugation at 10000×g for 10 minutes at 2–8 °C is recommended. Analyze immediately or aliquot and store frozen at −20 °C.
4. **Cell lysate:** For adherent cells: remove culture medium, wash once with PBS, normal saline, or serum- free medium. Add an appropriate volume of lysis buffer and pipette up and down several times to ensure full contact with cells. Cells are typically lysed within 10 seconds.For suspension cells: collect cells by centrifugation, wash once with PBS, normal saline, or serum- free medium. Add an appropriate volume of lysis buffer, pipette to disperse cells, and tap gently to ensure complete lysis.After thorough lysis, centrifuge at 10000–14000×g for 3–5 minutes and collect the supernatant. Analyze immediately or aliquot and store frozen at −20 °C.
5. **Urine:** Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If precipitates form, centrifuge again.

Assay procedure

Restore all reagents and samples to room temperature 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 negative control, 2 wells for positive control and 1 well for blank control respectively. **Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **positive/negative control** to the control wells, add 50 μL of sample to the sample wells, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
3. **Incubate:** gently tap the plate to ensure thorough mixing, cover the plate sealer, incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 20 s intervals/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).
5. **HRP conjugate:** add 100 μL of **HRP Conjugate** to each well except the blank control well.
6. **Incubate:** cover the plate sealer and incubate at 37℃ for 30 min in shading light.
7. **Wash:** repeat step 4 for washing.
8. **Color Development:** add 100 μL of **Substrate Reagent A** and **Substrate Reagent B** mixture. (**Substrate Reagent A** and **Substrate Reagent B** are mixed 1:1 according to volume, must be fully mixed, the mixture is used within 15 minutes, avoid the use of metal container, avoid stirring reagents.) Gently oscillate for 10 s to mix thoroughly. Incubate 37℃ for 15 min at in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. **OD Measurement:** set the Micro-plate Reader wavelength at 450 nm to detect A value of each well.

Reference value

Normally, average A value of positive control (PC): $A_{450} > 0.80$ and average A value of negative control (NC): $A_{450} < 0.25$.

Interpretation of test results

Cut Off (C.0) = 0.35 + negative control (NC) average A value.

1. Positive result: average A value of sample > Cut Off.
2. Negative result: average A value of sample < Cut Off.
3. Negative result indicates no ATR-IgG detected in samples, while positive result means the opposite.

Limitations of test method

1. This test is only used as the qualitative detection of ATR-IgG 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.

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