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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Human Aspergillus Thioredoxin Reductase (ATR) IgG ELISA Kit

Catalog No: E-HD-E140

96T/96T*2

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 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	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

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-E140. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E140 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.

Sample preparation

1. **Serum**: Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated. Avoid of samples with hyperlipidemia, hemolysis or jaundice, although the negative effects were not found in our tests. Obviously contaminated samples can't be detected.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment in general.
3. Do not use heated inactivated samples. Heat inactivation will degrade antibodies.
4. Samples can be stored at 2-8℃ for one week. If samples not tested in a week, store them at -20℃ and avoid freeze-thaw cycles.
5. Bring all reagents to room temperature (25℃) for more than 30 min before use. Freezing samples should be mixed fully before test.
6. **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.

Assay procedure

Restore all reagents and samples to room temperature (25℃) 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 3 wells for negative control, 1 well for positive control and 1 well for blank control respectively. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 100 μL of **positive/negative control** respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
   b) Dilute the tested serum with **Sample Diluent** at 1:20 (add 100 μL of Sample Diluent to the reaction well, and then add 5 μL of sample), mix fully.
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, 30-60 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 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37℃ 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 (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min**.

Reference value

Normally, blank well (just substrate agent and stop solution) absorbance: A450 ≤ 0.08;
positive control (PC): A_{450} > 0.80 and average A value of negative control (NC): A_{450} < 0.1.

Interpretation of test results

Cut Off (C.0) = 0.10 + negative control (NC) average A value (when NC average A_{450} < 0.05, calculate at 0.05; while NC average A_{450} ≥ 0.05, calculate at the actual 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.