Alpha-Toxin gene of clostridium perfringens Antibodies ELISA Kit

Catalog No: E-AD-E141

96T/96T*3/96T*5

Version Number:	V1.2
Replace version:	V1.1
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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
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

Alpha-Toxin gene of clostridium perfringens (CP-α) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect CP-α-Ab in serum of sheep. During the experiment, add control and samples into the ELISA Microtiter plate. If CP-α-Ab exist in the samples, it will be bound with the antigen on the ELISA Microtiter plate. Then wash the plate to remove unbound components, add the HRP conjugate to specifically bind with the compound of antibody and antigen on the microtiter plate. The unbound HRP Conjugate will be removed by washing. Substrate Reagent is added into the well, it will react with the enzyme and become blue. The color shade is positive correlation with CP-α-Ab levels in the samples. At last, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well by using a Microplate Reader with 450 nm wavelength, then we can judge whether CP-α-Ab exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Negative Control	0.5 mL
Positive Control	0.5 mL
HRP Conjugate	10 mL
20×Concentrated Wash Buffer	25 mL
Sample Diluent	6 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.

Experimental instrument

Microplate 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 performed. 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 contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the E-AD-E141. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E141 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

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.

Reagent preparation

1. **Serum:** Use the conventional method to prepare animal serum, the serum collection tubes must be pyrogen-free and endotoxin-free.
2. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000 rpm at 2-8℃, then Carefully collect the supernatant.
3. **Cell culture supernates:** Remove particulates by centrifugation.
4. **Tissue homogenization:** Add an appropriate amount of saline to the tissue and mash. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant. Restore samples to room temperature and ensure the sample is thawed evenly and thoroughly before use.
5. **Preservation:** Aliquot samples and store at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.
6. **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℃) 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/positive control respectively. **Samples need test in duplicate.**
2. **Add sample:** add Positive control and Negative control 50 μL to Positive and Negative control well. Add testing sample 10 μL then add **Sample Diluent** 40 μL to testing sample well.
3. **HRP Conjugate:** then add 100 μL of **HRP Conjugate** into each well.
4. **Incubate**: cover the plate sealer and mix thoroughly, and incubate at 37℃ for 60 min in shading light.
5. **Wash:** remove the liquid in each well. Immediately add 350 μL of Wash Buffer to each well and wash. Repeat wash procedure for 5 times, 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).
6. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well and mix thoroughly. Cover the plate sealer and incubate at 37℃ for 15 min in shading light.
7. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
8. **OD Measurement:** measure the absorbance value (A-value) of each well by using a micro-plate reader with 450 nm wavelength. **Note: Read the results within 15 min.**

Reference value

Normally, the average absorbance of negative control ≤ 0.15 and average absorbance of positive control ≥ 1.00.

Interpretation of the results

Cut off = the average of negative control + 0.15.

1. Positive result: sample OD > Cut off;
2. Negative result: sample OD < Cut off.

Limitations of this test method

1. This test is only used for the qualitative detection of CP-α-Ab in sample of goat.
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.