Canine Echinococcus Granulosus Antigen ELISA Kit

Catalog No: E-AD-E068

96T

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2025.12.18

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
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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

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with Echinococcus granulosus (Eg) antibodies. Apply the principle of Sandwich-enzyme-linked immunoassay (ELISA) to detect Eg-Ag in feces of canine. During the experiment, add control and samples into the ELISA Microtiter plate, Eg-Ag will be bound with the antibodies on the ELISA Microtiter plate. Then wash the plate to remove unbound components, horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a positive correlation between the OD value of samples and the concentration of Eg-Ag. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether Eg antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
10×Concentrated Wash Buffer	100 mL
5×Sample Diluent	50 mL
Substrate Reagent	11 mL
Stop Solution	10 mL
Positive Control	1 mL
Negative Control	1 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

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
6. 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.
7. The results shall depend on the readings of the micro-plate Reader.
8. **Each reagent is optimized for use in the E-AD-E068. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E068 with different lot numbers.**
9. 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. **Solution preparation**

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

   Sample Diluent: The **5×Sample Diluent** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:4.

   Wash Buffer: The **10×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9.

2. **Sample preparation**

   Feces solution: Take 1±0.05 g of canine feces to centrifuge tube and add 2 mL of **Sample Diluent**, oscillate for 5 min, and centrifuge at 2000g for 10 min, then take the supernatant for analysis.

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 100 μL of **positive/negative control** to positive/negative control well. Add 100μL of **Feces solution** to the sample wells.
3. **Incubate:** cover the plate sealer and gently shake to mix evenly, incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 250μL **Wash Buffer** to fill each well and wash. Repeat wash procedure for 4-6 times. 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** into each well. cover the plate sealer and incubate at 37℃ for 30 min in shading light.
6. **Wash:** repeat Step 4 for washing.
7. **Color Development:** add 100 μL of **Substrate Reagent** into each well and mix thoroughly. Cover the plate sealer and mix thoroughly, incubate at 37℃ for 10 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** Measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength).

Reference value

Normally, average absorbance of negative control < 0.2 and Average absorbance of positive control > 0.5.

Interpretation of the results

S/P value = $OD_{Sample}/OD_{Average\ positive}$

1. Positive result: S/P value ≥ 0.45
2. Negative result: S/P value < 0.45
3. Negative result indicates no Eg-Ag detected in samples, while positive result means the opposite.

Limitations of this test method

1. This test is only used for the qualitative detection of Eg-Ag in samples of Canine.
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.