Rabies Virus Antibodies ELISA Kit

Catalog No: E-AD-E142

96T

Version Number:	V1.6
Replace version:	V1.5
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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
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 kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant Rabies Virus (RBV) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect RBV-Ab in serum or plasma of canine and cat. During the experiment, add control and samples into the ELISA Microtiter plate, RBV-Ab will be bound with the antigen on the ELISA Microtiter plate. Then horseradish peroxidase (HRP) conjugate is added to each ELISA microtiter plate well, and substrate reagent is added for color development, the blue signal by Enzyme catalysis is in positive correlation of antibody content in sample. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether RBV antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
10×Concentrated Wash Buffer	100 mL
Substrate Reagent	11 mL
Sample Diluent	100 mL
Stop Solution	15 mL
Positive Control	1.6 mL
Negative Control	2 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

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 or distilled 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 th e 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-E142. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E142 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.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

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

   Solution 1:**Wash Buffer**

   Dilute the **10×Concentrated Wash Buffer** with deionized water. (10×Concentrated Wash Buffer (V): Deionized water (V) =1:9).

Sample preparation

1. **Serum:** Use the conventional method to prepare serum, the serum must be clear, no hemolysis and no pollution .Samples can be conserved at 2-8℃ in 1 week, and it should be stored at - 20℃ for a long term storage.
2. **Diluted serum:** Dilute the sample with the **Sample Diluent** at 1:25 (6 μL sample and 144 μL of **Sample Diluent**, mix fully). The positive/negative control do not need to be diluted.

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 standard in order (multiple well), and keep a record of standard wells and sample wells. Set 2 wells for positive/negative control respectively. **Samples need test in duplicate**.
2. **Add sample:**
   a) Add 100 μL of **Positive/Negative control** to positive/negative control well.
   b) Add 100 μL of **Diluted serum** to sample well .
3. **Incubate:** Gently tap the plate to mix thoroughly, cover the plate sealer, incubate at 37℃ for 30 min in shading light.
4. **Wash:**remove the liquid in each well. Immediately add 250μL **Wash Buffer**(Solution 1) to fill each well and wash. Repeat wash procedure for 5 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** to each well. Gently oscillate for 10 s to mix thoroughly. Incubate at 37℃ for 15 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently oscillate to mix thoroughly.
9. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min after stop reaction.

Reference value

Normally, the OD of positive control (P) > 0.50 and the OD of negative control (N) < 0.20.

Interpretation of the results

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

1. Positive result: S/P value ≥ 35%
2. Negative result: S/P value < 35%.

Limitations of this test method

1. This kit is only used as the qualitative detection of RBV antibodies in serum of animals.
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.