Technical Manual

Rabies Virus Antibodies ELISA Kit

- Catalogue Code: ADES0028
- Antibody ELISA Kit
- Research Use Only

1. 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.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
10×Concentrated Wash Buffer	100 mL
Sample Diluent	25 mL
HRP Conjugate (100 x concentrate)	120 µl
HRP Conjugate (Red)	15 mL
TMB Substrate	11 mL
Stop Solution	6 mL
Rabies Positive Serum National Standard	2 vial
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.

3. 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° C incubator or water bath
- Deionized or distilled water
- Absorbent paper

4. 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. A void 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 ADES0028. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0028 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.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Sample Diluent:** Dilute the serum or plasma samples with Sample Diluent (1:100) before test. The suggested 100-fold dilution can be achieved by adding 2μl sample to 198 μl of **Sample Diluent**.
2. **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 (10×Concentrated Wash Buffer (V): Deionized water (V) = 1:9). Wash Buffer can be conserved at 4° C in 1 week.
3. **Anti-Rabies Virus Standard**: Reconstitute the **Rabies Positive Serum National Standard** (CVCC Z53) with 1.0 mL **Sample Diluent**. Do not substitute other diluents. This reconstitution produces a stock solution of 4IU/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Dilution method: Take 6 EP tubes, add 500 μL of Reference Standard & Sample Diluent to each tube. Pipette 500 μL of the 4 IU/mL **Sample Diluent** to the first tube and mix up to produce a 2 IU/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one according to this step. The illustration on the next page is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.

4. **HRP conjugate:** Centrifuge the HRP Conjugate (100 x) before opening. HRP- conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 μl of HRP-conjugate (100 x) + 990 μl of HRP- conjugate Diluent.

7. Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA microplate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** number the standard, control or sample in order (multiple well), and keep a record of these wells. Set 2 wells for standard or control respectively.

Samples need test in duplicate.

2. **Add sample:** add 100 μL of **standard** and **control** to standard control well, add 100 μL of **diluted Sample** to other sample well.

3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37°C for 1 hour in shading light.

4. **Wash:** remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 3 times, 30 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** into each well, cover the plate sealer and incubate at 37°C for 1 hour in shading light.

6. **Wash:** repeat step 4 for five times.

7. **Color Development:** add 100 μL of **Substrate Reagent** into each well, Cover the plate sealer and mix thoroughly, incubate at room temperature for 15 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). **This step should be finished within 10 minutes after stop reaction.**

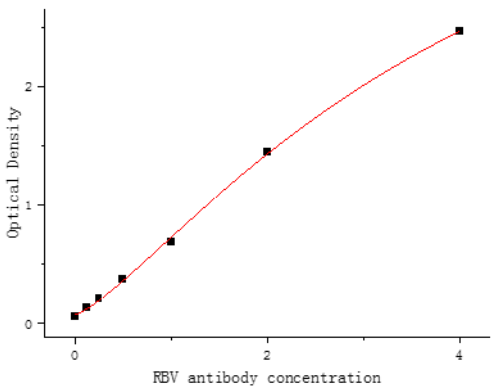
Calculation of Results

Average the duplicate readings for each standard and sample. Plot a four-parameter logistic curve, with standard concentration on the X-axis and OD values on the Y-axis.

If the OD value of the unknown sample exceeds the mean OD value of the 4 IU/ml standard, precise quantification cannot be performed. In this case, if precise quantification is required, dilute the sample by a factor of 1/10 or more and repeat the assay to ensure that the OD value falls within the range of the standard curve. The final concentration should be multiplied by the dilution factor.

8. Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.



9. Limitations

1. This kit is only used as the quantitative detection of RBV antibodies in serum of animals. The antibody concentration can be calculated according to the values (International Unit , IU).
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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Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com