Canine Rabies antibody ELISA Kit

C. Rabies Ab Test

Product Number: KE0011

Product Unit: 1 plate, 96T

FOR Professional and Laboratory use only, Not for use in therapeutic procedures.

Manufacturer Information

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1. Introduction

Rabies is a deadly virus spread to people from the saliva of infected animals. The rabies virus is usually transmitted through a bite. Animals most likely to transmit rabies in the United States include bats, coyotes, foxes, raccoons and skunks.

2. Description of Test

The Rabies antibody ELISA kit is designed to detect rabies specific antibody in canine serum/plasma. The 96well microtiter plate was pre-coated with recombinant protein. During test, samples are added into the microplate wells, in which the pre-coated antigen will capture the rabies antibody in sample. None specific antibody are discarded by a washing step. Then HRP conjugated anti-canine IgG is added into well. After another wash step to remove unreacted conjugate, Substrate is added and a blue color will be developed if rabies antibody is present. The enzyme reaction is stopped and OD450nm value is measured. The measured intensity is positively proportional to the amount of antibody present in the sample.

This ELISA kit can be used to detect rabies specific antibody level in canine serum/plasma.

3. Precautions

- Store the kit at 2-8℃,Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations. After experiment, all lab materials shall be handled properly according to local regulations.
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4. Limitations of Test

This ELISA kit is currently designed for research purpose. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

5. Reagent Provided

The kit contains the following items.

Item No.	Description	Quantity
1	Microplate pre-coated with recombinant antigen	1 plates
2	Positive Control	0.5ml
3	Negative Control	0.5ml
4	Sample buffer	30 ml
5	HRP enzyme conjugate	12 ml
6	TMB substrate	12 ml
7	Stop solution -- Not provided	20 ml
8	20X Wash buffer	20 ml
9	Kit instruction	1set

Note: Stop solution is 2M sulfuric acid, which is not provided due to air cargo shipment, which shall be prepared by client.

6. Instrument Required

- ELISA reader
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Wash buffer:** dilute the 20xWash buffer provided in the kit with deionized water in the volume ratio of 1:19. For example, 10ml 20xWash buffer + 190ml deionized water.
- The diluted wash buffer can be stored at room temperature for 3 days.
- No other reagent is required. Please remember to return all kit component to room temperature before use.

8. Sample Preparation

Serum: collect canine blood sample and then let it separate and coagulate naturally to obtain clear serum. You can also centrifuge at 4000 r/min for 10min after coagulation to get the serum.

Plasma: take canine blood into a tube and centrifuge at 4000 r/min for 10min to get the upper layer for testing.

Note: please freshly prepare before assay. The prepared serum/plasma shall be clear without any hemolyzed.

In the assay, 2ul serum/plasma sample will be used, thus a minimum of 5ul serum/plasma sample is required.

dilute with serum/plasma with sample buffer in the volume ratio of 1:99. For example: 2ul sample + 198ul sample buffer. Mix thoroughly for assay.

9. Assay Procedure

1) Make sure the kit and all test samples are returned to room temperature (18-26°C) before use. Shake each reagent gently before adding into the well.

2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.

3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. Running the test in duplicated wells is recommended to minimize operational error.

4) **Add Positive control**: add **100ul positive control** into the wells.

5) **Add Negative control**: add **100ul negative control** into the wells.

6) **Add Sample**: add **100ul sample** into the other wells.

7) **Incubation**: cover the plate with plate cover and incubate at **37℃ for 30min**.

8) **Washing**: pour the liquid out from the wells and wash with wash buffer (300ul per well) for 5 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.

9) **Add enzyme conjugate**: add **100ul of HRP conjugate** into each well. Cover the plate again and then incubate at **37℃ for 30min**.

10) **Washing**: repeat the washing step again.

11) **Add substrate**: add **TMB substrate** into each well, **100ul** per well. Cover the plate again and then incubate at **room temperature again for 10min**. Color reaction will occur in the plate.

12) **Stop the reaction**: add **50ul stop solution** into each well, the color will turn yellow from blue.

13) **Read the plate**: using ELISA reader to read the plate at 450nm.

10. Result Determination

1) Criteria of valid tests

PC = **Mean OD of Positive Control**

NC = **Mean OD of Negative Control**

The test results are valid if **PC ⩾ 0.6 and NC ⩽ 0.2.** Otherwise please run the analysis again with new kit.

2) Criteria of Positive and Negative results.

SP = (Mean OD of Sample – NC) / (PC – NC)

Positive : **SP / NC ⩾ 0.3**
Indicating the antibody level in the sample is above 0.5IU/ml.

Negative: **SP / NC < 0.3**
Indicating the antibody level in the sample is below 0.5IU/ml. Extra vaccination may require.

11. Storage and expiration

The kit shall be store at 2-8℃, avoid direct sunlight.

The valid period is 12 months.

12. References

(1) Wasniewski, Marine, and Florence Cliquet. "Evaluation of ELISA for detection of rabies antibodies in domestic carnivores." Journal of virological methods 179.1 (2012): 166-175.

(2) Fournier-Caruana, Jacqueline, et al. "Inactivated rabies vaccine control and release: use of an ELISA method." Biologicals 31.1 (2003): 9-16.