Rabies Virus IgG Ab (Dog) ELISA

Enzyme Immunoassay for the determination of IgG antibodies against Rabies Virus in serum or plasma

REF DE2486

96 wells
Please use only the valid version of the Instructions for Use provided with the kit.

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A virus based ELISA, to detect IgG antibodies against Rabies Virus in serum or plasma samples in canine species.

1 INTRODUCTION

Rabies virus can infect all warm-blooded species and in many species the disease can present itself in two different forms. Furious rabies, in which predominantly the brain is infected and paralytic rabies in which predominantly the spinal cord is involved. When cells of the limbic system are infected the first changes in behavior characteristic of rabies may be observed. It has been suggested that the phase before infecting cells of the nervous system may take a considerable length of time, causing a variable incubation period from 10 days to several years. Hence the virus is present in the saliva, which favors the most natural way of transmission by biting in the various stages of the disease, also sporadic cases of aerosol infections have been documented. Carnivores, especially domestic dogs and cats, and also rodent and recently bats, are usually involved in transmission of infections to dogs and man. Infections of dogs with rabies virus seem to be invariably fatal. Persistent in apparent infection accompanied by virus shedding has been documented in several human and animal species including cats and raccoons. This standardized ELISA test system based on semi purified virus is intended to use as a rapid screening test for the detection of rabies antibodies in serum or plasma samples of dogs.

2 INTENDED USE OF THE TEST KIT

This diagnostic test-system for the establishment of Rabies infection is intended to identify antibodies against epitopes of rabies virus, in serum or plasma samples. In contrast to other test systems this standardized ELISA based on whole-inactivated virus, has a very high sensitivity and specificity.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of semi purified virus with polyclonal dog antibodies. To this end purified rabies antigen has been coated to a 96-well microtiter strip plate.

- **Qualitative**
  The sample is added (diluted 1:250) to the wells of the coated plate.

- **Quantitative**
  The sample also can be titrated using a 3-step dilution, starting with a dilution 1:50 (1:150 → 1:450 → 1:1350).

After washing, the bound dog antibodies are detected by HRPO conjugated anti-species conjugate. The color reaction in the wells is directly related to the concentration of rabies virus antibodies in the serum or plasma sample.

4 CONTENTS

- **SORB MT** 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 18 mL **BUF** ELISA buffer (green cap)
- 1 x 12 mL **ENZ CONJ** HRPO conjugated anti-species antibodies, ready to use (red cap)
- 1 x 0.5 mL **CONTROL 1** Positive control (freeze dried) (purple cap)
- 1 x 1.0 mL **CONTROL 2** Negative control (freeze dried) (silver cap)
- 1 x 20 mL **WASH SOLN 200x** Wash-solution (200x concentrated) (black cap), diluted in de-ionized water before use!
- 1 x 8 mL **SUB A** Substrate A (white cap)
- 1 x 8 mL **SUB B** Substrate B (blue cap)
- 1 x 8 mL **STOP SOLN** Stop-solution (yellow cap)
- 1 x Plastic cover seal
- 1 x Instructions for Use
4.1 Supplies needed (not included)
- Round bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes
- ELISA plate reader

5 HANDLING AND STORAGE OF SPECIMENS
The kit should be stored at 4 °C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20 °C before use.
After first use, ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20 °C.
Avoid repeated freezing and thawing as this increase non-specific reactivity.

6 WASH PROTOCOL
In ELISA’s, un-complexed components must be removed efficiently between each incubation step.
This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing
1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer
2. Fill all the wells with 250 µL wash solution
3. This washing cycle (step 1 and 2) should be carried out at least 5 times
4. Turn the plate upside down and empty the wells with a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment
When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7 PREPARATIONS
- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min at room temperature (± 21 °C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4 °C - 8 °C immediately after use.
8 TEST PROTOCOL QUALITATIVE
Before starting this test read “PREPARATIONS”

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

Wash microtiter strip(s) with washing solution, according to washing protocol.

   **The wash solution provided must be diluted 200x in aqua bidest. (5 MΩ water)!**

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. **Reconstitute** directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

3. **Reconstitute** directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

4. **Dilute the positive control** (purple cap) **1:50** in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

   **Example:**
   - Add 147 µL buffer to **well 1A**, add 3 µL of the positive control to the **well 1A** and mix well.

5. **Dilute the negative control** (silver cap) **1:50** in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

   **Example:**
   - Add 147 µL buffer to **well 1B**, add 3 µL of the negative control to the **well 1B** and mix well.

6. **Dilute the samples** **1:250** in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

   **Example:**
   - A pre-dilution is needed:
     - Add 90 µL buffer to **well 1C**, add 10 µL of the sample to the **well 1C** and mix well.
     - Add 144 µL buffer to **well 1D**, add 6 µL of pre-dilution **well 1C** in the well **1D** and mix well **(only transfer this dilution to the coated plate at step 8)**.

7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these wells.

8. Transfer **100 µL** of all dilutions to the virus-coated microtiter strips.

9. Seal and incubate for **60 min** at **37 °C**.

10. Wash the plate according to the wash protocol **see sub 6**.

11. Dispense **100 µL** conjugated anti-species antibody to all wells.

12. Seal and incubate for **60 min** at **37 °C**.

13. Wash the plate according to the wash protocol **see sub 6**.

14. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking.

   **Prepare immediately before use!**

   **Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

15. Dispense **100 µL** substrate solution to each well.

16. Incubate **10 - 20 min** in the dark (e.g. cover the wells with a sheet of paper) at room temperature (**21 °C**).

   Make sure the negative control does not become too dark.

17. Add **50 µL** stop solution to each well; mix well.

18. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

   **N.B.:**

   *if you pipet directly into the coated ELISA plate with only a small number of samples (< 6), make sure the first dilution is done in round bottom microtiter plate, second step can be done directly in the coated ELISA plate.*
9 TEST PROTOCOL QUANTITATIVE

Before starting this test read “PREPARATIONS”

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4 °C and use them within 10 days.

Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

The washing solutions provided must be diluted 200x in aqua bidest. (5 MΩ water)!

Use validated precision pipettes and use a clean pipette tip before pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the negative control (silver cap) in 1.0 mL aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

3. Reconstitute directly before use the positive control (purple cap) in 0.5 mL aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

4. Make a pre-dilution of the positive control (purple cap) in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

Example: Add 90 µL ELISA buffer to well 1A and add 10 µL of the positive control to the well 1A.

5. Make a pre-dilution of the negative control (silver cap) in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

Example: Add 90 µL ELISA buffer to well 1B and add 10 µL of the negative control to the well 1B.

6. Make a pre-dilution of each sample in ELISA buffer (green cap) in a round-bottomed plate (not supplied).

Example: Add 90 µL ELISA buffer to well 1C and add 10 µL of the sample to the well 1C.

7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these wells.

8. Add for dilution of the positive control 120 µL ELISA buffer to row 1A.

And 100 µL to 1B, 1C, 1D of the coated microtiter strip.

9. Add for dilution of the negative control 120 µL ELISA buffer to row 1E.

And 100 µL to 1F, 1G, 1H of the coated microtiter strip.

10. Add for dilution of the samples 120 µL ELISA buffer to the other well 2A and 2E.

And 100 µL to 2B, 2C, 2D and 2F, 2G, 2H (depending on the number of samples) of the coated microtiter strip.


Example: - Dispense 30 µL positive control from step 4 to the well 1A of the microtiter strip.

- Mix well and transfer 50 µL to the well 1B

- Mix well and transfer 50 µL to the well 1C

- Mix well and transfer 50 µL to the well 1D

- Mix well and discard 50 µL.


Example: - Dispense 30 µL negative control from step 5 to the well 1E of the microtiter strip.

- Mix well and transfer 50 µL to the next well 1F

- Mix well and transfer 50 µL to the next well 1G

- Mix well and transfer 50 µL to the well 1H

- Mix well and discard 50 µL.


Example: - Dispense 30 µL of each sample from step 6 to the well 2A and/or 2E of the microtiter strip.

- Mix well and transfer 50 µL to the well 2B and/or 2F

- Mix well and transfer 50 µL to the well 2C and/or 2G

- Mix well and transfer 50 µL to the well 2D and/or 2H

- Mix well and discard 50 µL.
14. Dispense 100 µL of the substrate control of step 7 to the last 2 wells of the microtiter strip.
15. Seal and incubate for 60 min at 37 °C.
16. Wash the plate according to the wash protocol see sub 6.
17. Dispense 100 µL conjugated anti-species antibody to all wells.
18. Seal and incubate for 60 min at 37 °C.
19. Wash the plate according to the wash protocol see sub 6.
20. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking.

**Prepare immediately before use!**

Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.

21. Dispense 100 µL substrate solution to each well.
22. Incubate 10 - 20 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C).
Make sure the negative control does not become too dark.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

10 PRECAUTIONS

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11 VALIDATION OF THE TEST

**Qualitative:**

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 1.000
  - The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.400
In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

**Calculation**

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

\[
S/P = \frac{OD_{sample} - MV \, OD_{NC}}{MV \, OD_{PC} - MV \, OD_{NC}}
\]

**Quantitative:**

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 1.000 OD units (450 nm) and give an endpoint titer of ≥ 150.
The negative control should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 50.
12 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

**Qualitative:** Positive – Negative
- A sample with the S/P ratio < 0.34 is negative
  - Specific antibodies to Rabies could not be detected.
- A sample with the S/P ratio ≥ 0.34 is positive
  - Specific antibodies to Rabies were detected.

**Quantitative:** End point titre
- The ELISA titre can be calculated by constructing a curve and using cut-off line (dilution 1:50 – 150 – 450 – 1350 – 4050 – 12150, etc., total 8 dilutions of 3 steps) OD on Y-axis and Titre on X-axis.
  
  ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1.50.

The FAVN titre of the positive control is 1.83 IU. Knowing this the K factor can be calculated by dividing the obtained ELISA titre by 1.83 (example: ELISA titre positive control = 1350; 1350/1.83 = 737.7 → K factor = 737.7.

All ELISA titres obtained in the constructed graphic can in this way be divided by K to obtain FAVN titres in IU.

The IU titre obtained by this way will be close to the FAVN/RIFFIT titre in the original tissue culture test but final correlation depends on the Lab performing the FAVN/RIFFIT test.

Small Lab to Lab variation in FAVN/ RIFFIT titre will always be seen due to the nature of biological material, in this case cells and virus and Fetal Calf serum used.

The entire risk as to the performance of these products is assumed by the purchaser. Demeditec shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products In case of problems or questions contact Demeditec.

SYMBOLS USED WITH DEmeditec ASSAYS

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