Instructions for Use

Rabies Virus Antibody (human) ELISA

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REF EIA-5900

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Table of Contents

A virus based ELISA to detect IgG antibodies against Rabies Virus in serum or plasma samples in human

For research use only.

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 cased 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 men. 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 human.**

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 antibodies.

To this end purified rabies antigen have 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 1:100 → 1:300 → 1:900 → 1:2700).

After washing, the bound 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

- 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 18 ml ELISA buffer (white bottle + green cap)
- 1 x 12 ml HRPO conjugated anti-species antibodies, **READY TO USE** (black bottle + red cap)
- 1 x 0,5 ml Positive control (freeze dried) (purple cap)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 20 ml Wash solution (200x concentrated) (whits bottle + black cap), **dilute in de-ionized water before use!**
- 1 x 8 ml Substrate buffer A (white bottle + white cap)
- 1 x 8 ml Substrate buffer B (black bottle + blue cap)
- 1 x 8 ml Stop solution (white bottle + yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

4.1 Supplies needed (not included)

- ELISA plate reader
- Pipette tips and clean containers/tubes
- Validated precision pipettes (1-10 µL, 10-200 µL, 200-1000 µL)
- Round-bottomed microtiter plate

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 4 °C.

An open packet should be used within 20 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 increases non-specific reactivity.

6 WASH PROTOCOL

In ELISA, 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 results.

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. Make sure that none of the wells dries 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 minutes 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 2 °C – 8 °C immediately after use.

8 TEST PROTOCOL QUALITATIVE

1. Before starting this test read “preparations”
2. 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 2 °C – 8 °C and use them within 20 days.

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use).**
5. Reconstitute directly before use the **positive control** (purple cap) in 0,5 ml aquabidest (5 mega Ohm water), divide into aliquotes, and store after complete dissolving immediately at -20°C until use, avoid freeze and thaw cycles.
6. Reconstitute directly before use the **negative control** (silver cap) in 1,0 ml aquabidest (5 mega Ohm water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
7. **Dilute** the **positive control** (purple cap) **starting 1:100 → 1:300 → 1:900 → 1:2700 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: A **pre-dilution** is needed:
   - Add 90 µL ELISA buffer (green cap) to **well 1A**, add 10 µL of the positive control to the **well 1A** and mix well.
   - Add 180 µL ELISA buffer (green cap) to **well 2A**,
   - Add 120 µL ELISA buffer (green cap) to **2B, 2C, 2D**
   - Add 20 µL of pre-dilution **well 1A** in the well **2A** and mix well
   - Mix well 2A and transfer 60 µL to the well **2B**
   - Mix well 2B and transfer 60 µL to the well **2C**
   - Mix well 2C and transfer 60 µL to the well **2D**
   - Mix well 2D and discard 60 µL.
8. **Dilute** the **negative control** (silver cap) **1:100 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example:
   - Add 200 µL ELISA buffer (green cap) to **well 2E**, add 2 µL of the negative control to the **well 2E** and mix well.
9. Dilute **the sample 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 1F**, add 10 µL of the sample to the **well 1F** and mix well.
   - Add 144 µL ELISA buffer (green cap) to **well 2F**, add 6 µL of pre-dilution **well 1F** in the well **2F** and mix well.
10. Take 2 wells as **substrate control** add only **120 µL ELISA buffer** (green cap) to these wells.

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11. Transfer 100 µL of all dilutions of **row 2** to the **virus-coated microtiter strips**, including the substrate controls.
12. Seal and incubate for 60 minutes at 37 °C.
13. Wash the strips 5x according to the wash protocol see sub 6.
14. Add **100 µL HRPO conjugated anti-species antibodies** (black bottle + red cap) to all wells.
15. Seal and incubate for 60 minutes at 37 °C.
16. Wash the strips 5x according to the wash protocol see sub 6.
17. 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.
18. Add **100 µL substrate solution** to each well.
19. Incubate 10 - 15 minutes 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.
20. Add **50 µL stop solution** to each well; mix well.
21. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. **Use the substrate controls as blank.**

NB: 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

1. Before starting this test read “preparations”
2. 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 20 days.

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature.
4. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use).**
5. **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.
6. **Reconstitute** directly before use the **negative control** (silver cap) in **1.0 ml aquabidest** (**5 MΩ** water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.
7. 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.**
8. 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.**
9. 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.**
10. Take 2 wells as **substrate controls** add only **120 µL ELISA buffer** (green cap) to these wells.

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11. Add for dilution of the **positive control 135 µL ELISA buffer** (green cap) to **well 1A**, and 100 µL to **1B, 1C, 1D** of the **coated** microtiter strip.
12. Add for dilution of the **negative control 135 µL ELISA buffer** (green cap) to **well 1E,** and 100 µL to **1F, 1G, 1H** of the **coated** microtiter strip.
13. Add for dilution of the **samples 135 µL ELISA buffer** (green cap) to the other **row 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.
14. Make a 3-step dilution of the **positive control** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example:
    - Add 15 µL positive control from step 7 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.
15. Make a 3-step dilution of the **negative control** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example:
    - Add 15 µL negative control from step 8 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.
16. Make 3-step dilution of **each sample** in the coated microtiter strip,
    starting 1:100 → 1:300 → 1:900 → 1:2700.
    Example:
    - Add 15 µL of each sample from step 9 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.
17. Add **100 µL** of the **substrate control** of step 10 to the last 2 wells of the microtiter strip.
18. Seal and incubate for 60 minutes at 37 °C.

19. Wash the strips 5x according to the wash protocol see sub 6.
20. Add **100 µL HRPO conjugated anti-species antibodies** to all wells.
21. Seal and incubate for 60 minutes at 37 °C.
22. Wash the strips according to the wash protocol see sub 6.
23. 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.
24. Add **100 µL substrate solution** to each well.
25. Incubate 10-15 minutes 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.
26. Add **50 µL stop solution** to each well; mix well.
27. Read the absorbency values immediately (within 10 minutes!) at 450nm using 620 nm as reference on the ELISA reader. **Use the substrate controls as blank.**

10 PRECAUTIONS

- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- Do not pipette by mouth.
- Do not use components past the expiry date and do not mix components from different serial lots.
- 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.
- Handle all biological material as though capable of transmitting infectious diseases.
- 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.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.

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), diluted 1:100, must be ≥ 1.000.
- The MV of the measured OD value for the negative control (NC), diluted 1:100, must be ≤ 0.300.

In case of invalid assays, the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean value (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, diluted 1:100, should be ≥ 1.000 OD Units (450 nm) and give an endpoint titer of ≥ 150.

The negative control, diluted 1:100, should be ≤ 400 OD Units (450 nm) and give an endpoint titer of ≤ 100.

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 1:100 – 1:300 – 1:900 – 1:2700 etc. total 8 dilutions of 3 steps) **OD** on Y-as and **titre** on X-as

ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:100.

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 1350/1,83 = 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 in this 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. DRG 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 DRG.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité