Instructions for Use

Rabies Virus Antibody ELISA (human)

RUO

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.

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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 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 whole-inactivated virus is intended to use as a rapid screening test for the detection of rabies antibodies in serum 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 samples.

In contrast to other test systems this standardized ELISA based on whole-inactivated virus, has a very high sensitivity and specificity.

For human samples this test is for research use only.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of whole-inactivated virus with polyclonal antibodies.

To this end purified inactivated virus has been coated to a 96-well microtiter strip plate. The serum is added to the wells of the coated plate.

- **Qualitative**

The serum sample is added (diluted 1:100) to the wells of the coated plate.

- **Quantitative**

The serum 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 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 sample.

4 CONTENTS

- 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 18 mL ELISA buffer (green cap)
- 1 x 12 mL HRPO conjugated anti-species antibodies (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) (black cap), **diluted in de-ionized water before use!**
- 1 x 8 mL Substrate A (white cap)
- 1 x 8 mL Substrate B (blue cap)
- 1 x 8 mL Stop-solution (yellow cap)
- 1 x Plastic cover seal

4.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Precision pipette 10 - 200 µL
- Precision pipette 200 - 1000 µL
- 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.

Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date.

Avoid repeated freezing and thawing as this increases 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 washing 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 washing 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 washing 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 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, store them at +4 °C, and use them within 10 days. Wash microtiter strip(s) with washing solution, according to washing protocol.
   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ) water!
   Use the precision pipette 10 - 200 µL & 200 - 1000 µL 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) 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Dilute the **negative control** (silver cap) 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
6. Dilute the **serum or plasma samples** 1:100 in ELISA buffer (green cap) in a round bottomed plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
7. Take 2 wells as substrate controls, add only 100 µL ELISA buffer (green cap) to these well.
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 buffer A (white cap) and buffer B (blue cap) with gentle shaking.
    Prepare immediately before use!
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 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 by using an ELISA reader. Use the substrate controls as blank.

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, store them at +4 °C, and use them within 10 days. Wash microtiter strip(s) with washing solution, according to washing protocol.
   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ) water!
   Use the precision pipette 10 - 200 µL & 200 - 1000 µL 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 1.0ml 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 3-step dilution of the **positive control** (purple cap) in ELISA buffer (green cap)
   starting with 1:50 → 1:150 → 1:450 → 1:1350 in a round-bottomed microtiter plate (not supplied).
   Make sure to make minimal 120 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Make a 3-step dilution of the **negative control** (silver cap) in ELISA buffer (green cap)
   starting with 1:50 → 1:150 → 1:450 → 1:1350 in a round-bottomed microtiter plate (not supplied).
   Make sure to make minimal 120 µL of every dilution to be able to transfer 100 µL to the coated plate.
6. Make 3-step dilutions of **each sample** in ELISA buffer (green cap)
   starting with 1:50 → 1:150 → 1:450 → 1:1350 in a round-bottomed microtiter plate (not supplied).
   Make sure to make minimal 120 µL of every dilution to be able to transfer 100 µL to the coated plate.
7. Take 2 wells as substrate controls add only 100 µL ELISA buffer (green cap) to these well.
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 buffer A (white cap) and buffer B (blue cap) with gentle shaking.
    Prepare immediately before use!
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 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 by using an 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 ≤ 0.350

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 ≤ 0.350 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 < 0.4 are negative
  - Specific antibodies to Rabies could not be detected.
- A sample with the S/P ratio ≥ 0.4 are positive
  - Specific antibodies to Rabies were detected.

Quantitative: End point titre

- The ELISA titer 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 titer on X-axis. ELISA titers can be calculated using as cut-off 2.5 times OD value of negative control at 1:50.

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
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Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité