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User's Manual

Rabies Virus IgM Ab (Dog) ELISA

Enzyme Immunoassay for the quantitative determination of rabies antibodies in serum samples of dogs

VET

REF **DE2487**

Σ **96 wells**

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Please use only the valid version of the package insert provided with the kit.

Table of Contents

1 INTRODUCTION

For Veterinary use only!

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

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.

3 PRINCIPLE

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

The dog serum sample is added (diluted 1:100) to the wells of the coated plate. The serum sample also can be titrated using a 3-step dilution, starting with a dilution 1:50 (150; 450; 1350).

After washing, the bound dog antibodies are detected by HRPO conjugated anti-species conjugate.

The colour 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
- 1 x 13 mL HRPO conjugate
- 1 x 1 mL Positive control (ready to use)
- 1 x 1 mL Negative control (freeze dried)
- 1 x 20 mL Wash solution (200x concentrated), **dilute in de-ionised water before use!**
- 1 x 8 mL Substrate A
- 1 x 8 mL Substrate B
- 1 x 8 mL Stop solution
- 1 x Plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 2 °C - 8 °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 ELISAs, 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 washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 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 4 washing cycles.

7 TEST PROTOCOL

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 2 °C - 8 °C. Use them within 10 days. Wash the microtiter strip(s) with washing solution, according to wash protocol.
   The washing solution provided must be diluted 200x in de-ionised water!
   Samples should preferable be adsorbed with protein A or anti dog IgG magnetic beads, to adsorb IgG antibodies which otherwise might block the large IgM and course competition.
2. Reconstitute the negative control in 1.0 mL deionized water. Store in aliquots at -20 °C.
3. **Qualitative:**
   Dilute the serum or plasma samples and the negative control 1:50 in ELISA buffer.
   Make a **1:2 dilution** of the positive control (starting 1:2; 1:4; 1:8, etc.)

   Quantitative:
   Make 3-step dilutions of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round-bottomed microtiter plate. Make also a 3-step dilution of the negative control starting 1:30
   Make also a 3-step dilution of the positive control but starting with a **dilution of 1:3**
4. Transfer 100 µL of these dilutions to the (virus-coated) microtiter strips.
5. Incubate for **60 minutes** at 37 °C.
6. Wash as pointed out in the wash protocol.
7. Dispense 100 µL HRPO conjugate to all wells.
8. Seal and incubate **60 minutes** at 37 °C.
9. Wash as pointed out in the wash protocol.
10. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use!
    Dispense 100 µL substrate solution to each well.
    Incubate **10-15 minutes** at room temperature (21 °C).
11. Add 50 µL stop solution to each well; mix well.
12. Read the absorbency values immediately (within 10 min.!) at 450 nm.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting Rabies.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the 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.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 0.700 OD units (450 nm).

The negative control should be lower than 0.350 OD units (450 nm) and give an endpoint titer of ≤ 30.

10 INTERPRETATION OF TEST RESULTS

This test can be used in 2 ways.

1. **Qualitative:** positive – negative

A sample is scored positive if the OD is higher than the OD of the negative control plus 0,200.

2. **Quantitative:** end point titre

The end-point titre of the sample is the dilution which gives an extinction just above the OD of the negative control plus 0.150.

The RIFFIT titre is still seen as the standard but final correlation with ELISA depends on the laboratory performing the RIFFIT test.

Small lab to lab variation in RIFFIT titre will always been seen due to the nature of biological material (cells and virus)

The purchaser assumes the entire risk as to the performance of these products.

Demeditec shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformität-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	For veterinary use only				
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità