DRG® Rabies Virus IgG Ab (Canine) ELISA (EIA-2486)

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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

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 STANDARDISATION

To standardize the rabies ELISA, positive and negative controls have to be tested.
The positive control should give an extinction higher than 0.500 OD units measured by 450 nm.

4 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 color reaction in the wells is directly related to the concentration of rabies virus antibodies in the serum sample.

5 CONTENTS

12 x 8 **microtiter strips**.

1 x **strip holder**.

1 x 18 ml **ELISA buffer**.

1 x 12 ml **HRPO conjugated anti-species antibodies**.

1 x 0,5 ml **Positive control**, reconstitute in aqua-bidest before use.

1 x 0,5 ml **Negative control**, reconstitute in aqua-bidest before use.

1 x 20 ml **Wash-solution** (200x concentrated), **dilute in de-ionized 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.

6 HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8°C. An unopened package can be used until the expiry date. Avoid repeated freezing and thawing as this increases non-specific reactivity.
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.

7 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 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 downward 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 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 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.

8 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, store them at +4°C and use them within 10 days.
Wash the microtiter strip(s) with washing solution, according to washing protocol.
The washing solution provided must be diluted 200x in de-ionized water!

2. **Qualitative:**
   Dilute the serum or plasma 1:100 in ELISA buffer.
   Make also a 1:100 dilution of the positive and negative control.
   (reconstitute the controls with 0,5 ml aqua bidest)
   Quantitative:
   Make 3-step dilutions of each sample in ELISA buffer, starting 1:50 (150; 450; 1350) in a round-bottomed microtiter plate. Make also a 3-step dilution of the positive and negative control.
3. Transfer 100 µl of these dilutions to the (virus-coated) microtiter strips.
4. Incubate 60 min. at 37°C.
5. Wash as in 1.
6. Dispense 100 µl HRPO conjugated anti-species antibodies to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash as in 1.
9. 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 min. at room temperature (21°C).
10. Add 50 µl stop solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 1.000 OD units (450nm).
The negative control should be lower than 0.350 OD units (450nm) and give an endpoint titer of ≤ 50.

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

The titre in IU according RIFFIT can be calculated by dividing the ELISA titre by 122.3 . The IU titre obtained in this will be close to the RIFFIT titre but final correlation depends on the Lab 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)

11 PRECAUTIONS

- Handle all biological materials as though capable of transmitting Rabies.
- Do not pipette by mouth.

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- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots together.
- 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.

The purchaser assumes the entire risk as to the performance of these products.
DRG shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.

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SYMBOLS USED WITH DRG 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
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..