DRG® Rabies Virus (nucleoproteins) Elisa (EIA-2490)

REVISED 28 JULY 2010 RM (VERS. 2.0) **FOR VETERINARY USE ONLY**

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

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1 INTRODUCTION

Rabies vaccines for use in man or animal consist of inactivated antigen suspensions of in vitro cultured rabies virus. The potency virus test for inactivated rabies vaccines as recommended by the World Health Organization is the NIH mouse protection test (NIH test, Seligmann, 1973). This NIH test suffers from several drawbacks (e.g. animal distress, time consuming, poor reproducibility), therefore the introduction of alternative testing methods is highly recommended.

Enzyme immunoassays (ELISA) are being developed to quantify assay the nucleoprotein content of rabies vaccines. ELISAs are rapid and highly reproducible, and the use of standardized test kits enables the user to determine the nucleoprotein contents of inactivated antigen preparations according to the WHO standard. For this purpose the test kit includes a positive standard extensively tested in the NIH test.

2 INTENDED USE OF THE TESTKIT

This diagnostic test is intended for the detection of rabies virus nucleoprotein in inactivated antigen samples. This standardized ELISA is based on monoclonal antibodies, with high sensitivity and specificity for the nucleoprotein of rabies virus. The use of this ELISA kit is restricted to participants of the collaborative study organized by the National Institute of Health and Environmental Protection (RIV, The Netherlands).

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of rabies nucleoproteins with biotinylated monoclonal antibodies. To this end rabies nucleoproteins have been coated to a 96-well microtiter strip plate.

The antigen sample (diluted 1:16 for qualitative assay or in serial two-fold dilutions for quantitative assay) are dispensed to the wells of a preincubation plate. Immediately the same volume of a biotinylated monoclonal antibody solution (MAb conjugate) is added. (The antigen sample can also be titrated using a 2-step dilution, starting with a dilution 1:4; 8; 16; 32; 64; 128 ;256 ;512)

After washing, the bound MAb conjugate is detected by Avidin HRPO conjugate.

After developing with substrate, the color reaction in the wells is reversely related to the concentration of rabies virus nucleoprotein in the sample

4 CONTENTS

- 1 x **round bottomed microtiter plate**
- 1 x 12x8-well **microtiter plate coated** with inactivated rabies virus
- 1 x 7 ml biotinylated monoclonal antibody (**MAb Conjugate**)
- 1 x 13 ml **Avidin-HRPO Buffer**
- 1 x 0,3 ml concentrated (100x) **Avidin HRPO Conjugate**
- 1 x 1,0 ml inactivated rabies **positive Standard** (1,6 IU) (freeze-dried)

- 1 x 1.0 ml inactivated rabies **negative Control** sample (freeze-dried)
- 1 x 20 ml **Wash Solution 200 x** concentrated (dilute in deionized water before use!)
- 1 x 22 ml **ELISA Buffer**
- 1 x 7 ml **Substrate Buffer A**
- 1 x 7 ml **Substrate Buffer B**
- 1 x 13 ml **Stop Solution**
- 1 x plastic cover seals

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 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 by a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution from 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. Reconstitute directly before use the positive standard and negative control in 1.0 ml deionized water, for long time storage (>10 days) store at -20C avoid freezing and thawing cycles !!
2. Sample preparation:
   Make a 1:16 dilution or 2-step dilutions of each sample in ELISA buffer,
   starting 1:4 (8, 16, 32, 64, 128, 256, 512) in a round bottomed microtiter plate with a **final volume of 60 µl**.

   Make also a 2-step dilution of the positive control, starting undiluted 1:2/4/8/16 to row A1 - G1.

   Add the negative control diluted 1:2 to wells H1/H2.
3. After all samples and controls are added; add immediately 60 µl MAB Biotin conjugate (ready to use) to the wells already containing 60 µl sample.
   Incubate this mixture of sample + Mab conjugate for 1 hour at 37°C.
4. Open the package with the coated ELISA plate and wash this extensively according to washing protocol. The washing solution provided, must be diluted 200x in deionized water.
5. Transfer 100 µl of the antigen/biotin conjugate mixture to the precoated ELISA plate
   and incubate for 1 hour at 37°C.
6. Wash as in 4.
7. Dilute the concentrated Avidin HRPO conjugate 1:100 in conjugate buffer
   (this is stable for only 2 days, prepare what is needed).
   Dispense 100 µl (of this diluted) Avidin HRPO conjugate to all wells.
8. Seal and incubate for 25 min. at 37°C.
9. Wash as in 4.
10. Mix equal parts of buffer A and buffer B with gentle shaking in polypropylene/polycarbonate unused clean centrifuge tube (15/50 ml, not supplied). Prepare this immediately before use!

    Prepare for each well 105 µl substrate solution.
    Make for e.g. 20 wells 2.1 ml substrate solution (= 1.05 ml A + 1.05 ml B.)
    Do not make more than necessary.

    Dispense 100 µl substrate solution to each well.
    Incubate for 15-25 min. 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. Use 620 nm as reference wavelength.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting Rabies virus (high mortality virus).
- 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 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.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions the OD of the negative control should be approximately 1.100 OD units (450nm).

The highest [conc.] of the positive control should be about 0.200 OD units (450nm).

To standardize the rabies ELISA, positive and negative standards have to be tested.

The positive standard contains 1.6 IU according to NIH potency test (standardized against WHO standard.)

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways

a. qualitative: positive - negative

b. quantitative: ELISA units which can be transformed into mouse assay units

Qualitative:

A sample is scored negative if the OD is higher than 80% of the negative control.

A sample between 60% and 80% of the OD of the negative control is considered weak positive.

A sample below 60% of the negative OD is considered to be positive

Quantitative:

The value in NIH units of the samples can be calculated by comparison to their OD-values with a curve which is constructed from the OD-values of the dilutions of the standard (Y-axis) and their corresponding NIH I.U. (X-axis) on LIN/LOG paper. With this graphic presentation it is possible to determine the value in units of the samples.

N.B.

Do not forget to multiply this concentration with the dilution factor to obtain the final concentration in the sample.
According to WHO standards values below 2.5 I.U./dose (for human use) or 1.0 I.U./dose (for veterinary use) are considered not usable (will induce too less antibody after immunisation).

11 REFERENCE

Seligmann Jr.E.B., The NIH test for potency. In Laboratory Techniques in Rabies, 3rd ed., chap. 33, pp 279-285 Geneve, WHO (1973).

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