DRG® Rabies Virus Antigen ELISA (EIA-2489)

Revised 26 May 2011 rm (Vers. 5.1) **USA:RUO**

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

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE OF THE TESTKIT

This test is intended for the detection of rabies virus glycoprotein in inactivated antigen samples. This standardized ELISA is based on monoclonal antibodies, with high sensitivity and specificity for the glycoprotein of rabies virus.

PRINCIPLE OF THE TEST KIT

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

The antigen sample (diluted 1:8 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 undiluted sample/standard (4; 8; 16; 32; 64,128,256).

After preincubation the antigen/conjugate mixture is transferred to the coated microtiter plate to bind un-complexed MAb conjugate.

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

After developing with substrate, the colour reaction in the wells is reversed related to the concentration of rabies virus glycoprotein in the sample.

CONTENTS

- 1 x 12 x 8 microtiter strips
- 1 x strip holder
- 1 x 18 ml **ELISA buffer**
- 1 x 6 ml **biotinylated** monoclonal antibody (MAb **conjugate**)
- 1 x 11 ml **Avidin-HRPO**
- 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 8 ml **Substrate buffer A**
- 1 x 8 ml **Substrate buffer B**
- 1 x 8 ml **Stop Solution**
- 1 x plastic cover seals

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Supplies needed (not included):

- Round bottomed microtiter plate

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

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.

TEST PROTOCOL

1. Reconstitute directly before use the positive and negative control in 1.0 ml deionized water.
2. Sample preparation:
   Make a 1:25 dilution or 2-step dilutions of each sample in ELISA buffer, starting undiluted 1: (2; 4, 8,16, 32; 64, 128, 256) **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, 1:2/4/8/16/32/64 to row A1 - F1.

   Add the negative control diluted 1: 2 to wells G1/H1.
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 it extensively according to washing protocol.
   The washing solution provided must be diluted 200x in deionized water.

   Transfer 100 µl of the incubated (in round bottom plate) antigen/conjugate mixture to the precoated ELISA plate and incubate for 1 hour at 37°C.
5. Wash as pointed out in wash protocol.
6. Dispense 100 µl (ready to use) Avidin HRPO conjugate to all wells.
7. Seal and incubate for 25 min. at 37°C.
8. Wash as pointed out in wash protocol.
9. Mix equal parts of buffer A and buffer B with gentle shaking in clean centrifuge Prepare this immediately before use! Dispense 100 µl substrate solution to each well.
10. 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.

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.

VALIDATION OF THE TEST

In order to confirm appropriate test conditions the OD of the negative control should be approximately 1.600 OD units (450 nm).

The highest [conc.] of the positive control should be about 0.200 OD units (450 nm) and give an endpoint titer of 16.

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).

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.

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