DRG® FIV-p24/p17 (EIA-2463)

Revised 24 Aug. 2009 (Vers. 4.1) **For Veterinary Use Only**

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

INTRODUCTION

FIV-p24/p17 are both core proteins of FIV. Infected cats produce antibodies against these FIV antigens, which can be detected in an ELISA using a horseradish peroxidase (HRPO)-anti-species-conjugate.

INTENDED USE OF THE TEST KIT

The FIV-p24/p17 ELISA kit is designed to detect antibodies against these proteins. To this end recombinant p24/p17 products are attached to the solid phase. After washing the plates are incubated with the cat sera to be tested. The plates are washed after incubation to remove unbound materials. An anti-species conjugate is added to detect bound cat antibodies to FIV-p24/p17. After incubation and rinsing, the substrate is added and the optical density is measured at 450 nm.

PRINCIPLE OF THE TEST

The test is based on the reaction of p24/p17 proteins with cat antibodies. To this end, p24/p17 expression proteins have been coated to a 96 well microtiter strip plate.
The cat serum sample is added (diluted 1:100) to the wells of the coated plate.
After washing, the bound cat antibodies are detected by an anti-species conjugate.
Bound anti-species conjugate is made visible by adding substrate/chromagen mix.
The intensity of the color reaction in the wells is directly related to the concentration of anti-FIV-p24/p17 antibodies in the serum sample.

CONTENTS

- 12 x 8-well microtiter strips coated with recombinant FIV-p24 and p17
- 1 x Strip holder
- 1 x 18 ml Buffer
- 1 x 12 ml Anti-species conjugate
- 1 x 0.5 ml Positive control (freeze-dried)
- 1 x 1 ml Negative control (freeze-dried)
- 1 x 20 ml Wash solution 200 x concentrated, which must be diluted 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 seal

HANDLING AND STORAGE OF SPECIMENS.

The ELISA should be stored at 4-8˚C. An unopened package can be used until the expiry date.

An open package can be used if the requirements, mentioned in the validation (see chapter 9) are fulfilled. If not fulfilled the test can no longer be used.
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.

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. The wash solution is 200X concentrated, **dilute in deionized water before use!**
2. Empty each well by turning the microtitre plate upside down, followed by a firm vertical downward movement to remove the buffer.
3. Fill all the wells with 250 µl of diluted washing solution.
4. This washing cycle (2 and 3) should be carried out **at least 4 times**.
5. Turn the plate upside down and empty the wells by a firm short vertical movement.
6. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
7. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

The wash solution is 200X concentrated, **dilute in deionized water before use!** When using automatic plate washing equipment, check that all wells can be 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**.

PRECAUTIONS

- Handle all biological material as though capable of transmitting FeLV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
- TMB 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 is from damage and dirt.

TEST PROTOCOL

1. Reconstitute directly before use the positive control in 0.5 mL and negative control in 1 ml deionized water
2. Open the package 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 +4-8°C.
   Wash the microtiter strip(s) with washing solution, according to washing protocol.
   The provided washing solution must be diluted 200 x in deionized water.
3. Make a 1:100 dilution of the test sample in buffer and transfer 100 µl to one well of the microtiter strips.
   Also make a 1:100 dilution of the positive and negative control and transfer 100 µl of each to two wells.
4. Seal and incubate for 60 min. at 37°C.
5. Wash as in 2.
6. Dispense 100 µl anti-species conjugate to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash as in 2.
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.
10. Incubate 10-15 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 using 620 nm as reference.

VALIDATION OF THE TEST

To standardize the FIV-p24/p17 ELISA a positive control has to be tested.
The FIV positive control should give an extinction of ≥ 0.800 OD measured at 450 nm using 620 nm as reference. The OD (450 nm) of the sticky negative control must be ≤ 0.400.

INTERPRETATION OF TEST RESULTS

A sample is considered **positive** when the measured extinction is higher than 2 times the OD of the negative control.
When a sample is **negative > sample < 2 x negative**, it should be tested again within 2-4 weeks.

The OD of the positive control must be ≥ 0.700.

Important
Confirm a positive result always with a blocking ELISA test (Cat.no. EIA-2464) or with a virus blotting test or IFA test..

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.