DRG® Feline Panleucopenia Virus Antigen ELISA (EIA-2466)

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

INTRODUCTION

For diagnosis of Feline Panleucopenia Virus (FPV) infections in cats the demonstration of FPV antigen in feces is the most commonly used method. Possible false negative results caused by naturally occurring variants of the virus is minimized in this assay, since two monoclonal antibodies directed against two different well conserved epitopes were used in the assay.

PRINCIPLE OF THE TEST KIT

The principle of the test is based on the reaction of two monoclonal antibodies with 2 different antigenic determinants of FPV. One monoclonal antibody, coated to the plate, catches the Panleucopenia Virus in the feces sample after which the other, enzyme-labelled antibody detects the bound virus.

CONTENTS

- 8 x 12 **microtiter strips** coated with monoclonal anti-FPV antibody.
- 1 x **Strip holder**
- 1 x 10 ml **Buffer**
- 1 x 11 ml **HRPO-conjugated monoclonal antibodies**
- 1 x 1 ml FPV **Positive Control**
- 1 x 1 ml FPV **Negative Control** (Freeze Dried)
- 20 ml **washing 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

Products needed that are not provided:

- Phosphate Buffered Saline (PBS)

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. Open the packet of strips. Take out the strips to be used, cover the remaining strips with a part of the seal provided; store at +4°C and use within 10 days.
2. Wash according to the washing protocol. Dilute washing solution 200 x before use!
   Reconstitute the negative control in 1 ml deionized water.
3. Dilute the faeces sample(s) at least 1+1 in a clean tube.
   Take a small sample of faeces/diarrhoea and add same amount/volume of PBS (not provided) to the tube, mix well.
   Let cloths of faeces sink or spin down 4 minutes at 4000 g, use only the supernatant.
   The supernatant has to be diluted 1+1 in buffer (50 µl supernatant + 50 µl buffer).
   Divide positive and negative controls into aliquots, and store immediately at -20°C until use.
4. Add 100 µl positive control to the first well.
   Add 100 µl negative control to the second well.
5. Add 100 µl diluted sample to other wells.
6. Incubate 60 minutes at 37°C.
7. Wash as pointed out in wash protocol.
8. Add 100 µl HRPO-conjugated monoclonal antibodies to each well.
9. Incubate 60 min. at 37°C.

10. Wash as pointed out in wash protocol.
11. Mix equal parts of buffer A and B with gentle shaking. Prepare immediately before use!
    Dispense 100 µl substrate mixture to each well. Incubate for 10-15 minutes at room temperature (21°C).
12. After incubation the reaction is stopped by adding 50 µl stopping solution to each well.
13. Read the absorbency values immediately (within 10 min.!) at 450 nm.
    Use as a reference wavelength 620nm

PRECAUTIONS

- Handle all biological materials as though capable of transmitting FPV.
- 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

To standardize the Feline Panleucopenia Virus ELISA, positive and negative controls have to be tested.
The FPV positive control should give an OD (450nm) > 0,900.
The OD (450nm) of the negative control must be lower than 0,300.

INTERPRETATION OF TEST RESULTS

The test samples are considered FPV positive if the absorbency is above 3 times the absorbency of the negative control.
These animals will shed the Panleucopenia Virus and will be infectious to other animals.
When a sample is negative > sample < 3 x negative, it should be tested again within 5 days.