DRG® Canine Parvo Virus IgM Ab ELISA (EIA-2476)

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INTRODUCTION

For diagnosis of Canine Parvo Virus (CPV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. Antibodies induced through infection or vaccination are caught by the virus which is attached to the solid phase by use of monoclonal antibodies.
IgM antibody titers above a dilution of 1:50 are considered to be recently infected with CPV.
The antibody response of these animals have to be checked several times at IgM and IgG level and (re)vaccinated if the developed IgG titer is too low.

PRINCIPLE OF THE TEST KIT

The principle of the CPV test kit is based on the detection of antibodies against parvo virus. The parvo virus is attached to the solid phase by use of a monoclonal antibody. After the attachment of the antigen (parvo virus) sera containing antibodies are able to react with the antigen. After the antigen/antibody reaction, the attached antibodies can be detected by use of a polyclonal conjugate.

CONTENTS

- 12 x 8-well **microtiter strips** coated with monoclonal anti parvo virus antibody
- 1 x strip holder
- 2 x 6 ml **anti dog IgM HRPO conjugate**
- 3 x 5 ml **ELISA buffer**
- 1 x 100 ml **wash solution, 10 x** *concentrated*. Dilute in deionized water before use!
- 3 x 4 ml parvo **antigen solution**
- 1 x 0.5 ml **positive control** serum *(freeze-dried)*
- 1 x 1 ml **negative control** serum *(freeze-dried)*
- 1 x 8 ml **substrate A**
- 1 x 8 ml **substrate B**
- 1 x 8 ml **stop solution**
- 1 x plastic cover seal

HANDLE AND STORAGE OF SPECIMENS

The reagents should be stored at +4°C. They are stable until the date given on the label.
Positive and negative control may be stored after reconstitution in aliquots at -20°C. and used until the expiration date.
Repeated freezing and thawing must be avoided.
Once a packet of strips is opened it should be used within 10 days.

WASHING PROTOCOL

In ELISA's, between each immunological incubation step un-complexed components have to be removed efficiently. 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 advised to carefully follow the washing procedures outlined below. Both manual washing and washing with automatic equipment can be performed. (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 movement.
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 short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove 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 using automatic plate wash 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.

TEST PROTOCOL

1. Open the packet of strip(s), take out the strips to be used, cover the remaining strips with a part of the provided seal; store at 4°C and use within 10 days.
   Wash according to washing protocol, with washing solution (dilute washing solution 1:10 in deionized water before use.)
2. Add 100 µl parvo antigen to each well.
3. Seal and incubate one hour at 37°C.
4. Wash as 1.
5. Make 3 steps serum dilution of each sample (1:50; 1:150; 1:450; 1:1350) with ELISA Buffer in another round bottomed microtiter plate.
   Directly before use reconstitute the positive control with 0.5 ml and negative control with 1 ml PBS.
   Make the following dilutions of the positive and negative controls (1:50; 1:150; 1:450 and 1:1350).

   Add 100 µl of each dilution to one coated well.
6. Seal and incubate one hour at 37°C.
7. Wash as in 1.
8. Add 100 µl conjugate to each well.
9. Seal and incubate one hour at 37°C.
10. Wash as in 1.
11. Mix equal parts of buffer A and B with gentle shaking. Prepare immediately before use!
    Dispense 100 µl substrate solution to each well.
    Incubate 10-20 min. at room temperature (21°C.)
12. Add 50 µl stop solution to each well.
13. Read the absorbency values immediately (within 10 min!) at 450 nm. Use 620 nm as a reference wavelength.

PRECAUTIONS

- Handle all biological materials as though capable of transmitting CPV.
- Do not pipette by mouth.
- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.

- TMB is toxic by inhalation, in contact with skin and when swallowed; observe care when handling the substrate.
- Do not use components past expiration date and do not intermix 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 prevent it from damage and dirt.

VALIDATION OF THE TEST

The negative control should give an OD < 0.350.
The end point titer of the positive control should be between 1:150 and 1:450 according to the instructions for interpretation of test results.

INTERPRETATION

The endpoint titer of the sample is the dilution which gives an extinction above 3 times the OD value of the negative control.
The test is valid if the first two dilutions of the positive control are above 0,350 (OD 450 nm).

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.