Canine Distemper Virus Antibody ELISA

A monoclonal mediated antibody ELISA, to detect IgG antibodies against Canine Distemper Virus in serum or plasma

REF EVL-EIAD1002-AB01
Please use only the valid version of the package insert provided with the kit. 
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung. 
Si prega di usare la versione valida dell’inserto del pacco a disposizione con il kit. 
Por favor, se usa solo la version valida de la metodico técnico incluido aquí en el kit.

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1 INTRODUCTION
For diagnosis of Canine Distemper Virus (CDV) 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. IgG antibody titers above dilutions of 1:150 are considered protected. After reaching peak values within two or three weeks, antibody titers fall back to a threshold level at which they persist. Re-exposure results in an anamnestic response.

2 INTENDED USE OF THE TESTKIT
The CDV test kit is based on monoclonal antibodies against a common epitope of CDV, which are coated to the solid phase. The distemper virus is attached to the solid phase by the monoclonal antibody. After the attachment of the antigen (distemper virus) sera containing antibodies are able to react with the attached antigen. After the antigen/antibody reaction, the attached antibodies can be detected by use of a polyclonal conjugate.

3 PRINCIPLE OF THE TEST KIT
The test is based on the reaction of CDV proteins with CDV antibodies. To this end purified CDV proteins have been coated to a 96-well microtiter plate.

The diluted dog serum sample is added to the wells of the coated plate. After washing the bound CDV antibodies are detected by a HRPO conjugated anti-species conjugate.

The color reaction in the wells is directly related to the concentration of CDV antibodies in the serum.

4 CONTENTS

- 12 x 8 microtiter strips
- 1 x strip holder
- 1 x 18 ml ELISA buffer
- 1 x 12 ml HRPO conjugated anti-species antibodies
- 1 x 0.5 ml Positive control (Freeze dried)
- 1 x 1 ml Negative control (Freeze dried)
- 1 x 20 ml Wash-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

Supplies needed (not included): Round bottomed micro titer plate, pipet, tips and clean containers/tubes, reader 1 x 12 8-well microtiter plate coated with inactivated rabies virus

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 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.

7 TEST PROTOCOL
1. Open the packet 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°C. and use them within 10 days. Wash the microtiter strip(s) with washing solution, according to washing protocol. **The washing solution provided must be diluted 200x in de-ionized water!**
2. Reconstitute directly before use the positive control in 0,5 ml and the negative control in 1 ml de-ionized water, divide into aliquots, and store immediately at -20°C until, use avoid freeze and thaw cycles.
3. Make 3-step dilution of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate (not supplied). Make also a 3-step dilution of the positive and negative control.
4. Transfer 100 µl of this dilution to the CDV coated microtiter strips. Seal and incubate for 60 min. at 37°C.
5. Wash the plate according to the wash protocol.
6. Dispense 100 µl conjugated anti-species antibody to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash the plate according to the wash protocol.
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. Incubate 10-20 min. at room temperature (21°C).(Make sure the negative does not become to dark)
10. Add 50 µl stop solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.) at 450 nm.

8 PRECAUTIONS
- Handle all biological material as though capable of transmitting CDV.
- 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.
9 VALIDATION OF THE TEST
The negative control should give an OD < 0.500 (at 450 nm). The end point titer of the positive control should between 1:90 and 1:270 according to the instructions for interpretation of test results.

10 INTERPRETATION OF TEST RESULTS
The end point titer of the sample is the dilution that gives an extinction just above 0.350 OD units at 450 nm.

Antibody titers of 270 and higher in diseased animals showing signs suggestive of CDV are considered positive.

In summary:
- ≤ 30 = no antibodies found.
- 90-270 = antibodies found, retest in 3 months.
- ≥ 810 = high titer of antibodies found.

Diseased animal: suggestive for CDV. Healthy animal: Retest in 3 months.

SYMBOLS USED WITH EVL ASSAYS

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<th>English</th>
<th>Deutsch</th>
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