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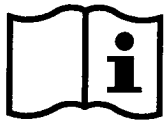

Useŕs Manual

CDV (Canine Distemper Virus) IgG ELISA

Enzyme immunoassay for the detection of IgG antibodies against Canine Distemper Virus (CDV) in serum or plasma

VET

REF **DE2478**

96 wells

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Please use only the valid version of the Instructions for Use provided with the kit.

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

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 1:250 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 TEST KIT

The CDV ELISA 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 antigen/antibody reaction, the attached antibodies can be detected by use of a polyclonal conjugate.

3 PRINCIPLE OF THE TEST KIT

The CDV ELISA test kit 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.

- **Qualitative**

The dog sample is added (diluted 1:150) to the wells of the coated plate.

- **Quantitative**

The dog sample also can be titrated using a 3-step dilution,
starting with a dilution 1:50 (→ 1:150→ 1:450 →1:1350).

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 serum or plasma.

4 CONTENTS

- **SORB MT** 12 x 8 Microtiter strips
- 1 x Strip holder
- **BUF** 1 x 18 mL ELISA buffer (green cap)
- **ENZ CONJ** 1 x 12 mL HRPO conjugated anti-species antibodies (red cap)
- **CONTROL 1** 1 x 0.5 mL Positive control (freeze dried) (purple cap)
- **CONTROL 2** 1 x 1.0 mL Negative control (freeze dried) (silver cap)
- **WASH SOLN 200x** 1 x 20 mL Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- **SUB A** 1 x 8 mL Substrate A (white cap)
- **SUB B** 1 x 8 mL Substrate B (blue cap)
- **STOP SOLN** 1 x 8 mL Stop solution (yellow cap)
- 1 x Plastic cover seal
- 1 x Instructions for Use

4.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes
- ELISA plate reader

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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.
After first use, ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20 °C. 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 wash solution.
3. This washing cycle (1 and 2) should be carried out at least 5 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 wash 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 wash solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7 PREPARATIONS

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min at room temperature (± 21 °C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4 °C - 8 °C immediately after use.

8 TEST PROTOCOL <u>QUALITATIVE</u>

Before starting this test read “PREPARATIONS”

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.

   <u>Wash microtiter strip(s) with wash solution, according to wash protocol.</u>

   <u>The wash solution provided must be diluted 200x in aqua bidest (5 MΩ water)</u>!
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

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2. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
3. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
4. Dilute the **positive control** (purple cap) **starting 1:50 → 1:150 → 1:450 → 1:1350** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - A **pre-dilution** is needed:
   - Add 80 µL ELISA buffer to **well 1A**, add 20 µL of the positive control to the **well 1A** and mix well.
   - Add 180 µL ELISA buffer to **well 2A**, and 120 µL to **wells 2B, 2C, 2D**
   - Add 20 µL of **pre-dilution** from **well 1A** in the **well 2A** and mix well
   - Mix well and transfer 60 µL to the well **2B**
   - Mix well and transfer 60 µL to the well **2C**
   - Mix well and transfer 60 µL to the well **2D**
   - Mix well and discard 60 µL.
5. Dilute the **negative control** (silver cap) **1:50** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - Add 147 µL ELISA buffer to **well 2E**, add 3 µL of the negative control to the **well 2E** and mix well.
6. Dilute the **sample 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - A pre-dilution is needed:
   - Add 90 µL ELISA buffer to **well 1F**, add 10 µL of the sample to the **well 1F** and mix well.
   - Add 140 µL ELISA buffer to **well 2F**, add 10 µL of pre-dilution **well 1F** in the **well 2F** and mix well
7. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.

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8. Transfer 100 µL of all dilutions of **column 2 (round bottomed plate)** to the **CDV coated microtiter strips**.
9. Seal and incubate for 60 min at 37 °C.
10. Wash the plate according to the wash protocol see sub 6.
11. Dispense 100 µL conjugated anti-species antibody to all wells.
12. Seal and incubate for 60 min at 37 °C.
13. Wash the plate according to the wash protocol see sub 6.
14. Mix equal parts of Substrate A (white cap) and Substrate B (blue cap) with gentle shaking.
    Prepare immediately before use!
    Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.
15. Dispense 100 µL substrate solution to each well.
16. Incubate 10 - 20 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C).
    Make sure the negative control does not become too dark.
17. Add 50 µL stop solution to each well; mix well.
18. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

NB: if you pipet directly into the coated ELISA plate, with only a small number of samples (< 6), make sure the first dilution is done in round bottom microtiter plate, second step can be done directly in the coated ELISA plate.

9 TEST PROTOCOL QUANTITATIVE

Before starting this test read "PREPARATIONS"

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 microtiter strip(s) with wash solution, according to wash protocol.

   The wash solution provided must be diluted 200x in aqua bidest (5 MΩ water)!
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
3. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
4. Make a pre-dilution of the **positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - Add 80 µL ELISA buffer to **well 1A** and add 20 µL of the positive control to the well **1A.**
5. Make a pre-dilution of the **negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - Add 80 µL ELISA buffer to **well 1B** and add 20 µL of the negative control to the well **1B.**
6. Make a pre-dilution of **each sample** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - Add 80 µL ELISA buffer to **well 1C** and add 20 µL of the sample to the **well 1C.**
7. Take 2 wells as substrate controls add only 120 µL ELISA buffer (green cap) to these wells.

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8. Add for dilution of the **positive control** 135 µL ELISA buffer to **well 1A**, and 100 µL to **1B, 1C, 1D** of the coated microtiter strip.
9. Add for dilution of the **negative control** 135 µL ELISA buffer to **well 1E**, and 100 µL to **1F, 1G, 1H** of the coated microtiter strip.
10. Add for dilution of the **samples** 135 µL ELISA buffer to the other **well 2A and 2E**, and 100 µL to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.
11. Make a 3-step dilution of the **positive control** in the coated microtiter strip, starting **1:50 → 1:150 → 1:450 → 1:1350**.
    Example: **-** Dispense 15 µL positive control from step 4 to the **well 1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.
12. Make a 3-step dilution of the **negative control** in the coated microtiter strip, starting**1:50 → 1:150 → 1:450 → 1:1350**.
    Example: **-** Dispense 15 µL negative control from step 5 to the **well 1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well and transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.
13. Make 3-step dilution of **each sample** in the coated microtiter strip, starting**1:50 → 1:150 → 1:450 → 1:1350**.
    Example: **-** Dispense 15 µL of each sample from step 6 to the well **2A and/or 2E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **2B and/or 2F**
    - Mix well and transfer 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.
14. Dispense 100 µL of the substrate control of step 7 to the last 2 wells of the microtiter strip.

15. Seal and incubate for 60 min at 37 °C.
16. Wash the plate according to the wash protocol see sub 6.
17. Dispense 100 µL conjugated anti-species antibody to all wells.
18. Seal and incubate for 60 min at 37 °C.
19. Wash the plate according to the wash protocol see sub 6.
20. Mix equal parts of Substrate A (white cap) and Substrate B (blue cap) with gentle shaking.
    Prepare immediately before use!
    Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.
21. Dispense 100 µL substrate solution to each well.
22. Incubate 10 - 20 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21 °C).
    Make sure the negative control does not become too dark.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the ELISA reader. Use the substrate controls as blank.

10 PRECAUTIONS

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling 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.

11 VALIDATION OF THE TEST

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC), diluted 1:50, must be ≥ 0.850
  - The MV of the measured OD value for the Negative Control (NC) , diluted 1:50, must be ≤ 0.400

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control, diluted 1:50, should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 90. The negative control, diluted 1:50, should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 30.

12 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio **< 0.25** is negative
  - Specific antibodies to Distemper virus could not be detected.
- A sample with the S/P ratio **≥ 0.25** is positive
  - Specific antibodies to Distemper virus were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using a cut-off line (dilution 1:50 → 1:150 → 1:450 → 1:1350 → 1:4050→ 1:12150, etc., total 8 dilutions of 3 steps) OD on Y-axis and titre on X-axis. ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:50.

The entire risk as to the performance of these products is assumed by the purchaser. Demeditec shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products In case of problems or questions contact Demeditec.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore

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