Product information

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CAV (Canine Adeno Virus) Ab ELISA

VET

REF **DE2480**

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

Table of Contents

An ELISA to detect antibodies against Canine Adeno Virus in serum or plasma samples.

1 INTRODUCTION

Canine Adeno Virus (CAV) is an important and complex disease of both wild and domestic dogs. The great majority of dogs that become infected only have a subclinical infection and recover completely. Upon today we recognize two sub groups: CAV1 and CAV2.
We distinguish 3 types of infection:

- Per acute: mostly young animals, which die within 1 or 2 days.
- Acute: 3 - 5 days increase in temperature, increase in kidney volume, diarrhea with blood.
- Sub-acute: 6 - 14 days, as acute, only milder symptoms.

Eye changes are mostly an indication of the recovery phase. These are induced by antibody-antigen-interactions which results in fluid between lamina propria and cornea. Approximately 10 days after infection antibodies are detectable. The highest antibody level is reached after 8-10 weeks. The titer then degrees to a threshold level within about 6 months.
Caution! Even after clinical recovery the dog can still shed virus in the urine (CAV is very resistant).
Important in the diagnosis of CAV are:

- antibody detection
- clinical history
- clinical signs

2 INTENDED USE OF THE TEST KIT

The CAV ELISA test kit is designed to detect antibodies against purified CAV proteins.

CAV proteins are attached to the solid phase. After washing the strips are incubated with the dog serum/plasma sample to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labeled anti-species conjugate is added to detect bound antibodies to CAV proteins. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of CAV proteins with polyclonal dog antibodies. To this end CAV proteins have been coated to a 96-well microtiter plate.

➢ **Qualitative**

The dog sample is added (diluted 1:250) 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 dog antibodies are detected by a HRPO conjugated anti-species conjugate.

The color reaction in the wells is directly related to the concentration of CAV antibodies in the serum/plasma sample.

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 LYO** 1 x 0.5 mL Positive control (freeze dried) (purple cap)
- **CONTROL 2 LYO** 1 x 1.0 mL Negative control (freeze dried) (silver cap)
- **WASH SOLN 200x** 1 x 20 mL Wash solution (200x concentrated) (black cap), **diluted 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

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 increase non-specific reactivity.

6 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 result.

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 (step 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 added.

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 added, 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 QUALITATIVE

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 washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ water) !
   Use validated precision pipettes and use a clean pipette tips **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 after complete dissolving 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 after complete dissolving 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 **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 **each sample** 1:250 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 120 µL ELISA buffer to **well 2F**, add 5 µL of pre-dilution from **well 1F** in the well **2F** and mix well.
7. Take 2 wells as substrate control, add only 120 µL ELISA buffer (green cap) to these wells.

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8. Transfer 100 µL of all *dilutions of row 2* to the CAV **coated** microtiter strips, including the substrate controls.
9. Seal and incubate for 60 min at 37 °C.
10. Wash the strips according to the wash protocol see sub 6.
11. Add 100 µL HRPO conjugated anti-species antibodies to all wells.
12. Seal and incubate for 60 min at 37 °C.
13. Wash the strips 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. Add 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 washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ water) !
   Use validated precision pipettes and use a clean pipette tips **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 after complete dissolving 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 after complete dissolving 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 90 µL ELISA buffer to **well 1A** and add 10 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 90 µL ELISA buffer to **well 1B** and add 10 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 90 µL ELISA buffer to **well 1C** and add 10 µL of the sample to the well **1C**.
7. Take 2 wells as substrate control, add only 140 µL ELISA buffer (green cap) to these wells.

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8. Add for dilution of the **positive control** 120 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** 120 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** 120 µL ELISA buffer to the other **wells 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 30 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: - Add 30 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: - Add 30 µL of each sample from step 6 to the well **2A and/or 2E** of 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. Add 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 strips according to the wash protocol see sub 6.
17. Add 100 µL HRPO conjugated anti-species antibodies to all wells.

18. Seal and incubate for 60 min at 37 °C.
19. Wash the strips 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. Add 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 ≥ 1.000
  - The MV of the measured OD value for the Negative Control (NC) , diluted 1:50,
    must be ≤ 0.350

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 ≥ 1.000 OD units (450 nm) and give an endpoint titer of ≥ 150.

The negative control, diluted 1:50, should be < 0.350 OD units (450 nm) and give an endpoint titer of ≤ 50.

12 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio **< 0.27** is negative.
  - Specific antibodies to Adeno could not be detected.
- A sample with the S/P ratio **≥ 0.27** is positive.
  - Specific antibodies to Adeno 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	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-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	utilisation 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 catalogo
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	Température 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	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta