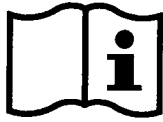


Product information

Information about other products is available at: www.demeditec.com



User's Manual

CAV (Canine Adeno Virus) Ab ELISA

*Enzyme Immunoassay for the detection of antibodies against
Canine Adeno Virus in serum or plasma.*

VET

REF DE2480

Σ 96 wells

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

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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. 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 degrades to a threshold level within about 6 months.

Take care! Even after clinical recovery the dog can still shed virus in the urine (CAV is very resistant).

Important in the diagnosis of CAV are:

- clinical history
- clinical signs
- laboratory findings: antibody detection

2 INTENDED USE OF THE TEST KIT

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

CAV proteins are attached to the solid phase. After washing the strips are incubated with the dog sera to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labeled anti-species conjugate is added to detect bound dog 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.

The diluted dog serum/plasma sample is added to the wells of the coated plate.

After washing the bound dog antibodies are detected by a HRPO conjugated antispecies conjugate.

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

4 CONTENTS

- 12 x 8-well **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 1.0 mL **Negative Control** (freeze dried)
- 1 x 20 mL **Wash solution 200 x concentrated, dilute in deionized water before use!**
- 1 x 8 mL **Substrate Buffer A**
- 1 x 8 mL **Substrate Buffer B**
- 1 x 8 mL **Stop Solution**
- 1 x Plastic cover seal

Supplies needed not included:

- Round bottomed microtiter plate

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at 2 °C - 8 °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, 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 200 x in deionized water!
2. Dilute the positive control in 0.5 mL deionized water and the negative control in 1 mL deionized water.
3. **Qualitative:** Make a dilution 1:100 of each sample in ELISA buffer in a round bottomed microtiter plate (not supplied). Make a dilution 1:100 of the positive and negative control.

Quantitative: Make 3-step dilutions of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate.

Make also a 3-step dilution of the positive and negative control.

4. Transfer 100 µL of these dilutions to the CAV coated microtiter strips.
5. Seal and incubate for 60 min. at 37 °C.
6. Wash the plate according to the in wash protocol.
7. Dispense 100 µL conjugated anti-species antibody to all wells.
8. Seal and incubate 60 min. at 37 °C.
9. Wash the plate according to the in wash protocol.
10. 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 10-15 min. at room temperature (21 °C).
11. Add 50 µL stop solution to each well; mix well.
12. Read the absorbency values immediately (within 10 min.!) at 450 nm. Use 620 nm as a reference wavelength.

8 PRECAUTIONS

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

In order to confirm appropriate test conditions, the positive control should give an extinction ≥ 0.1000 OD units and an end point titer ≥ 270 .

The negative control should give an OD ≤ 0.350 and an end point titer ≤ 30 .

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

a. qualitatively: positive or negative

A sample is scored positive if the OD is higher than 2.5 x OD of the negative control.

b. quantitatively: end point titer

The end point titer of the sample is the dilution which gives an extinction just above 0.350 OD units (450 nm)












Antibody titers of 90 and higher in diseased animals showing signs suggestive of CAV are considered positive and the dog will be suspected of shedding CAV. A rise in antibody titer in a dog with CAV represents an exaggerated, effective immune response.

In summary:

- ≤ 30 = no antibodies found
- 90 - 270 = diseased: antibodies found, probably shedding CAV, retest in 2 months.
not diseased: low titer normally found incompletely recovered dogs but they still might be virus carriers.
- ≥ 810 = high titer of antibodies found 8-10 weeks after infection or vaccination.

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.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	For veterinary use only				
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità