User's Manual

# Canine Corona Virus Antibody ELISA

An ELISA test to detect antibodies against Canine Corona Virus in serum or plasma samples

REF D1005-AB01

Σ 96

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***Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.***
***Please use only the valid version of the package insert provided with the kit.***
***Verwenden Sie nur die jeweils gültige, im Test kit 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 aqui en el kit.***

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## 1. Introduction

Canine Corona Virus (CCV) is an important disease of both wild and domestic dogs. The great majorities of dogs that become infected recover completely and develop immunity to CCV. Some of the recovered dogs become carriers of the virus and can infect other dogs. A few infected dogs do not build up immunity to CCV and the disease progress to a fatal form. The fatal form of CCV is a progressive disease characterized by diarrhea, intestinal disease, weakness and loss of appetite.

Important in the diagnosis of CCV is:
- Clinical history
- Clinical signs
- Laboratory findings:
- Antibody detection
- Antigen detection

This test measures corona virus antibodies that are present in the blood or plasma. Most low antibody positive dogs are possible virus carriers and may shed CCV.

## 2. Intended use of the test kit

The CCV ELISA test kit is designed to detect antibodies against CCV proteins (mostly glycoproteins). CCV proteins are attached to the solid phase. After washing the strips are incubated with the canine 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 canine antibodies to CCV 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 CCV proteins (mostly glycoproteins) with polyclonal dog antibodies. To this end CCV proteins have been coated to a 96-well microtiter plate.

- **Qualitative**
  The canine sample is added (diluted 1:150) to the wells of the coated plate.
- **Quantitative**
  The canine sample also can be titrated using a 3-step dilution, starting with a dilution 1:30 (→ 1:90→ 1:270 →1:810).

After washing the bound canine antibodies are detected by a HRPO conjugated anti-species conjugate.
The color reaction in the wells is directly related to the concentration of CCV antibodies in the serum/plasma sample.

## 4. Contents

- 12 x 8 microtiter strips
- 1 x strip holder
- 1 x 18 ml ELISA buffer (green cap)
- 1 x 12 ml HRPO conjugated anti-species antibodies (red cap)
- 1 x 0,5 ml Positive control (freeze dried) (purple cap)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 20 ml Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop solution (yellow cap)
- 1 x plastic cover seal
- 1 x User's manual

**Supplies needed (not included)**

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- 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 increases 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 4-8°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 aquabidest (5 mega Ohm) 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 **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
3. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm 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 1:30 → 1:90 → 1:270 → 1:810 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   ***Example:*** - A **pre-dilution** is needed:
   - Add 80µl ELISA buffer to **row 1A**, add 20µl of the positive control to the **well 1A** and mix well.
   - Add 150µl ELISA buffer to **row 2A**,
   - And 120µl to **2B, 2C, 2D**
   - Add 30µl of pre-dilution **well 1A** in the well **2A** and mix well
   - Mix well and transfer 60µl to the well **2B**
   - Mix well and transfer 60µl to the well **2C**
   - Mix well and transfer 60µl to the well **2D**
   - Mix well and discard 60µl.
5. Dilute the **negative control** (silver cap) **1:30 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   ***Example:*** - Add 145µl ELISA buffer to **well 2E**, add 5µl of the negative control to the **well 2E** and mix well.
6. Dilute **each sample 1:150 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   ***Example:*** - A pre-dilution is needed:
   - Add 90µl ELISA buffer to **row 1F**, add 10µl of the sample to the **well 1F** and mix well.
   - Add 140µl ELISA buffer to **row 2F**, add 10µl of pre-dilution **well 1F** in the well **2F** and mix well.
7. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.

8. Transfer 100µl of all dilutions of **row 2** to the CCV 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 **100µl HRPO conjugated anti-species antibodies** to all wells.

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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 **100µl 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 **50µl stop solution** to each well; mix well.
18. Read the absorbency values immediately (within 10 min!) at 450 nm by using an 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.***

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# 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 aquabidest (5 mega Ohm) 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 aquabidest** (5 mega Ohm 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 1,0ml aquabidest** (5 mega Ohm 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 80µl ELISA buffer to **row 1A** and add 20µl 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 80µl ELISA buffer to **row 1B** and add 20µl 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 80µl ELISA buffer to **row 1C** and add 20µl of the sample to the well **1C.**
7. Take 2 wells as **substrate controls** add only **140µl ELISA buffer** (green cap) to these wells.

8. Add for dilution of the **positive control 125µl buffer** to **row 1A.**
   And 100µl to **1B, 1C, 1D** of the coated microtiter strip.
9. Add for dilution of the **negative control 125µl buffer** to **row 1E.**
   And 100µl to **1F, 1G, 1H** of the coated microtiter strip.
10. Add for dilution of the **samples 125µl buffer** to the other **row 2A and 2E.**
    And 100µl 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 1:30 → 1:90 → 1:270 → 1:810**.
    ***Example:*** - Add 25µl 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 50µl to the well **1C**
    - Mix well and transfer 50µl to the well **1D**
    - Mix well and discard 50µl.

12. Make a 3-step dilution of the **negative control** in the coated microtiter strip, **starting 1:30 → 1:90 → 1:270 → 1:810**.
    ***Example:*** - Add 25µl negative control from step 5 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50µl to the next well **1F**
    - Mix well transfer 50µl to the next well **1G**
    - Mix well and transfer 50µl to the well **1H**
    - Mix well and discard 50µl.
13. Make 3-step dilution of **each sample** in the coated microtiter strip, **starting 1:30 → 1:90 → 1:270 → 1:810**.
    ***Example:*** - Add 25µl 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 50µl to the well **2C and/or 2G**
    - Mix well and transfer 50µl to the well **2D and/or 2H**
    - Mix well and discard 50µl.
14. Add **100µl** 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 **100µl 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 **100µl 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 does not become too dark.

23. Add **50µl stop solution** to each well; mix well.
24. Read the absorbency values immediately (within 10 min!) at 450 nm by using an 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 1:30 must be ≥1.000.
  - The MV of the measured OD value for the Negative Control (NC) diluted 1:30 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:

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

**Quantitative:**

In order to confirm appropriate test conditions the OD of the positive control, diluted 1:30, should be ≥ 0.800 OD units (450nm)and give an endpoint titer of ≥270.
The negative control, diluted 1:30, should be lower than 0.400 OD units (450nm) 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 <0.24 is negative.
  - Specific antibodies to Canine Corona virus could not be detected.
- A sample with the S/P ratio ≥0.24 is positive.
  - Specific antibodies to Canine Corona virus were detected.

**Quantitative:**End point titre

- The ELISA titre can be calculated by constructing a curve and using cut-off line(dilution 1:30-90-270 -810-2430 -7290 etc total 8 dilutions of 3 steps) OD on Y-as and Titre on X-as.
  ELISA titres can be calculated using as cut-off 2,5 times OD value of negative control at 1:30.

## 13. Symbols used with EVL 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 |
| CE | European Conformity | CE-Konfirmitäts-kennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
| IVD | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso 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. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
| LOT | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
| Σ | 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 | Mindesthaltbarkeits-datum | 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 |

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