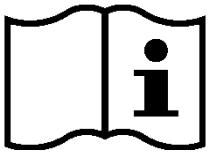

Userś Manual

Canine Parvo Virus Antigen ELISA

A monoclonal antibody-mediated capture ELISA to detect Canine Parvo Virus in faeces samples

REF D1001-AG01

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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 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 aqui en el kit.

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1. Introduction

For diagnosis of Canine Parvo Virus (CPV) infections in dogs the demonstration of CPV antigen in faeces is the most commonly used method. Possible false-negative results caused by naturally occurring variants of the virus is minimized in this assay, since two monoclonal antibodies directed against two different well conserved epitopes were used in the assay.

2. Principle of the test kit

The test is based on the reaction of CPV proteins with monoclonal antibodies. To this end monoclonal antibodies proteins have been coated to a 96-microwell plate.
The diluted dog faeces sample is added to the wells of the coated plate.

- **Qualitative**
  The dog faeces sample is added (diluted 1:2) to the wells of the coated plate.
- **Quantitative**
  The dog faeces sample also can be titrated using a 3-step dilution, starting with undiluted (→ 1:3 → 1:9 → 1:27)

After washing, the bound dog antigens are detected by HRPO conjugated anti-CPV conjugate.
The color reaction in the wells is directly related to the concentration of CPV antigen in the faeces sample.

3. Contents

- 12 x 8 Microtiter strips coated with monoclonal anti-CPV antibody
- 1 x Strip holder
- 1 x 18 ml ELISA buffer (green cap)
- 1 x 12 ml HRPO conjugated anti-CPV antibodies (red cap)
- 1 x 1,0 ml Positive control (ready to use) (yellow cap)
- 1 x 1,0 ml Negative control (ready to use) (brown 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)
- Aquabidest
- ELISA plate reader

4. 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.

5. 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.

6. 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.

7. 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 the 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. Take a small sample of **faeces** and add same amount of **PBS** (0,01M) or **aqua bidest** (not provided) to a clean tube (dilution 1:1), mix well.
   Example: 250µl faeces + 250µl PBS.
   Let cloths of faeces sink or spin down 4 minutes at 4000 g, use only the supernatant.

3. Make a three-step dilution of the **positive control** (yellow cap) **in ELISA buffer** (green cap) starting undiluted → 1:3 → 1:9 → 27 in a round-bottomed plate (not supplied).
   Example: - Add 180µl positive control to the well **1A.**
   - Add 120µl ELISA buffer to all other wells **1B, 1C, 1D.**
   - Transfer 60µl from well **1A** to well **1B.**
   - Mix well and transfer 60µl from well **1B** to the well **1C.**
   - Mix well and transfer 60µl from **1C** to the well **1D.**
   - Mix well and discard 60µl.
4. Add **125µl negative control** (brown cap) to the well **1E** second well of a round-bottomed plate (not supplied).
5. Add 70µl ELISA buffer (green cap) to all other wells of a round-bottomed plate (not supplied) and thereafter **70µl supernatant of each centrifuged sample.**
6. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.

7. Transfer 100µl of all dilutions to the coated microtiter strips, including the substrate controls.
8. Seal and incubate for 60 min at 37°C.

9. Wash the strips according to the wash protocol see sub 5.
10. Add **100µl HRPO conjugated anti-CPV antibodies** to all wells.
11. Seal and incubate for 60 min at 37°C.

12. Wash the strips according to the wash protocol see sub 5.
13. 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.

14. Add **100µl substrate solution** to each well.
15. 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.
16. Add **50µl stop solution** to each well; mix well.
    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 pre-dilution step is done in round bottom plate, second step can be done directly in the coated ELISA plate all other steps are done before pipetting directly into the ELISA plate.

8. 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 the microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Take a small sample of **faeces** and add same amount of **PBS** (0,01M) or **aqua bidest** (not provided) to a clean tube (dilution 1:1), mix well.
   Example: 250 µl faeces +250ul PBS
3. Let cloths of faeces sink or spin down 4 minutes at 4000 g, use only the supernatant.

4. Make a three-step dilution of the **positive control** (yellow cap) **in ELISA buffer** (green cap) starting undiluted → 1:3 → 1:9 → 27 in a round-bottomed plate (not supplied).
   Example: - Add 180µl positive control to the well **1A.**
   - Add 120µl ELISA buffer to all other wells **1B, 1C, 1D.**
   - Transfer 60µl from well **1A** to well **1B.**
   - Mix well and transfer 60µl from well **1B** to the well **1C.**
   - Mix well and transfer 60µl from **1C** to the well **1D.**
   - Mix well and discard 60µl.
5. Make a three-step dilution of the **negative control** (brown cap) **in ELISA buffer** (green cap) starting undiluted → 1:3 → 1:9 → 27 in a round-bottomed plate (not supplied).
   Example: - Add 180µl negative control to the well **1E.**
   - Add 120µl ELISA buffer to all other wells **1F, 1G, 1H.**
   - Transfer 60µl from well **1E** to well **1F.**
   - Mix well and transfer 60µl from **1F** to well **1G.**
   - Mix well and transfer 60µl from well **1G** to the well **1H.**
   - Mix well and discard 60µl.
6. Make a three-step dilution of **each faeces sample in ELISA buffer** (green cap) **starting undiluted (=supernatant step 3) → 1:3 →1:9 →1:27** in a round-bottomed plate (not supplied).
   Example: - Add 180µl of the sample to the well **2A and/or 2E.**
   - Add 120µl ELISA buffer to all other wells **2B, 2C, 2D and/or 2F, 2G, 2H.**
   - Transfer 60µl from well **2A and/ or 2E** to well **2B and/or 2F.**
   - Mix well and transfer 60µl from well **2B and/or 2F** to the well **2C and/or 2G.**
   - Mix well and transfer 60µl from **2C and/or 2G** to the well **2D and/or 2H.**
   - Mix well and discard 60µl.
7. Take 2 wells as **substrate controls** add only **140µl ELISA buffer** (green cap) to these wells.

8. Transfer 100µl of all dilutions to the 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 5.

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11. Add **100µl HRPO conjugated anti-CPV 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 5].
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.
    Read the absorbency values immediately (within 10 min!) at 450 Nm by using an ELISA reader. **Use the substrate controls as blank.**

9. 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.

10. 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), undiluted, must be ≥0.500.
  - The MV of the measured OD value for the Negative Control (NC), undiluted, must be ≤0.250.

  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, undiluted, should be ≥ 0.500 OD units (450nm) and give an endpoint titer of ≥2.
The negative control, undiluted, should be ≤ 0.250 OD units (450nm) and give an endpoint titer of ≤2.

11. Interpretation of the test results

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio <0.29 is negative.
  - Parvo virus antigen could not be detected.
- A sample with the S/P ratio ≥0.29 is positive.
  - Parvo virus antigen was detected.

Quantitative: End point titre

- The viral antigen titre can be calculated by constructing a curve and using a cut-off line, with OD values on Y-axis and antigen dilutions on X-axis (undiluted 3- 9-27 - dilutions of 3 steps). ELISA titres can be calculated using as cut-off, 2.5 times OD value of the undiluted negative control.

12. 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
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	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	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

The entire risk as to the performance of these products is assumed by the purchaser. EVL 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 EVL.

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