Instructions for Use

Canine Parvo Virus Antigen ELISA

VET

REF EIA-2477

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Table of Contents

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

1 INTRODUCTION

For diagnosis of canine parvovirus (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 INTENDED USE OF THE TEST KIT

The principle of the canine parvo virus antigen ELISA test kit is based on the detection of antigen in faeces of dogs. Monoclonal antibodies against CPV is attached to the solid phase. After the attachment of the parvovirus antigen samples containing antigen are able to react with the monoclonal antibody attach to the microtiter plate. After the HRPO antibody/antigen reaction, the attached antigen can be detected by use of a monoclonal conjugate.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of CPV antigen in faeces with monoclonal antibodies. To this end monoclonal antibodies have been coated to a 96-microwell plate.

The diluted dog faeces sample is added to the wells of the monoclonal 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 faeces antigens are detected by HRPO conjugated anti-CPV monoclonal antibody.

The color reaction in the wells is directly related to the concentration of CPV antigen in the faeces samples.

4 CONTENTS

- 12 x 8 Microtiter strips coated with monoclonal anti-CPV antibody
- 1 x Strip holder
- 1 x 18 mL ELISA buffer (white bottle + green cap)
- 1 x 12 mL HRPO conjugated anti-CPV antibodies (black bottle + red cap)
- 1 x 0,5 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) (white bottle + black cap), **dilute in de-ionized water before use!**
- 1 x 8 mL Substrate buffer A (white bottle + white cap)
- 1 x 8 mL Substrate buffer B (black bottle + blue cap)
- 1 x 8 mL Stop-solution (white bottle + yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

4.1 Supplies needed (not included)

- ELISA plate reader
- Pipette tips and clean containers/tubes
- Precision pipette 10-200µl (EVL)
- Precision pipette 1-10µl (EVL)
- Precision pipette 200-1000µl (EVL)
- Round bottomed microtiter plate
- Validated precision pipettes

5 HANDLING AND STORAGE OF SPECIMENS

- After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20 °C.
- An open packet should be used within 20 days.
- Avoid repeated freezing and thawing as this increases non-specific reactivity.
- Samples may be used fresh or may be kept frozen below -20 °C before use.
- The kit should be stored at 4 °C.

6 WASH PROTOCOL

In ELISA, 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 (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. Make sure that none of the wells dries 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 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 minutes 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, store them at +4 °C, and use them within 20 days.

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Before testing make sure all reagents are at room temperature.
3. Wash the wells as pointed out in wash protocol. (Dilute the washing fluid 1:200 in aquabidest before use).
4. 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 + 250 µL PBS.
5. Let clots of faeces sink or spin down 4 minutes at 4000 *g*, use only the supernatant.
6. 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 180 µL positive control to the well **1A**.
   - Add 120 µL ELISA buffer to all other wells **1B, 1C, 1D**.
   - Transfer 60 µL from well **1A** to well **1B**.
   - Mix well and transfer 60 µL from well **1B** to the well **1C**.
   - Mix well and transfer 60 µL from **1C** to the well **1D**.
   - Mix well and discard 60 µL.
7. Add **125 µL negative control** (brown cap) to the well **1E** of a round bottomed plate (not supplied).
8. Add **70 µL ELISA buffer** (green cap) to all other wells of a round bottomed plate (not supplied) and thereafter **70 µL supernatant** of each centrifuged sample.
9. Take 2 wells as **substrate controls**, add only **120 µL ELISA buffer** (green cap) to these wells.

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10. Transfer **100 µL of all dilutions** to the **coated microtiter strips**, including the substrate controls.
11. Seal and incubate for 60 minutes at 37 °C.
12. Wash the strips 5x according to the wash protocol see sub 6.
13. Add **100 µL HRPO conjugated anti-CPV antibodies** (red cap) to all wells.
14. Seal and incubate for 60 minutes at 37 °C.
15. Wash the strips 5x according to the wash protocol see sub 6.
16. 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.
17. Dispense **100 µL substrate solution** to each well.
18. Incubate 10 - 15 minutes 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.
19. Add **50 µL stop solution** to each well; mix well.
    Read the absorbency values immediately (within 10 minutes!) 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, store them at ±4 °C, and use them within 20 days.

   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Before testing make sure all reagents are at room temperature.
3. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use).**
4. Take a small sample of faeces and add same amount of PBS (0.01 M) or aqua bidest (not provided) to a clean tube (dilution 1:1), mix well.
   Example: 250 µL faeces + 250 µL PBS.

5. Let cloths of faeces sink or spin down 4 minutes at 4000 g, use only the supernatant

6. Make a three-step dilution of the **positive control** (yellow cap) in ELISA buffer (green cap) starting undiluted → 1:3 → 1:9 → 1:27 in a round bottomed plate (not supplied).
   Example: **-** Add 180 µL positive control to the well **1A.**
   - Add 120 µL ELISA buffer (green cap) to all other wells **1B, 1C, 1D.**
   - Transfer 60 µL from well **1A** to well **1B.**
   - Mix well and transfer 60 µL from well **1B** to the well **1C.**
   - Mix well and transfer 60 µL from well **1C** to the well **1D.**
   - Mix well and discard 60 µL.

7. Make a three-step dilution of the **negative control** (brown cap) in ELISA buffer (green cap) starting undiluted → 1:3 → 1:9 → 1:27 in a round bottomed plate (not supplied).
   Example: **-** Add 180 µL negative control to the well **1E.**
   - Add 120 µL ELISA buffer to all other wells **1F, 1G, 1H.**
   - Transfer 60 µL from well **1E** to well **1F.**
   - Mix well and transfer 60 µL from well **1F** to the well **1G.**
   - Mix well and transfer 60 µL from **1G** to the well **1H.**
   - Mix well and discard 60 µL.

8. Make a three-step dilution of each **faeces sample** in ELISA buffer (green cap) starting undiluted (supernatant of step 5) → 1:3 → 1:9 → 1:27 in a round bottomed plate (not supplied).
   Example: **-** Add 180 µL of the sample to the well **2A and/or 2E.**
   - Add 120 µL ELISA buffer (green cap) to all other wells **2B, 2C, 2D and/or 2F, 2G, 2H.**
   - Transfer 60 µL from well **2A and/ or 2E** to well **2B and/or 2F.**
   - Mix well and transfer 60 µL from well **2B and/or 2F** to the well **2C and/or 2G.**
   - Mix well and transfer 60 µL from **2C and/or 2G** to the well **2D and/or 2H.**
   - Mix well and discard 60 µL

9. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these wells.

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10. Transfer **100 µL of all dilutions** to the **coated microtiter strips**, including the substrate controls.
11. Seal and incubate for 60 minutes at 37 °C.
12. Wash the strips according to the wash protocol see sub 6.
13. Add **100 µL HRPO conjugated anti-CPV antibodies** (red cap) to all wells.
14. Seal and incubate for 60 minutes at 37 °C.
15. Wash the strips 5x according to the wash protocol see sub 6.
16. 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.
17. Add **100 µL substrate solution** to each well.
18. Incubate 10 - 15 minutes 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.
19. Add **50 µL stop solution** to each well; mix well.
20. Read the absorbency values immediately (within 10 minutes!) at 450 nm by using an ELISA reader.
    Use the substrate controls as blank.

10 PRECAUTIONS

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

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

12 INTERPRETATION OF TEST RESULTS

This test can be used in 2 ways.

Qualitative: positive – negative

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

Quantitative: End point titre

- The viral antigen titre can be calculated by constructing a curve and using cut-off line, with OD values on Y-axis and antigen dilutions on X-axis (undiluted – 1:3 – 1:9 – 1:27 etc. total 8 dilutions).

  ELISA titres can be calculated using as cut-off the 2.5 times OD value of the undiluted negative control.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
VET	for veterinary use				
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité