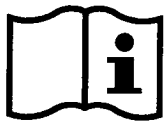
Product information

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



Useŕs Manual

CPV (Canine Parvo Virus) antigen in faeces ELISA

Enzyme immunoassay for the detection of Canine Parvo Virus in feces samples

VET

REF **DE2477**

Σ **96 wells**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
info@demeditec.de • www.demeditec.com

Version 08-01/18
DMC/DL
Updated 180201

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 for the detection of Canine Parvo Virus in feces samples

1 INTRODUCTION

For diagnosis of Canine Parvo Virus (CPV) infections in dogs the demonstration of CPV antigen in feces 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 antibody proteins have been coated to a 96-microwell plate. The diluted dog feces sample is added to the wells of the coated plate.

- **Qualitative**
  The dog feces sample is added (diluted 1:1) to the wells of the coated plate.

- **Quantitative**
  The dog feces 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 feces sample.

3 CONTENTS

- **SORB MT** 12 x 8 Microtiter strips coated with monoclonal anti-CPV antibody
- 1 x Strip holder
- **BUF** 1 x 18 mL ELISA buffer (green cap)
- **ENZ CONJ** 1 x 12 mL HRPO conjugated anti-CPV antibodies (red cap)
- **CONTROL 1** 1 x 1,0 mL Positive control (ready to use) (yellow cap)
- **CONTROL 2** 1 x 1,0 mL Negative control (ready to use) (brown cap)
- **WASH SOLN 200x** 1 x 20 mL Wash-solution (200x concentrated) (black cap), **diluted in deionized 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 User's manual

3.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Precision pipette 10 µL - 200 µL
- Precision pipette 200 µL - 1000 µL
- Pipette tips and clean containers/tubes
- ELISA plate reader
- Aqua bidest
- Phosphate buffered saline (PBS), 0.01 M

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. 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.

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 washing 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 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 5 washing cycles.

6 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 resuspend 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, 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 MΩ) water!
   Use the precision pipette 10 µL - 200 µL & 200 µL - 1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Take a small sample of feces 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 feces + 250 µL PBS.
3. Let clots of faeces sink or spin down 4 minutes at 4000 *g*, use only the supernatant.
4. Add 125 µL **positive control** (yellow cap) to the first well of a round bottomed plate (not supplied).
5. Add 125 µL **negative control** (brown cap) to the second well of a round bottomed plate (not supplied).
6. Add 70 µL **buffer** (green cap) to all other wells of a round bottomed plate (not supplied) and thereafter 70 µL **supernatant** of each centrifuged sample.
7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these well.
8. Transfer 100 µL of all dilutions to the coated microtiter strips.
9. Seal and incubate for 60 minutes at 37 °C.
10. Wash the plate according to the wash protocol see sub 5.
11. Dispense 100 µL conjugated anti-CPV antibody to all wells.
12. Seal and incubate for 60 minutes at 37 °C.
13. Wash the plate according to the wash protocol see sub 5.
14. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking.
    Prepare immediately before use!
15. Dispense 100 µL substrate solution to each well.
16. Incubate 10 - 20 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.
17. Add 50 µL stop solution to each well; mix well.
    Read the absorbency values immediately (within 10 minutes!) 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 all other steps are done before pipetting directly into the ELISA plate.
In this case only use 100 µL controls and only add 50 µL ELISA buffer and 50 µL of each sample (= supernatant) to the test wells.

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, 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 MΩ) water!
   Use the precision pipette 10 µL - 200 µL & 200 µL - 1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Take a small sample of feces 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 feces + 250 µL 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 → 1:27 in a round bottomed plate (not supplied).
   Example: **-** Add 180 µL positive control to the well **1A.**
   - Add 120 µL 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.
5. 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 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.
6. Make a three-step dilution of each **feces sample** in ELISA buffer (green cap) starting
   undiluted (= supernatant of step 3) → 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 buffer 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
7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these well.
8. Transfer 100 µL of all dilutions to the coated microtiter strips.
9. Seal and incubate for 60 minutes at 37 °C.
10. Wash the plate according to the wash protocol see sub 5.
11. Dispense 100 µL conjugated anti-CPV antibody to all wells.
12. Seal and incubate for 60 minutes at 37 °C.
13. Wash the plate according to the wash protocol see sub 5.
14. Mix equal parts of buffer A (white cap) and buffer B (blue cap) with gentle shaking.
    Prepare immediately before use!
15. Dispense 100 µL substrate solution to each well.
16. Incubate 10 - 20 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.
17. Add 50 µL stop solution to each well; mix well.
    Read the absorbency values immediately (within 10 minutes!) 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 undiluted Positive Control (PC) must be ≥ 0.500
- The MV of the measured OD value for the Negative Control (NC) 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 undiluted positive control should be ≥ 0.500 OD units (450 nm) and give an endpoint titer of ≥ 2.
The negative control should be ≤ 0.250 OD units (450 nm) 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 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 the 2.5 times OD value of the undiluted negative control.

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	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-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	Ussage 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 Cat.
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	Temperature 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	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore