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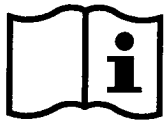

Useŕs Manual

CPV (Canine Parvo Virus) IgG ELISA

Enzyme immunoassay for the detection of IgG antibodies against Canine Parvo Virus in serum or plasma

VET

REF **DE2475**

96 wells

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DMC
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Please use only the valid version of the Instructions for Use provided with the kit.

Table of Contents

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An ELISA to detect IgG antibodies against Canine Parvo Virus in serum or plasma samples.

1 INTRODUCTION

For diagnosis of Canine Parvo Virus (CPV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. The virus that is attached to the solid phase by use of monoclonal antibodies catches antibodies induced through infection or vaccination.
IgG antibody titers above a dilution of 1:810 are considered protected.

2 INTENDED USE OF THE TEST KIT

The principle of the CPV test kit is based on the detection of antibodies against Parvo virus. The Parvo virus is attached to the solid phase by use of a monoclonal antibody. After the attachment of the antigen (Parvo virus) sera or plasma containing antibodies are able to react with the antigen. After the antigen/antibody reaction, the attached antibodies can be detected by use of a monoclonal HRPO conjugate.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of CPV proteins with dog antibodies. To this end CPV proteins have been coated to a 96-well-microtiter plate by use of monoclonal antibodies.

- **Qualitative**

The dog sample is added (diluted 1:250) to the wells of the coated plate.

- **Quantitative**

The dog sample also can be titrated using a 3-step dilution,
starting with a dilution 1:100 (→ 1:300 → 1:900 → 1:2700).

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

4 CONTENTS

- **SORB MT** 12 x 8 microtiter strips
- 1 x strip holder
- **BUF** 1 x 18 mL ELISA buffer (green cap)
- **Ag** 1 x 11 mL Inactivated Canine Parvo Virus antigen (lila cap)
- **ENZ CONJ** 1 x 12 mL HRPO conjugated anti-species antibodies (red cap)
- **CONTROL 1** 1 x 0.5 mL Positive control (freeze dried) (purple cap)
- **CONTROL 2** 1 x 1.0 mL Negative control (freeze dried) (silver cap)
- **WASH SOLN 200x** 1 x 20 mL Wash-solution (200x concentrated) (black cap), **dilute in de-ionized 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 Instructions for Use

4.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes
- ELISA plate reader

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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.
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 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 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 dispensed, 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 °C - 8 °C immediately after use.

8 TEST PROTOCOL <u>QUALITATIVE</u>

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.

   <u>Wash microtiter strip(s) 5x with washing solution, according to washing protocol.</u>

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

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2. Dispense 100 µL of inactivated Canine Parvo Virus antigen to all required wells of the CPV coated microtiter strips.
3. Incubate 75 minutes at 37 °C.

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4. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest. (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
5. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest. (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
6. Dilute the **positive control** (purple cap) **starting 1:100 → 1:300 → 1:900 → 1:2700** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - A **pre-dilution** is needed:
   - Add 90 µL ELISA buffer to **well 1A**, add 10 µL of the positive control to the **well 1A** and mix well.
   - Add 180 µL ELISA buffer to **well 2A**, and 120 µL to **2B, 2C, 2D**
   - Add 20 µL **pre-dilution** of **well 1A** in the well **2A** and mix well
   - Mix well and transfer 60 µL to the well **2B**
   - Mix well and transfer 60 µL to the well **2C**
   - Mix well and transfer 60 µL to the well **2D**
   - Mix well and discard 60 µL.
7. Dilute the **negative control** (silver cap) **1:250** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - A pre-dilution is needed:
   - Add 45 µL ELISA buffer to **well 1E**, add 5 µL of the negative control to the **well 1E** and mix well.
   - Add 96 µL ELISA buffer to **well 2E**, add 4 µL of pre-dilution from **well 1E** in the well **2E** and mix well.
8. Dilute each **sample 1:250** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example:
   - A pre-dilution is needed:
   - Add 45 µL ELISA buffer to **well 1F**, add 5 µL of sample to the **well 1F** and mix well.
   - Add 96 µL ELISA buffer to **well 2F**, add 4 µL of pre-dilution from **well 1F** in the well **2F** and mix well.
9. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.

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10. Wash the with antigen incubated plate according to the wash protocol see sub 6.
11. Transfer 100 µL of all dilutions of **column 2 (round bottomed plate)** to the **CPV coated microtiter strips**.
12. Seal and incubate for 60 min at 37 °C.
13. Wash the plate according to the wash protocol see sub 6.
14. Dispense 100 µL conjugated anti-species antibody to all wells.
15. Seal and incubate for 60 min at 37 °C.
16. Wash the plate according to the wash protocol see sub 6.
17. 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.**
18. Dispense 100 µL substrate solution to each well.
19. 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.
20. Add 50 µL stop solution to each well; mix well.
21. Read the absorbency values immediately (within 10 min!) 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 and store them at +4 °C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest. (5 MΩ water) !
   Use validated precision pipettes and and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Dispense 100 µL of inactivated Canine Parvo Virus antigen to all required wells of the CPV coated microtiter strips.
3. Incubate 75 minutes at 37 °C.

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4. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest. (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
5. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest. (5 MΩ water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
6. Make a **pre-dilution** of the **positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1A** and add 10 µL of the positive control to **well 1A** and mix well.
7. Make a **pre-dilution** of the **negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1B** and add 10 µL of the negative control to **well 1A** and mix well.
8. Make **pre-dilutions** of **each sample** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer to **well 1C** and add 10 µL of sample to **well 1C** and mix well.
9. Take 2 wells as substrate controls add only 120 µL ELISA buffer (green cap) to these well.

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10. Wash the with antigen incubated plate according to the wash protocol see sub 6.
11. Add for dilution of the **positive control** 135 µL ELISA buffer to **well 1A** and 100 µL ELISA buffer to the **wells 1B, 1C, 1D** of the coated microtiter strip.
12. Add for dilution of the **negative control** 135 µL ELISA buffer to **well 1E** and 100 µL ELISA buffer to the **wells 1F, 1G, 1H** of the coated microtiter strip.
13. Add for dilution of **the samples** 135 µL ELISA buffer to **wells 2A and 2E** (depending on the number of samples) and
    100 µL ELISA buffer to the **wells 2B, 2C, 2D** and **wells 2F, 2G, 2H** of the coated microtiter strip.
14. Make 3-step dilution of the **positive control** in the coated microtiter strip starting 1:100 → 1:300→ 1:900 →1:2700.
    Example: **-** Dispense 15 µL positive control from step 6 to **well 1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.
15. Make 3-step dilution of the **negative control** in the coated microtiter strip starting 1:100 → 1:300→ 1:900 →1:2700.
    Example: **-** Dispense 15 µL negative control from step 7 to **well 1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1F**
    - Mix well and transfer 50 µL to the well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.

16. Make 3-step dilution of **each sample** in the coated microtiter strip starting 1:100 → 1:300→ 1:900 →1:2700.
    Example: **-** Dispense 15 µL of each sample from step 8 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 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.
17. Dispense 100 µL of the substrate control of step 9 to the last 2 wells of the microtiter strip.
18. Seal and incubate for 60 min at 37 °C.
19. Wash the plate according to the wash protocol see sub 6.
20. Dispense 100 µL conjugated anti-species antibody to all wells.
21. Seal and incubate for 60 min at 37 °C.
22. Wash the plate according to the wash protocol see sub 6.
23. 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.
24. Dispense 100 µL substrate solution to each well.
25. 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.
26. Add 50 µL stop solution to each well; mix well.
27. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the 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 1:100, must be ≥ 1.000
  - The MV of the measured OD value for the Negative Control (NC), diluted 1:250, 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:

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

Quantitative:

In order to confirm appropriate test conditions, the OD of the positive control, diluted 1:100, should be ≥ 1.000 OD units (450 nm). The negative control, diluted 1:100, should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 50.

12 INTERPRETATION OF THE TEST RESULTS

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio **< 0.22** is negative
  - Specific antibodies to Parvo virus could not be detected.
- A sample with the S/P ratio **≥ 0.22** is positive
  - Specific antibodies to Parvo virus were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using a cut-off line (dilution 1:100 → 1:300 → 1:900 → 1:2700 → 1:8100→ 1:24300, etc., total 8 dilutions of 3 steps) OD on Y-axis and titre on X-axis.

  ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:100.

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
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
	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