

Product information

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User's 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

***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) infection or vaccination control, demonstration of antibody titer is the most commonly used method. The virus has 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 TESTKIT

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 containing antibodies are able to react with the antigen. After the antigen/antibody reaction, the attached antibodies can be detected by use of a polyclonal 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. The diluted dog serum/plasma sample is added to the wells of the coated plate. After washing the bound dog antibodies are detected by a HRPO conjugated anti-species conjugate. The colour reaction in the wells is directly related to the concentration of CPV antibodies in the serum/plasma sample.

4 CONTENTS

- 12 x 8 microtiter strips
- 1 x strip holder
- 1 x 11 ml Inactivated Canine Parvo Virus antigen
- 1 x 18 ml ELISA buffer
- 1 x 12 ml HRPO conjugate
- 1 x 0.5 ml Positive control (freeze dried)
- 1 x 1 ml Negative control (freeze dried)
- 1 x 20 ml Wash-solution (200 x concentrated), dilute in de-ionised water before use!
- 1 x 8 ml Substrate A
- 1 x 8 ml Substrate B
- 1 x 8 ml Stop-solution
- 1 x Plastic cover seal

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, uncomplexed 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 washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 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 4 washing cycles.

7 TEST PROTOCOL

1. Open the packet of strips and take out the strips to be used (see 4). 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 solution provided must be diluted 200x in de-ionised water!
2. Reconstitute the positive control in 0.5 ml deionised water and negative control in 1 ml deionised water, store in aliquots at -20°C.
3. Dispense 100 µl of inactivated Canine Parvo Virus antigen to all wells to be used.
4. Incubate 75 min. at 37°C.
5. Make 3-step dilutions of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate.
Make also a 3-step dilution of the positive and negative control
6. Wash the wells as pointed out in the wash protocol.
7. Transfer 100 µl of this dilution to the CPV coated microtiter strips.
Seal and incubate for 60 min. at 37°C.
8. Wash as pointed out in wash protocol.
9. Dispense 100 µl conjugated anti-species antibody to all wells.
10. Seal and incubate 60 min. at 37°C.
11. Wash as pointed out in wash protocol.
12. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use!
Dispense 100 µl substrate solution to each well.
Incubate 10-15 min. at room temperature (21°C).
If temperature is higher (above 28°C) than incubate 5-10 min instead of 10-15 min.
13. Add 50 µl stop solution to each well; mix well.
14. Read the absorbency values immediately (within 10 min.!) at 450 nm (ref 620 nm).

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting CPV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the 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.

9 VALIDATION OF THE TEST

The negative control should give an OD < 0.400.

The end point titer of the positive control should be between 1:150 and 1:450 according to the instructions for interpretation of test results.

10 INTERPRETATION OF TEST RESULTS

The titer of the sample is the dilution which gives an extinction above 2 times the OD value of the negative control.











The test is valid if the first two dilutions of the positive control are above 0.700 OD (450 nm).

In summary:

30	= no antibodies found.
90-270	= antibodies found.
≥ 810	= high titer of antibodies found.

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	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-Konformitätskennzeichnung	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	Mindesthaltbarkeitsdatum	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