Product information

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User´s Manual

Canine Parvo Virus Ag ELISA

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

VET

REF **DE2477**

Σ **96 wells**

12/05

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

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1 INTRODUCTION

For Veterinary use only!

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

The principle of the test is based on the reaction of two monoclonal antibodies with 2 different antigenic determinants of CPV. One monoclonal antibody, coated to the plate, catches the parvovirus in the faeces sample after which the other, enzyme-labeled antibody detects the bound virus.

3 CONTENTS

- 1 x 12 **microtiter strips** coated with monoclonal anti-CPV antibody
- 1 x strip holder
- 1 x 12 ml Buffer
- 1 x 0.3 ml **Conjugate**-HRPO, dilute 1:50 in conjugate diluent.
- 1 x 12 ml **Conjugate diluent**
- 1 x 1 ml CPV **positive control**
- 1 x 1 ml CPV **negative control**
- 60 ml **washing solution, 200x concentrated**. Dilute in de-ionized 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

Products needed that are not provided:

- Phosphate Buffered Saline (PBS)

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 of 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 increase non-specific reactivity.

5 WASHING 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 results.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical movement.
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 by a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

When using automatic plate wash equipment, check that all wells can be 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.

6 TEST PROTOCOL

1. Open the packet of strips. Take out the strips to be used, cover the remaining strips with a part of the seal provided; store at +4°C and use within 10 days.
   Wash according to the washing protocol. **Dilute washing solution 200 x before use!**
2. Dilute the faeces sample(s) at least 1:1 in a clean tube.
   Take a small sample of faeces/diarrhoea and add same amount/volume of PBS (0,01 M) or aqua bidest (not provided) to the tube, mix well.
   Let cloths of faeces sink or spin down 4 minutes at 4000 g, use only the supernatant.
   The supernatant has to be diluted 1:1 in buffer (50 µl supernatant + 50 µl buffer).
   Divide positive and negative controls into aliquots, and store immediately at -20°C until use.
3. Add 100 µl positive control to the first well.
   Add 100 µl negative control to the second well.
   Add 50 µl to all other wells and thereafter 50 µl of each centrifuged sample
4. Incubate 60 minutes at 37°C.
5. Wash as in 1.
6. Dilute the Conjugate-HRPO 1:50 in conjugate diluent, don't make more than is needed this diluted conjugate is only stable for one week
   Add 100 µl diluted Conjugate-HRPO to each well.
7. Incubate 60 min. at 37°C.
8. Wash as in 1.
9. Mix equal parts of buffer A and B with gentle shaking. Prepare immediately before use!
   Dispense 100 µl substrate mixture to each well.
   Incubate for 10-15 minutes at room temperature (21°C).
10. After incubation the reaction is stopped by adding 50 µl stopping solution to each well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm.
    Use as a reference wavelength 620nm.

7 VALIDATION OF THE TEST

To standardize the Canine Parvo Virus ELISA, positive and negative controls have to be tested.

The CPV positive control should give an OD (450nm) ≥ 0.500.

The OD (450nm) of the negative control must be lower than 0.150.

8 INTERPRETATION OF TEST RESULTS

The test samples are considered CPV positive if the absorbency is above 3 times the absorbency of the negative control.

These animals will shed the Parvo Virus and will be infectious to other animals.

When a sample is negative > sample < 3 x negative, it should be tested again within 5 days.

9 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 work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expire date and do not mix components from different serial lots together.
- 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 is from damage and dirt.

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.

SYMBOLS USED WITH DEMEDITEC ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de usoo	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
FRO	For research use only	Nur für Forschungszwecke			
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Numero de catálogo	Numero di Catalogo
LOT	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	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	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
FRO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..