

# Product information

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## Users Manual

# FPV (Feline Panleukopenia Virus) Ab ELISA

Enzyme Immunoassay for the detection of antibodies against feline panleucopenia virus in serum or plasma

**VET**

**REF**

**DE2467**



**96 wells**

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## 1 INTRODUCTION

### **For Veterinary use only!**

For diagnosis of Feline Panleucopenia Virus (FPV) 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. Antibody titers above dilutions of 1:1350 are considered protected.

## 2 INTENDED USE

The principle of the FPV testkit is based on the detection of antibodies against Panleucopenia virus. The Panleucopenia antigen is attached to the solid phase by use of a monoclonal antibody. After the attachment of the antigen (Panleucopenia 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

The test is based on the reaction of FPV proteins with feline antibodies. To this end FPV proteins have been coated to a 96-well microtiter plate by use of monoclonal antibodies. The diluted feline serum/plasma sample is added to the wells of the coated plate. After washing the bound feline antibodies are detected by a HRPO conjugated anti-species conjugate. The colour reaction in the wells is directly related to the concentration of FPV antibodies in the serum/plasma sample.

## 4 CONTENTS

- 12 x 8 **microtiter strips**
- 1 x **strip holder**
- 1 x 12 ml Inactivated Feline Panleucopenia Virus **antigen**
- 1 x 18 ml **ELISA buffer**
- 1 x 12 ml **HRPO conjugated anti-species antibodies**
- 1 x 0,5 ml **Positive control**
- 1 x 0,5 ml **Negative control**
- 1 x 20 ml **Wash-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

## 5 HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8°C. An unopened package can be used until the expiry date. 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. Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.

## 6 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 short 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.

## 7 TESTPROTOCOL

1. Open the packet of strips and take out the strips to be used (see 5). Cover the remaining strips with a part of the provided seal and store them at 4-8°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-ionized water!
2. Dispense 100 µL of activated Feline Panleucopenia Virus antigen to all wells to be used.
3. Incubate 60 min. at 37°C
4. Wash as in 1.
5. Reconstitute the positive control in 0,5 and the negative control in 0,5 ml de-ionized water divide into aliquots and store -20°C  
Make 3-step dilutions of each sample in ELISA buffer, starting 1:50 (150; 450; 1350) in a round bottomed microtiter plate.  
Make also a 3-step dilution of the positive and negative control.
6. Transfer 100 µL of this dilution to the FPV coated microtiter strips.
7. Seal and incubate for 60 min. at 37°C.
8. Wash as in 1.
9. Dispense 100 µL conjugated anti-species antibody to all wells.
10. Seal and incubate 60 min. at 37°C.
11. Wash as in 1.
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 10-20 min. at room temperature (21°C).
13. Add 50 µL stop solution to each well; mix well.
14. Read the absorbency values immediately (within 10 min.!) at 450 nm.

## 8 VALIDATION OF THE TEST

To standardize the FPV ELISA positive and negative controls have to be tested.

The FPV positive control should give an OD (450nm)  $\geq 1,000$ .

The OD (450nm) of the negative control must be lower than 0,300.

## 9 INTERPRETATION

A sample is considered positive when the measured extinction is 0.150 higher than the OD of the negative control.

In summary:











< 1:50	= no antibodies found.
1:150 – 1:450	= antibodies found.
> 1:1350	= high titer of antibodies found.











## 10 PRECAUTIONS

- Handle all biological materials as though capable of transmitting FPV.
- 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 their 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 prevent it 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 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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europeaisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
				
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	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	Opbevarings-temperatur	Förvaringstemperatur	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..