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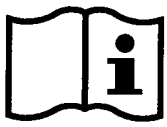

User´s Manual

FCV (Feline Calici Virus) Ab ELISA

Enzyme Immunoassay for the detection of antibodies against Feline Calici Virus in serum and plasma

VET

REF **DE2473**

Σ **96 wells**

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1 INTRODUCTION

For Veterinary use only!

Demonstration of serum antibodies is the most commonly used method for the diagnosis of Feline Calici Virus (FCV) infection or for monitoring the efficacy of vaccination. Feline Calici plays an important role in a complex disease of both wild and domestic cats ("sneezing disease").

Important in the diagnosis of Feline Calici infection:

- Clinical history
- Clinical signs
- Eye examination
- Laboratory findings

2 INTENDED USE

The FCV ELISA test is designed to detect antibodies against Feline Calici Virus proteins. To this end Feline Calici proteins are attached to the solid phase. The strips are washed after incubation to remove unbound materials. The cat sera to be tested are diluted and added to the wells and incubated. After incubation the strips are washed, a HRPO labeled anti-species conjugate is added to detect bound cat antibodies to FCV. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE

The test is based on the reaction of FCV proteins with polyclonal cat antibodies.

To this end FCV proteins have been attached to the solid phase.

The diluted cat serum/plasma sample is added to the wells of the pre-coated plate.

After washing, the bound cat antibodies are detected by a HRPO conjugated anti-species conjugate.

The colour reaction is directly related to the concentration of FCV antibodies in serum/plasma samples.

4 CONTENTS

- 12 x 8 **microtiter strips**.
- 1 x strip holder.
- 1 x 18 mL **ELISA Buffer**.
- 1 x 13 mL **HRPO conjugated anti-species antibodies**.
- 1 x 0.5 mL **Positive Control** (freeze dried).
- 1 x 0.5 mL **Negative Control** (freeze dried).
- 1 x 20 mL Wash-solution (200x concentrated), dilute in deionized 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 2 °C - 8 °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 of all the products as this increases non-specific reactivity.

6 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 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 downward 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 dries 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. Cover the remaining strips with a part of the provided seal and store them at 2 °C - 8 °C and use them within 10 days.
   Wash the microtiter strip(s) with washing solution, according to washing protocol.
2. The washing solution provided must be diluted 200x in deionized water
3. Reconstitute the positive and negative control with 0.5 mL deionized water.
   Divide into aliquots and store at -20 °C until use.
4. Make 3-step dilution 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.
5. Transfer 100 µL of this dilution to the FCV coated microtiter strips.
   Seal and incubate for 60 minutes at 37 °C.
6. Wash as pointed out in wash protocol.
7. Dispense 100 µL HRPO conjugated anti-species antibody to all wells.
8. Seal and incubate 60 minutes at 37 °C.
9. Wash as pointed out in wash protocol.
10. 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 minutes at room temperature (21 °C).
11. Add 50 µL Stop Solution to each well; mix well.
12. Read the absorbency values immediately (within 10 minutes!) at 450 nm (reference 620 nm).

8 PRECAUTIONS

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

In order to confirm appropriate test conditions,

the weak positive control should give an extinction ≥ 0.800 OD and an endpoint titer ≥ 90.

The negative control should give an OD ≤ 0.350 and an endpoint titer < 30.

10 INTERPRETATION OF TEST RESULTS

Intermediate antibody titers (90; 270) in diseased animals showing signs suggestive of FCV are considered positive and the cat will be suspected of shedding FCV. A rise in antibody titer in a non-vaccinated cat with a FCV infection represents an exaggerated, effective immune response.

A titer > 270 is considered to be protective, under normal virus pressure. If cat density increase viral pressure will also increase and the titer might not be high enough.

In summary:	< 30	=	no antibodies found.
	> 90	=	antibodies found, retest in 3 months (probably shedding FCV)
	> 270	=	high titer, considered to be protective.

The purchaser assumes the entire risk as to the performance of these products.

DEMEDITEC shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.

SYMBOLS USED WITH DEMEDITEC ASSAY´S

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
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso 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.	Numéro de catalogue	Número 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	Température de conservation	Temperatura de conservación	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	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità