Product information

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User´s Manual

Feline Immunodeficiency Virus p24/p17 Ab ELISA

Enzyme Immunoassay for the detection antibodies against Feline Immunodeficiency Virus (FIV) p24 and p17 antigen (expression protein) in serum and plasma samples.

VET

REF **DE2463**

Σ **96 wells**

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Please use only the valid version of the package insert provided with the kit.

Table of Contents

1 INTRODUCTION

FIV p17/p24 are both core proteins of FIV. Infected cats produce antibodies against these FIV antigens, which can be detected in an ELISA using an anti-species conjugate.

2 INTENDED USE OF THE TESTKIT

The FIV p17/p24 ELISA is designed to detect antibodies against these proteins. To this end recombinant p17/p24 proteins are attached to the solid phase. After washing the plates are incubated with the cat sera to be tested. The plates are washed after incubation to remove unbound materials. An anti-species conjugate is added to detect bound cat antibodies to FIV p17/p24. After incubation and rinsing, the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of FIV proteins with cat antibodies. To this end, p17/p24/TM expression proteins have been coated to a 96 well microtiter strip plate.

The cat serum sample is added (diluted 1:100) to the wells of the coated plate.

After washing, the bound cat antibodies are detected by an anti-species conjugate.

Bound anti-species conjugate is made visible by adding substrate/chromagen mix.

The intensity of the colour reaction in the wells is directly correlated to the concentration of anti-FIV p17/p24 antibodies in the serum sample.

4 CONTENTS

- 12 x 8 microtiter strips
- 1 x strip holder
- 1 x 18 mL Buffer
- 1 x 12 mL Anti-species 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-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 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 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. Reconstitute directly before use the positive (0,5 mL) and negative (1 mL) controls in 0,5 mL and 1 mL de-ionized water.
2. Open the packet of strips and take out the strips to be used. Leave the remaining strips covered in the plastic and store at 4 °C – 8 °C. Wash the microtiter strip(s) with washing solution according to the washing protocol.
   The provided washing solution must be diluted 200x in de-ionized water!
3. Make a 1:100 dilution of the test sample in the buffer and transfer 100 µL to one well of the microtiter strips. Also make a 1:100 dilution of the positive- and the negative control and transfer 100 µL of each to two wells.
4. Seal and incubate for 60 minutes at 37 °C.
5. Wash as pointed out in wash protocol.
6. Dispense 100 µL of anti-species conjugate to all wells.
7. Seal and incubate for 60 minutes at 37 °C.
8. Wash as pointed out in wash protocol.
9. Mix equal parts of substrate A and substrate B while gentle shaking.
   Prepare immediately before use!
10. Dispense 100 µL of substrate solution to each well.
    Incubate for 10-15 minutes at room temperature (21 °C).
11. Add 50 µL of 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 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

To standardize the FIV-p17/p24 ELISA positive and negative controls have to be tested.

The FIV positive control should give an extinction of ≥ 0,700 OD measured at 450 nm using 620 nm as reference. The OD (450 nm) of the sticky negative control must be ≤ 0,400.

10 INTERPRETATION OF TEST RESULTS

A sample is considered **positive** when the measured extinction is higher than 2 times the OD of the negative control.

When a sample is **negative > sample < 2 x negative**, it should be tested again within 2-4 weeks.

The OD of the positive control must be ≥ 0,700.

Important

A positive ELISA result must be confirmed by blocking ELISA or virus blotting test or IFA test.

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
CE	European Conformity	CE-Konfirmitäts-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