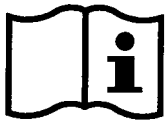

User´s Manual

Feline Leukaemia Virus-p27 Antigen ELISA

Enzyme Immunoassay for the detection of feline leukaemia virus-p27 antigen (FeLV p27) in serum and plasma samples.

VET

DE2469

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

FeLV-p27 antigen is the major core protein of FeLV. This antigen is found in the blood of FeLV-infected cats.

These cats are infectious for others through horizontal transmission. This test is an alternative for the widely used IFA test on blood smears, which is successfully used in control programs.

The detection levels are comparable with IFA, but the ELISA tends to detect FeLV-p27 antigen in some cases at an earlier stage.

IFA or virus-isolation must be used to confirm positive ELISA results!

2 INTENDED USE

The FeLV-p27 Antigen ELISA test kit is designed to detect p27 antigen in individual serum/plasma samples.

For this purpose monoclonal anti-FeLV antibodies attached to the plate will catch the viral antigen in the sample to be tested. After incubation, the bound antigen is detected by use of a polyclonal anti-FeLV conjugate. After incubation and washing the substrate is added. The color development is directly correlated with the quantity of the bound p27 antigen.

3 PRINCIPLE OF THE TEST

The test is based on the reaction of FeLV-p27 antigen with monoclonal anti-FeLV-p27 antibodies. To this end these monoclonal antibodies are coated to a 96 well microtiter strip plate.

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

After incubation, the bound p27 antigen is detected by a polyclonal anti-FeLV-p27 conjugate.

Bound conjugate is made visible by adding substrate/chromogen mix.

Intensity of the color reaction in the wells is directly correlated to the concentration of p27 antigen in the serum sample.

4 CONTENTS

- 12 x 8 **microtiter strips**
- 1 x strip holder
- 1 x 18 ml **ELISA buffer**
- 1 x 12 ml **HRPO conjugated anti-FeLVp27 antibodies**
- 1 x 1 ml **Positive control** serum (freeze-dried)
- 1 x 1 ml **Negative control** serum (freeze-dried)
- 1 x 20 ml **Wash solution 200 x** concentrated, dilute in deionized water before use!
- 1 x 7 ml **Substrate A**
- 1 x 7 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 microtitre plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl of diluted 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 short 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 TESTPROTOCOL

1. Reconstitute directly before use the positive control in 1 mL and the negative control in 1 mL deionized 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 4-8˚C. Wash the microtiter strip(s) with washing solution according to the washing protocol.
3. **The provided washing solution must be diluted 200x in de-ionized water!**
4. Add 50 µl of ELISA buffer to all wells to be used.
   To one well of the coated strip add 50 µl of positive control and to another well 50 µl of negative control.
   In addition, add 50 µl of each sample to an individual marked well of the strip.
5. Seal and incubate for 60 minutes at 37°C.
6. Wash as in 2.
7. Dispense 100 µl of anti-FeLV-p27 conjugate to all wells.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as in 2.
10. Mix equal parts of substrate A and substrate B with gentle shaking. Prepare immediately before use!
11. Dispense 100 µl of substrate solution to each well.
    Incubate for 15 minutes at room temperature (21°C.).
12. Add 50 µl of stop solution to each well; mix well.
13. Read the absorbency values immediately (within 10 minutes!) at 450 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 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 it from damage and dirt.

9 VALIDATION OF THE TEST

To standardize the FeLV-p27 ELISA a positive and negative controls have to be tested.

The FeLV positive control should give an OD (450nm) > 1.000.

The OD (450nm) of the negative control must be lower than 0.400.

10 INTERPRETATION OF TEST RESULTS

A sample is considered positive when the measured extinction is higher than 3 times the OD of the negative control.

When a sample is **negative > sample < 3 x negative**,
it should be tested again within 2-4 weeks.

The OD of the positive control must be > 1.000.

IMPORTANT: A positive ELISA-result must be confirmed by IFA or by virus isolation.

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
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
VET	For research use only				
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