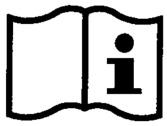

User´s Manual

FeLV-gp70 (Leukaemia Virus) Ab, virus coated ELISA

Enzyme Immunoassay for the detection of FeLV gp70 antibodies in serum or plasma

VET

REF DE2470

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1 INTRODUCTION

FeLV-gp70 is the glycoprotein of the envelope of FeLV. Following infection, cats may produce antibodies against gp70, which can be neutralizing. A cat with a neutralizing antibody titre above ± 32 is considered to be protected. In a more sensitive ELISA, this titre is equal or higher than 270.

2 INTENDED USE OF THE TESTKIT

The FeLV-gp70 antibody ELISA is designed to detect gp70 antibodies in serum and plasma samples. The kit procedure is based on a solid phase ELISA. When a standard gp70 antigen suspension is added, the gp70 molecule is bound by monoclonal antibodies attached to the solid phase. Unbound materials are removed by rinsing. A diluted serum/plasma sample is then added. After incubation and before the addition of peroxidase labelled anti-species conjugate, unbound materials are removed by rinsing. After incubation and rinsing, the substrate is then added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT

The test is based on the reaction of FeLV-gp70 antibodies present in the test sample with immobilized FeLV gp70 antigen. To this end, monoclonal anti-gp70 antibodies have been coated to the wells of a 96 well microtiter plate.

The FeLV-gp70 antigen suspension is added to the wells and is captured by the coated monoclonal antibodies. After washing, samples are added to the wells and will bind to the gp70 molecules, which have been caught. The bound antibody is detected by a horseradish peroxidase (HRPO) conjugated anti-species conjugate.

Color reaction in the wells is directly related to the concentration of FeLV-gp70 antibodies in the sample.

4 CONTENTS

- 12 x 8 **microtiter strips**.
- 1 x strip holder.
- 1 x 18 mL **ELISA Buffer**.
- 1 x 12 mL **Conjugate Buffer**.
- 1 x 0.16 mL concentrated HRPO **Conjugate**; *dilute 1:100 in conjugate buffer*.
- 1 x 12 mL **FeLV-gp70 antigen**.
- 1 x 0.3 mL **Positive Control** (Ready to use)
- 1 x 0.3 mL **Negative Control** (Ready to use)
- 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 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. 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!
2. Dispense 100 µL FeLV-gp70 antigen suspension to all wells.
3. Seal and incubate for **90 minutes** at **37 °C**.
4. Wash as pointed out in wash protocol.
5. *Make a 3-step dilution of the positive and negative control in ELISA buffer, starting 1:3 (9; 27; 81).*
6. *Make 3-step dilution of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate*.
7. Transfer 100 µL of the control dilutions to the FeLV coated microtiter strips.
   Transfer 100 µL of the sample dilutions to the FeLV coated microtiter strips.
8. Seal and incubate for **60 minutes** at **37 °C**.
9. Wash as pointed out in wash protocol.
10. *Dilute the concentrated HRPO conjugate 1:100 in conjugate buffer*. Dilute only the necessary amount.
    Dispense 100 µL diluted HRPO conjugate to all wells.
11. Seal and incubate for **60 minutes** at **37 °C**.
12. Wash as pointed out in wash protocol.
13. *Mix equal parts of buffer A and buffer B together with gentle shaking. Prepare immediately before use!*
    Dispense 100 µL substrate solution to each well.
14. Incubate **10-15 minutes** at **room temperature** (21 °C).
15. Add 50 µL stop solution to each well.
16. Read the absorbency values immediately (within 10 min.!) at 450 nm (Ref. 620 nm).

8 PRECAUTIONS

- Handle all biological materials as though capable of transmitting FeLV.
- 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 VALIDTION OF THE TEST

To standardize the FeLV ELISA, a positive and negative control has to be tested.

In order to confirm appropriate test conditions, the **positive control** should give an extinction higher than ≥ 0,750 OD units measured at 450 nm and an endpoint titre higher than 9.

The **negative control** should give an OD ≤ 0,400 units measured at 450 nm and an end point titer of ≤ 3.

10 INTERPRETATION OF TEST RESULTS

Using the OD results obtained with the dilutions of the gp70 standard (positive control) the concentration of test samples may be obtained by reference to the standard curve. This curve can be constructed from the OD values of the dilutions of the gp70 standard (y-axis) and the corresponding titres 30, 90, 270, 810, 2430 etc. (x-axis) on log/log paper. Draw a cut-off line at OD 0,400.

After putting all OD values in the graphic interpretation you can do extrapolation and just see where the curve crosses the 0,400 OD line. This is the final endpoint titre.

With this graphic presentation it is possible to determine the endpoint titre of the samples.

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
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