DRG® Feline Leukemia Virus-GP70 ELISA (EIA-2470)

Revised 10 Feb. 2009 (Vers. 4.0) **For Veterinary Use Only**

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 titer above >32 is considered to be protected. In a more sensitive ELISA, this titer is equal or higher than 270.

2 INTENDED USE OF THE TEST KIT

The FeLV-gp70 immunoassay (ELISA) test kit is designed to detect gp70 antibodies in serum 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 sample is then added. After incubation and before the addition of peroxidase labeled 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 microtitre strip-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 (HRP) 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-well **microtiter strips**
- 1 x stripholder
- 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 200 x** concentrated (must be diluted 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

4.1 Materials needed but not provided in the kit

- Deionized water

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 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 advised to carefully follow the washing procedures outlined below. Both manual washing and washing with automatic equipment can be performed. (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 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 washing 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 TEST PROTOCOL

1. Open the packet of strips, take out the strips to be used, cover the remaining strips with a part of the provided seal, store them at 4°C – 8°C.
   Wash the microtiter strips with washing solution, according to washing protocol.
   The washing solution provided must be diluted 200 x in deionized water !
2. Dispense 100 µl FeLV antigen suspension to all wells.
3. Seal the microtiter strips. Incubate for 90 min. at 37°C.
4. Wash as in 1.
5. Make a 3-step dilution of the positive and negative control in ELISA buffer, starting 1:3 (9; 27; 81)
6. Make a 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 min. at 37°C.
9. Wash as in 1.
10. Dilute the concentrated HRPO conjugate 1:100 in Conjugate Buffer. Dilute only the necessary amount.
11. Dispense 100 µl diluted HRPO conjugate to all wells.
12. Seal and incubate for 60 min. at 37°C.
13. Wash as in 1.
14. Mix equal parts of Substrate A and Substrate B together with gentle shaking. Prepare immediately before use!
    Dispense 100 µl substrate solution to each well.
    Incubate 10-15 min. 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 VALIDATION 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 ≥ 1.000 OD units measured at 450 nm and an endpoint titer higher than 9.

The negative control should give an OD ≤ 0.500 units measured at 450 nm and an endpoint titer ≤ 3.

9 INTERPRETATION OF TEST RESULTS

The end point titer can be determined by comparing the OD of each dilution with the following threshold:

OD negative control, at dilution 1:3 + 0.150.
If the OD of a dilution is above this threshold the dilution is considered to be positive.
Endpoint titers equal to or above 1:270 are considered to be protective in experimental situations.
Although other studies with lower virus pressure, protection has been shown with titers above 1:100.
It has been shown, by O. Jarret et al, that the relation between this type of ELISA and serum neutralization assays is above 94%.

Important
It is advised that positive test results should be confirmed by a serum neutralization test assay.

10 PRECAUTIONS

- Handle all biological material 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 work area.
- TMB is toxic by inhalation, through contact with skin and 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 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.

The entire risk as to the performance of these products is assumed by the purchaser. DRG shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

SYMBOLS USED WITH DRG 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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	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..