User's Manual

Feline Leukemia Virus-gp70 ELISA

A monoclonal-mediated ELISA to measure Feline Leukemia Virus-gp70

REF F1001-AG02

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

FeLV-gp70 is the glycoprotein of the envelope of FeLV. Cats are able to produce antibodies against gp70, which can be neutralizing. A cat with a neutralizing antibody titre >32 is considered to be protected. To obtain titres in this range a vaccine should minimal contain ca 200 ug per dose depending on the adjuvant used.

2. Intended use of the test kit

The FeLV gp70 enzyme immunoassay (ELISA) kit is designed to detect gp70 antigen in culture supernatants and virus/gp70-concentrates. The kit procedure is based on an indirect solid phase Enzyme Immuno Assay (EIA). The gp70 molecule is bound by monoclonal antibodies attached to the solid phase. Unbound materials are removed by rinsing. A diluted biotinilated anti gp70 serum is then added. After incubation, unbound materials are removed by rinsing. After streptavidin conjugate incubation and rinsing, the substrate is added and the optical density is measured at 45Onm.
To standardize the FeLV-gp70 EIA, a FeLV standard has to be tested. The concentration of FeLV-gp70 in the FeLV standard is 1000 µg/ml.

3. Principle of the test kit

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

- **Qualitative**
  The sample is added (diluted 1:1) to the wells of the coated plate.
- **Quantitative**
  The sample also can be titrated using a 3-step dilution, starting with undiluted (→ 1:3 → 1:9 → 1:27).

After washing biotinilated anti FeLV-gp70 antibodies are added to the wells and will bind to gp70 molecules which have been caught.

The complex is detected by a streptavidin horseradish peroxidase (HRPO) conjugate. Color reaction in the wells is directly related to the concentration of FeLV-gp70 in the sample.

4. Contents

- 12 x 8 Microtiter strips coated with monoclonal anti-FeLV-gp70 antibodies
- 1 x Strip holder
- 1 x 18 ml ELISA buffer (green cap)
- 1 x 18 ml HRPO conjugated anti-species antibodies buffer (red cap)
- 1 x 0,5 ml Concentrated streptavidin HRPO conjugate (dilute 1:100)
- 1 x 1,0 ml FeLV standard, 1000µg/ml (ready to use) (purple cap)
- 2 x 6 ml Polyclonal anti-FeLV antibodies biotinilated (freeze dried)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 20 ml Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop solution (yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

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.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing 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 result.

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 wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 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 wash 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 wash solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol qualitative

Before starting this test read "preparations"

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 +4°C. and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.
   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.

3. Make a 3-step dilution of the **positive control** (purple cap) in a round-bottomed plate (not supplied), **starting undiluted → 1:3 → 1:9 → 1:27**.
   Example: - Add 120µl ELISA buffer to wells **1B, 1C, 1D**
   - Add 180µl positive control to well **1A**
   - Transfer 60µl positive control from well **1A** to well **1B**
   - Mix well and transfer 60µl to well **1C**
   - Mix well and transfer 60µl to well **1D**
   - Mix well and discard 60µl.
4. Dilute the **negative control** (silver cap) **1:2 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 60µl ELISA buffer to **well 1E**, add 60µl of the negative control to the **well 1E** and mix well.
5. Dilute **the sample 1:2 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 60µl ELISA buffer to **row 1F**, add 60µl of the sample to the well **1F** and mix well.
6. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.
7. Transfer 100µl of all dilutions to the virus-coated microtiter strips, including the substrate controls.

8. Seal and incubate for 60 min at 37°C.

9. Reconstitute the freeze-dried biotinilated in 6 ml aqua bidest.

10. Wash the strips 5 times according to the wash protocol see sub 6.

11. Dispense **100µl** of this **anti-FeLVgp 70 conjugate** to all wells.
12. Seal and incubate for 60 min at 37°C.

13. Wash the strips 5 times according to the wash protocol see sub 6.

14. Dilute the streptavidin HRPO conjugate 1:100 in conjugate buffer.
15. Dispense **100µl diluted streptavidin HRPO conjugate** to all wells.
16. Seal and incubate for 30 min at 37°C.

17. Wash the strips according to the wash protocol [see sub 6].
18. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

19. Dispense **100µl substrate solution** to each well.
20. Incubate 10-13 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative does not become too dark.

21. Add **50µl stop solution** to each well; mix well.
22. Read the absorbency values immediately (within 10 min!) at 450 nm using 620nm as reference on the ELISA reader. **Use the substrate controls as blank.**

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round bottom microtiter plate second step can be done directly in the coated Elisa plate.

9. Test protocol quantitative

Before starting this test read "preparations"

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 +4°C. and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **negative control** (silver cap) in 1,0ml aquabidest (5 mega Ohm water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use, avoid freeze and thaw cycles.

3. Add for dilution of the **positive control** 100µl to **1B, 1C, 1D** of the coated microtiter strip.
4. Add for dilution of the **negative control** 100µl to **1F, 1G, 1H** of the coated microtiter strip.
5. Add for dilution of the **samples** 100µl to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.
6. Make a 3-step dilution of the **positive control** in the coated microtiter strip, **starting undiluted → 1:3 → 1:9 → 1:27**.
   Example: - Dispense 150µl positive control to the well **1A** of the microtiter strip.
   - Mix well and transfer 50 µl to the well **1B**
   - Mix well and transfer 50µl to the well **1C**
   - Mix well and transfer 50µl to the well **1D**
   - Mix well and discard 50µl.
7. Make a 3-step dilution of the **negative control** in the coated microtiter strip, **starting undiluted → 1:3 → 1:9 → 1:27**.
   Example: - Dispense 150µl negative control to the well **1E** of the microtiter strip.
   - Mix well and transfer 50µl to the next well **1F**
   - Mix well transfer 50µl to the next well **1G**
   - Mix well and transfer 50µl to the well **1H**
   - Mix well and discard 50µl.
8. Make 3-step dilution of **each sample** in the coated microtiter strip, **starting undiluted → 1:3 → 1:9 → 1:27**.
   Example: - Dispense 150µl of each sample to the well **2A and/or 2E** of the microtiter strip.
   - Mix well and transfer 50 µl to the well **2B and/or 2F**
   - Mix well and transfer 50µl to the well **2C and/or 2G**
   - Mix well and transfer 50µl to the well **2D and/or 2H**
   - Mix well and discard 50µl.
9. Take 2 wells as **substrate controls** add only **100µl ELISA buffer** (green cap) to these wells.
10. Seal and incubate for 60 min at 37°C.

11. Reconstitute the freeze-dried biotinilated in 6 ml aqua bidest.

12. Wash the strips 5 times according to the wash protocol [see sub 6].

13. Dispense **100µl of this anti-FeLVgp 70 conjugate** to all wells.

14. Seal and incubate for 60 min at 37°C.
15. Wash the strips 5 times according to the wash protocol see sub 6.

16. Dilute the streptavidin HRPO conjugate 1:100 in conjugate buffer.
17. Dispense **100µl diluted streptavidin HRPO conjugate** to all wells.
18. Seal and incubate for 30 min at 37°C.

19. Wash the strips according to the wash protocol [see sub 6].

20. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

21. Dispense **100µl substrate solution** to each well.
22. Incubate 10-13 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative does not become too dark.

23. Add **50µl stop solution** to each well; mix well.
24. Read the absorbency values immediately (within 10 min!) at 450 nm using 620nm as reference on the ELISA reader. **Use the substrate controls as blank.**

10. Precautions

- Handle all biological material 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 B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling 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.

11. Validation of the test

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC), diluted 1:2, must be ≥1.000.
  - The MV of the measured OD value for the Negative Control (NC), diluted 1:2, must be ≤0.400.

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation
Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:
In order to confirm appropriate test conditions the OD of the positive control, undiluted, should be ≥1.000 OD units (450nm) and give an endpoint titer of ≥18.
The negative control, undiluted, should be ≤0.400 OD units (450nm) and give an endpoint titer of ≤2.

12. Interpretation of the test results

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio <0.4 is negative.
  - Specific antibodies to FeLV-gp70 could not be detected.
- A sample with the S/P ratio ≥0.4 is positive.
  - Specific antibodies to FeLV-gp70 were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using cut-off line(dilution undiluted - 1:3 - 1:9 - 1:27 - 1:81 - 1:243 etc total 8 dilutions of 3 steps) OD on Y-as and Titre on X-as Elisa titres can be calculated using as cut-off 2,5 times OD value of negative control at 1:1.

13. Symbols used with EVL 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

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