Instructions for Use

Feline Leukaemia Virus-GP70 ELISA

EIA-2470

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso 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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A monoclonal mediated ELISA to detect FeLV gp70 antibodies in serum or plasma of cats

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 virus neutralizing antibody titre above ± 32 is considered to be protected. In a more sensitive ELISA, this titre is equal or higher than 300.

2 INTENDED USE OF THE TEST KIT

The FeLV-gp70 antibody ELISA is designed to detect FeLV-gp70 antibodies in serum and plasma samples. The kit procedure is based on a solid phase ELISA. When a standard FeLV-gp70 antigen suspension is added, the FeLV-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-FeLV-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.

- **Qualitative**

The sample is added (diluted 1:150) to the wells of the coated plate.

- **Quantitative**

The sample also can be titrated using a 3-step dilution,
starting with a dilution 1:50 → 1:150 → 1:450 → 1:1350.

After washing, samples are added to the wells and will bind to the FeLV-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 coated with FeLV-gp70 molecules
- 1 x Strip holder
- 1 x 18 mL ELISA buffer (white bottle + green cap)
- 1 x 12 mL HRPO conjugated anti-species antibodies (black bottle + red cap)
- 1 x 12 mL FeLV-gp70 antigen (lilac cap)
- 1 x 0,6 mL Positive control (**ready to use**) (yellow cap)
- 1 x 0,6 mL Negative control (**ready to use**) (brown cap)
- 1 x 20 mL Wash-solution (200x concentrated) (white bottle + black cap), **dilute in de-ionized water before use!**
- 1 x 8 mL Substrate buffer A (white bottle + white cap)
- 1 x 8 mL Substrate buffer B (black bottle + blue cap)
- 1 x 8 mL Stop-solution (white bottle + yellow cap)
- 1 x Plastic cover seal
- 1 x Instructions for Use

4.1 Supplies needed (not included)

- ELISA plate reader
- Pipette tips and clean containers/tubes
- Precision pipette 10-200µl
- Precision pipette 1-10µl
- Precision pipette 200-1000µl
- Round-bottomed microtiter plate
- Validated precision pipettes

5 HANDLING AND STORAGE OF SPECIMENS

- After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and store at -20 °C.
- An open packet should be used within 20 days.
- 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.
- The kit should be stored at 4 °C.

6 WASH PROTOCOL

In ELISA, 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 (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 washing solution in the wells
6. Make sure that none of the wells dries 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 5 washing cycles.

7 PREPARATIONS

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 minutes 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 °C - 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 20 days.

   Use validated precision pipettes, and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Before testing make sure all reagents are at room temperature.
3. Wash the wells as pointed out in wash protocol. (**Dilute the washing fluid 1:200 in aquabidest before use).**
4. Add 100 µL FeLV-gp70 antigen (lilac cap) suspension to all needed wells.
5. Incubate for 90 minutes at 37 °C.

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6. Dilute the **positive control** (yellow cap) **starting 1:3 → 1:9 → 1:27 → 1:81** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 120 µL ELISA buffer (green cap) to **well 1A**, and 120 µL to **wells 1B, 1C, 1D**
   - Add 60 µL of the positive control to **well 1A** and mix well
   - Mix well and transfer 60 µL to **well 1B**
   - Mix well and transfer 60 µL to **well 1C**
   - Mix well and transfer 60 µL to **well 1D**
   - Mix well and discard 60 µL.
7. Dilute the **negative control** (brown cap) **1:3** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 100 µL ELISA buffer (green cap) to **well 1E**, add 50 µL of the negative control to the **well 1E** and mix well.
8. Dilute the **sample 1:150** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 90 µL ELISA buffer (green cap) to **well 2F**, add 10 µL of the sample to the **well 2F** and mix well.
   - Add 140 µL ELISA buffer to **well 1F**, add 10 µL of pre-dilution from **well 2F** in the well **1F** and mix well.
9. Take 2 wells as substrate controls, add only 120 µL ELISA buffer (green cap) to these wells.

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10. Wash the with antigen incubated plate according to the wash protocol see sub 6.
11. Transfer 100 µL of all dilutions of **column 1** to the virus-coated microtiter strips, including the substrate controls.
12. Seal and incubate for 60 minutes at 37 °C.
13. Wash the strips 5x according to the wash protocol see sub 6.
14. Add 100 µL HRPO conjugated anti-species antibodies (red cap) to all wells.
15. Seal and incubate for 60 minutes at 37 °C.
16. Wash the strips 5x according to the wash protocol see sub 6.
17. 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.**
18. Add 100 µL substrate solution to each well.
19. Incubate 10 - 15 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (±21 °C). Make sure the negative control does not become too dark.
20. Add 50 µL stop solution to each well; mix well.
21. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm 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 20 days.

   Use validated precision pipettes, and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Before testing make sure all reagents are at room temperature.
3. Wash the wells as pointed out in wash protocol. **(Dilute the washing fluid 1:200 in aquabidest before use).**
4. Add 100 µL FeLV-gp70 antigen suspension to all needed wells.
5. Incubate for 90 minutes at 37 °C

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6. Make a pre-dilution of **each sample in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90 µL ELISA buffer (green cap) to **well 1A** and add 10 µL of the sample to the well **1A.**
7. Take 2 wells as substrate controls; add only 120 µL ELISA buffer (green cap) to these wells.

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8. Wash the with antigen incubated plate according to the wash protocol see sub 6.
9. Add for dilution of the **positive control 100 µL ELISA buffer** (green cap) to **wells 1A, 1B, 1C, 1D** of the coated microtiter strip.
10. Add for dilution of the **negative control 100 µL ELISA buffer** (green cap) to **wells 1E, 1F, 1G, 1H** of the coated microtiter strip.
11. Add for dilution of the **samples 120 µL ELISA buffer** (green cap) to the other **wells 2A and 2E;**
    and **100 µL** to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.
12. Make a 3-step dilution of the **positive control** (yellow cap) in the coated microtiter strip,
    starting 1:3 → 1:9 → 1:27 → 1:81.
    Example: - Add 50 µL positive control to the well **1A** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.
13. Make a 3-step dilution of the **negative control** (brown cap) in the coated microtiter strip,
    starting 1:3 → 1:9 → 1:27 → 1:81
    Example: - Add 50 µL negative control to the well **1E** of the microtiter strip.
    - Mix well and transfer 50 µL to the well **1F**
    - Mix well and transfer 50 µL to the well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.
14. Make 3-step dilution of **each sample** in the coated microtiter strip,
    starting 1:50 → 1:150 → 1:450 → 1:1350.
    Example: - Add 30 µL of each sample from step 6 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 50 µL to the well **2C and/or 2G**
    - Mix well and transfer 50 µL to the well **2D and/or 2H**
    - Mix well and discard 50 µL.
15. Add **100 µL** of the **substrate control** from step 7 to the last 2 wells of the microtiter strip.
16. Seal and incubate for 60 minutes at 37 °C.
17. Wash the strips 5x according to the wash protocol see sub 6.
18. Add 100 µL HRPO conjugated anti-species antibodies (red cap) to all wells.
19. Seal and incubate for 60 minutes at 37 °C.

20. Wash the strips 5x according to the wash protocol see sub 6.
21. 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.**
22. Add 100 µL substrate solution to each well.
23. Incubate 10 - 15 min in the dark (e.g. cover the wells with a sheet of paper) at room temperature (±21 °C). Make sure the negative control does not become too dark.
24. Add 50 µL stop solution to each well; mix well.
25. Read the absorbency values immediately (within 10 minutes!) at 450 nm using 620 nm as reference on the ELISA reader. **Use the substrate controls as blank**.

10 PRECAUTIONS

- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- Do not pipette by mouth.
- Do not use components past the expiry date and do not mix components from different serial lots.
- 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.
- Handle all biological material as though capable of transmitting infectious diseases.
- 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.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.

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 1:3, must be ≥ 0.850
  - The MV of the measured OD value for the negative control (NC), diluted 1:3, 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, diluted 1:3, should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 9.
  - The negative control, diluted 1:3, should be ≤ 0.400 OD units (450 nm) and give an endpoint titer of ≤ 3.

12 INTERPRETATION OF TEST RESULTS

This test can be used in 2 ways.

Qualitative: positive – negative

- A sample with the S/P ratio **< 0.31** is negative
    - Specific antibodies to FeLV-gp70 could not be detected.
- A sample with the S/P ratio **≥ 0.31** 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 1:50 → 150 → 450 → 1350 → 4050 → 12150, etc., total 8 dilutions of 3 steps) OD on Y-axis and titre on X-axis.
  ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:3.

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 In case of problems or questions contact DRG.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
VET	For veterinary use only				
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité