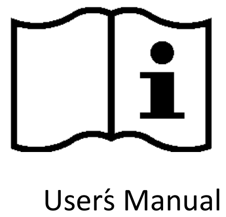
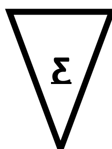


Userś Manual

Feline Leukaemia Virus-p27 Antigen SRE

A monoclonal antibody-mediated SRE to detect feline leukaemia virus-p27 antigen in serum and plasma samples

REF F3201-AG01



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Nov 2021

Please use only the valid version of the package insert provided with the kit.

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2. Introduction

FeLV p27-antigen is the major nucleo 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 SRE tends to detect FeLV-p27 antigen in some cases at an earlier stage. IFA or virus-isolation must be used to confirm positive SRE-results!

3. Intended use of the test kit

The FeLV-p27 antigen SRE 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 HRPO anti-FeLV conjugate. After incubation and washing the substrate is added.
The colour development is directly correlated with the quantity of the p27 antigen in serum or plasma samples.

4. Principle of the test kit

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 32-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 HRPO conjugate.
Bound conjugate is made visible by adding substrate/chromagen mix. Intensity of the colour reaction in the wells is directly correlated to the concentration of p27 antigen in the serum sample.

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5. Contents

- 4x 8 Microtiter strips coated with monoclonal anti-FeLV-p27 antibodies
- 1x Buffer (white bottle + green cap)
- 1x Negative control (ready to use) (brown cap)
- 1x Positive control (ready to use) (yellow cap)
- 1x Conjugate (black bottle + red cap)
- 1x Substrate A (white bottle + white cap)
- 1x Substrate B black bottle + (blue cap)

Supplies needed (not included)

- Fixed pipette 60µl (EVL)
- Pipette tips (EVL)
- ELISA plate reader (the results can be interpreted by eye, but for a more accurate and objective reading the use of the ELISA plate reader is strongly recommended)

6. Handling and storage of specimens

- The kit should be stored at 4°C.
- An open strip packet should be used within 28 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.

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 solutions.
- 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.

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8. Test protocol **qualitative**

Before starting this test read "preparations"

1. Before starting this test read **"preparations**".
2. Open the packet of strips and take out the amount of wells needed from the test strip, 1 for each sample and 2 extra wells for the controls. Cover the remaining strips with a part of the provided seal and store them at 4°C and use them within 10 days.
3. Use the fixed 60µl pipette and use a clean pipette tip **before** pipetting the buffer, samples, controls, conjugate and substrate.
4. Before testing make sure all reagents are at room temperature (±21°C).

5. Wash the test strips with running tap water:
    - Fill all wells to the rim.
    - Empty the wells.
    - <u>Repeat 5 times.</u>
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

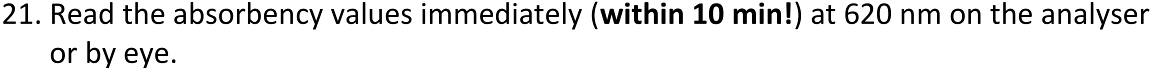
6. Add 60µl of buffer to all the wells.
7. Add 60µl of the negative control to the first well.
8. Add 60µl of the positive control to the second well.
9. Add 60µl of sample (serum/plasma) to the remaining wells.
10. Mix the reagents gently (see **"preparations"**).
11. Incubate for 40 minutes at room temperature (±21°C).

12. Wash the test strips with running tap water:
    - Fill all wells to the rim.
    - Empty the wells.
    - <u>Repeat 5 times.</u>
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

13. Add 120µl of conjugate to each well.
14. Incubate for 40 minutes at room temperature (±21°C).
15. Turn on the analyser (when available).

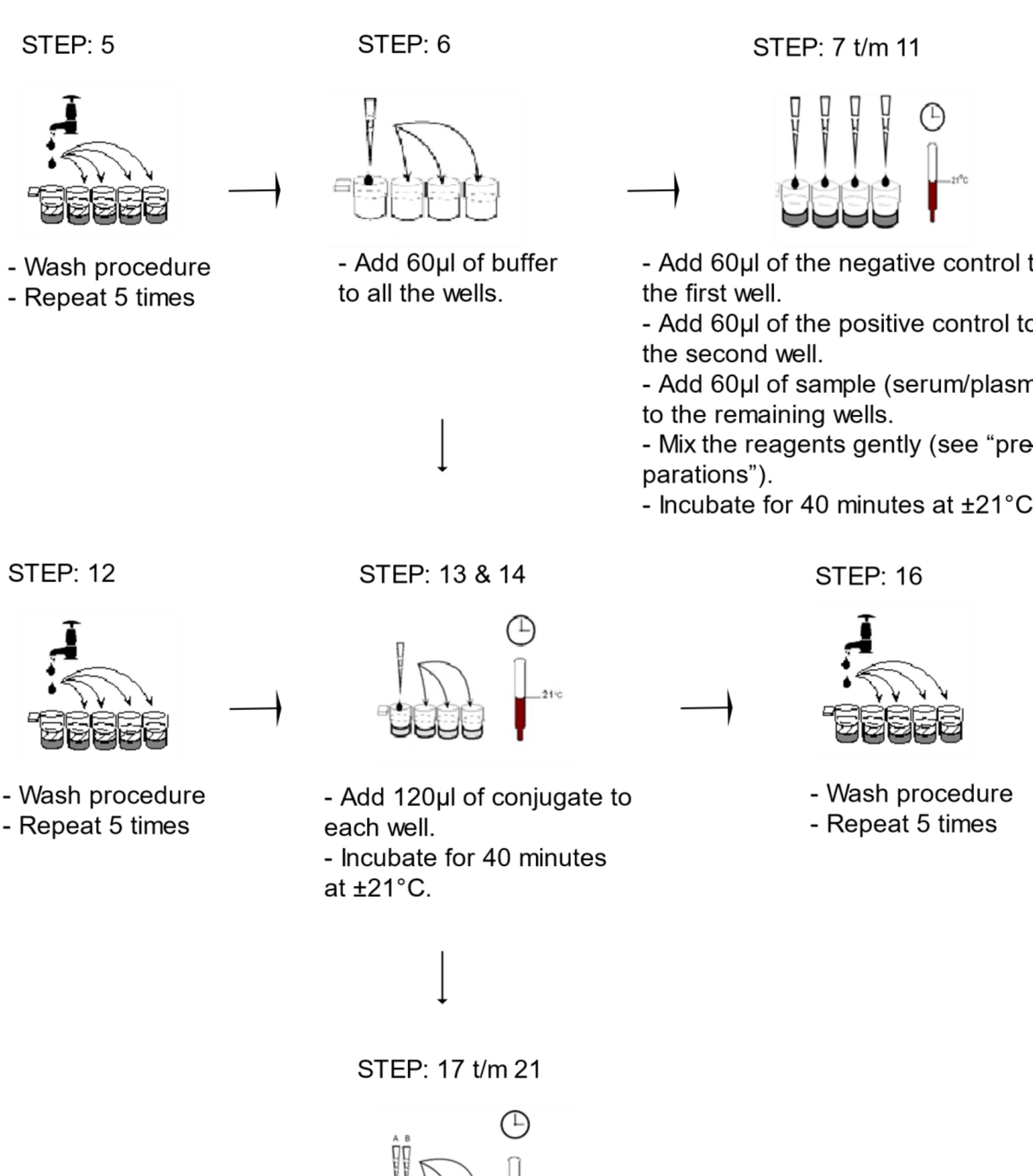
16. Wash the test strips with running tap water:
    - Fill all wells to the rim.
    - Empty the wells.
    - <u>Repeat 5 times.</u>
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

17. Add 60µl of substrate A to each well.
18. Add 60µl of substrate B to each well.
19. Mix the reagents gently (see **“preparations”**).
20. Incubate for 15 minutes in the dark (e.g. cover the wells with a sheet of paper).



21. Read the absorbency values immediately (**within 10 min!**) at 620 nm on the analyser or by eye.
    Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

9. Illustrated Test protocol



- Add 60µl of substrate A to each well.
- Add 60µl of substrate B to each well.
- Mix the reagents gently (see "preparations").
- Incubate for 15 minutes in the dark at ±21°C.

Read the absorbency values immediately (within 10 min!) at 620 nm on the analyser or by eye
Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

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10. Precautions

- Handle all biological material as through 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 optimal cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

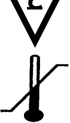
11. Interpretation of the test results

The analyser will give the results as positive, weakly positive or negative, but always double-check the outcome by observing the intensity of colour development.

- **Positive**
    - A sample is scored positive if the sample colour is dark blue, at least as blue as the positive control.
- **Weakly positive**
    - A sample is scored weakly positive if the sample colour is blue, with an intensity between that of the negative and positive control.
- **Negative**
    - A sample scored negative if the sample colour is equally blue or less blue than the negative control.

Note: diseased animal that are positive in this test (and are showing signs suggestive of FeLV) are considered positive and must be suspected of shedding FeLV.

12. Symbols used with EVL ASSAYS

Symbol	**Definition**
	Consult instructions for use
	European Conformity
IVD	In vitro diagnostic device
RUO	For research use only
REF	Catalogue number
LOT	Lot/ No. / Batch code
	Contains sufficient for <n> tests
	Storage Temperature
	Expiration Date
	Legal Manufacturer
Distributed by	Distributor
Content	Content
Volume/No.	Volume / No.

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