Feline Leukemia Virus DNA Molecular Detection Kit

Cat. No.30FLV104

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Feline Leukemia Virus DNA Molecular Detection Kit is intended for the detection of the Feline Leukemia pro Virus (FeLV) in DNA isolated from suitable feline biological samples such as blood, oralpharingeal and conjunctival swabs when using suitable extraction methods. The kit should used for the detection of progressive and regressive stages of the disease when the provirus is present. It contains all the disposable components required for performing an easy and accurate test.

PRINCIPLE

PCRun® is a molecular assay based on isothermal amplification of the 5' Long Terminal Repeat (5' LTR) FeLV gene. It is intended for the qualitative detection of the Feline Leukemia provirus associated with types A, B, and C. This kit is designed to be used with a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25^{0}C (room temperature or refrigerated).
- Avoid exposure to direct sunlight.
- Do not use beyond expiration date stated on the package label.
- Do not freeze!

Precautions:

- The PCRun® assay is not to be used on the specimen directly. An appropriate nucleic acid extraction method must be conducted prior to using this kit**.**
- In order to avoid the introduction of contaminating DNA into the reaction, clean laboratory examination gloves must be worn while performing the test.
- Open and remove the PCRun® reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRun® reaction tubes to the original aluminum packet together with the desiccator and seal the zip-lock tightly.**
- Do not use kit if the pouch or the components are damaged.
- Each component in this kit is suitable for use only with a specific lot number. Components have been quality control approved as a standard batch unit. Do not mix components from different lot numbers.
- Employ accepted sanitary procedures designated for biological and molecular waste when handling and disposing of the reaction tubes.

BACKGROUND

The Feline Leukemia Virus (FeLV) is a major pathogen of domestic and wild cats. It is a single stranded retrovirus which depends on a DNA intermediate for replication. If the cat's immune system is not able to neutralize the virus, the viral RNA is transformed and incorporated into the genome of the cat. The integrated DNA is termed "provirus". Once located in the genome, cell division will result in daughter cells that contain the viral DNA. The result is lifelong persistence of the virus. FeLV is transferred mostly by oronasal exposure to saliva and nasal secretions, commonly through mutual grooming and communal food and water bowls. Vertical transmission is considered of secondary importance[1]. The development of disease can be different for each cat. Once exposed, the felines can develop a progressive infection which leads to FeLV-associated diseases or a regressive infection which presents as an undetectable or transient viremia with antgenemia[2]. These latently infected cats have been shown to have persistence of the virus in bone marrow spleen, lymph nodes, small intestines and mammary glands[2]. Prognosis of the infected cat depends on its immune status and age as well as the pathogenicity of the virus, infection pressure and virus concentration[3].

DIAGNOSIS

During the early stages, the virus spreads into circulating lymphocytes and monocytes, distributing the virus to lymphoid tissues throughout the body. An important step in the disease process is infection of the bone marrow where precursor cells become virus-positive. Infected lymphocytes, granulocytes and monocytes are released into the blood, thereby spreading the virus throughout the body, infecting organs and tissues. FeLV can demonstrate various clinical signs. Cats may exhibit one or more of the following symptoms; anemia, enlarged lymph nodes, loss of weight, progressive weakness, lethargy and inflammation of the nose, cornea, gums and/or mouth[4]. Various immunological and molecular tests have been developed for the assistance in the diagnosis of FeLV infections. Virus isolation remains the gold standard. Serologically based fluorescent antibody test (FAT) and enzyme-linked immunosorbent assay (ELISA) are available, but the cats have to have been infected for 6 and 4 weeks respectively, to test positive[5]. Tests which detect the presence of the FeLV antigen are more diagnostically applicable. In order to define the progressive infection these tests should identify the presence of cell-associated antigens. Polymerase chain reaction (PCR) methods which target the FeLV proviral DNA or viral RNA have been developed for the diagnosis of FeLV in the leukocyte fraction of whole blood. RNA based tests are suitable for use during the early viremic stages, while DNA oriented molecular tests are applicable to determine previous exposure and latent disease Using these molecular tests, positive results can be obtained in less than 2 weeks after exposure and are therefore considered sensitive and accurate approaches for diagnosis[6].

KIT CONTENTS

Components	Amount
PCRun® strip of 4 lyophilized Feline Leukemia Virus single reaction tubes	1
Aluminium pouch with disposable nucleic acid detection device.	4
Disposable plastic capillary tubes - 20 µl*	5

***Accurate laboratory pipettes with aerosol barrier tips can be used in place of the plastic pipettes.**

EQUIPMENT TO BE SUPPLIED BY USER:

- Biogal PCRun® Sample Prep
- Heat block which maintains 60^{0}C – compatible with 0.2 PCR tubes Heat block can be supplied by Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from 50 µl of whole blood using PCRun® Sample Prep Kit (Cat No. 30PRE104). Samples can be collected in EDTA, heparin or sodium citrate. Blood anticoagulants have a substantial consequence on PCR reactions therefore it is imperative that the optimal volume of blood is collected as indicated on the tube.

For optimal results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRun® kit.

Unless otherwise recommended, samples and DNA extracts can be maintained at 2-8^{0}C for up to 24 hours or -20^0 C for an extended period of time.

PROTOCOL - PCRun® REACTION

1. Prepare a clean working area for the assay. Work area should be decontaminated with commercial household bleach (3.5%) diluted 1:10 with water.
2. Prepare all parts of the assay:

√ **Extracted DNA sample**
√ Pouch with reaction tubes
√ Capillary tubes for dispensing 20 µl volume
√ Fine tipped permanent marker

3. Switch on the heat block and adjust to 60°C. Once the block has reached the target temperature, continue with the reaction.

4. Remove the PCRun® strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with zip-lock to maintain a dry environment. Four individual reaction tubes are connected by a thin plastic spacer. Employing a small clean scissors, disconnect the required number of tubes without disturbing the lids. Tap the tubes lightly on a surface and observe that the small white pellet is located on the bottom of the tube.

5. Label the lid of the tubes clearly for sample identification.

6. Carefully open the lid of the reaction tubes, one at a time. Employing the 20 µl disposable capillary tube, dispense 20 µl of DNA extracted with PCRun® Sample Prep Kit into the reaction tube. Make sure that the entire content of the capillary tube has been emptied into the PCRun® reaction tube. Tap the tube on a surface to bring all the fluid to the bottom of the tube. Incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

7. Place the reaction tube into the appropriate hole in the pre heated block (60°C) and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

8. At the end of the incubation period (1 hr) remove the tube from the heat block and analyze immediately with the disposable nucleic acid detection device.

ANALYSIS OF PCRUN® REACTION WITH THE DISPOSABLE NUCLEIC ACID DETECTION DEVICE

One disposable nucleic acid detection device is needed for each test. Open and remove the components of the detection device. The device consists of two plastic parts, the Amplicon Cartridge containing a plastic buffer bulb and the Detection Chamber containing the lateral flow strip (Figure 1).

1. Verify the presence of fluid in the bulb.
2. Mark each chamber with the sample ID.
3. Align the lid section of the PCRun® reaction tube with the wide partition located beside the buffer bulb. Apply light pressure to attach the reaction tube to the Amplicon Cartridge (Figure 1).
4. Fold the Amplicon Cartridge in two and snap closed. Place the cartridge into the Detection Chamber with the bulb facing downwards and away from the chamber lever.
5. Push the lever downwards to lock the device.
6. Wait for 15-30 minutes to read the results. Results read after 30 minutes are invalid.

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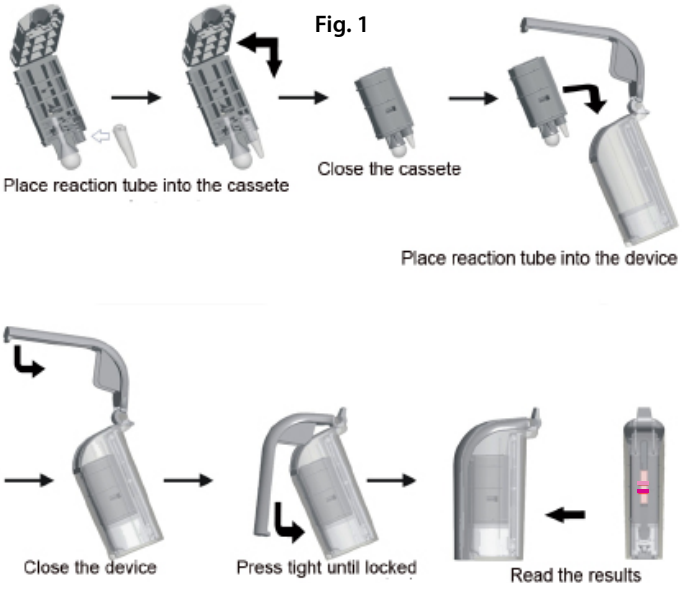
Fig. 1

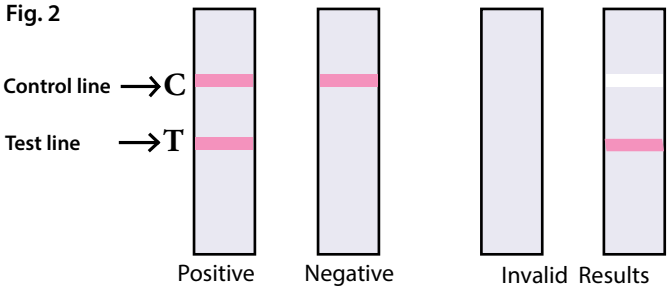


READING AND INTERPRETING THE RESULTS

A valid test must present a red control band. The control line must appear regardless of a positive or negative result.(Figure 2):

1. **Positive Result** - two bands appear , the upper control line and the lower test line. The appearance of both control line and test line indicates the presence of Feline Leukemia virus DNA.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the Feline Leukemia virus DNA or the that the copy number is below the detection limit.

Fig. 2



LIMITATIONS

As with any diagnostic kit, the results obtained must be used as an adjunct to other clinical and laboratory findings. The accuracy of the test results depends on the quality of the sample and adherence to protocol. A negative result may be obtained if the specimen is inadequate or target pathogen concentration is below the sensitivity of the test.

ANALYTICAL SENSITIVITY

The PCRun® reaction can detect 10^3 copies of the target gene in pure DNA.

REFERENCES

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