PCRun®

Feline Leukemia Virus DNA Molecular Detection Kit

Cat. No.30FLV116/30FLV148

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Feline Leukemia Virus DNA Molecular 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. The kit can be 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 PCRun® Reader.

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 antigenemia[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	16 Test Kit	48 Test Kit
PCRun® strip of 8 lyophilized Feline Leukemia Virus single reaction tubes	2	6
PCRun® buffer to re-dissolve lyophilized reaction pellets	2 Vials, 200 µl	6 Vials, 200 µl
PCRun® lyophilized Feline Leukemia Virus Positive Control	1 vial	1 vial
Buffer to reconstitute and dilute positive control.	1 vial, 800 µl	1 vial, 800 µl

EQUIPMENT TO BE SUPPLIED BY USER:

- DNA extraction kit designed for use with PCR reactions
- PCRun® Reader aquired from Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves
- Accurate laboratory pipettes with aerosol barrier tips

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for use with nucleic acid extracted from whole blood, oralpharingeal and conjuctival swabs employing most DNA extraction kits designed for use with PCR. Blood 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 best 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 diluted with water to a final concentration of 0.35%.

2. Prepare all parts of the assay:

- √ **Extracted DNA sample**
- √ Pouch with reaction tubes
- √ PCRun® buffer
- √ Pipettors for dispensing 5, 15 and 500 µl volume
- √ PCRun® Positive Control
- √ Positive Control Dilution Buffer
- √ Fine tipped permanent marker
- √ PCRun® Reader (Please refer to the PCRun® Reader Instruction Manual for operating directions)

3. **Positive Control**
A positive control is supplied with the kit. It is recommended that a positive control be run at the same time as the PCRun® reactions.

Dilution to final concentration of 10^6 copies/5 µl .

a. Add 500 µl Positive Control Dilution Buffer to the vial containing the lyophilized pellet. Vortex the vial and allow to stand 5 min at room temperature. Vortex again. The vial contains 10^6 copies of the target gene/5µl. Label the tube with the concentration. This dilution will be employed as the positive control.
b. Use 5 µl of the positive control in place of the DNA sample for PCRun® positive control reactions. It is not advisable to repeatedly freeze and defrost the Positive Control. The remainder of solution should be aliquoted into small volumes and maintained at -20^0 C for later use.

The positive control can be a source of contamination therefore maximum attention must be applied to ensure that the positive control does not come in contact with any other kit components. The positive control should be added to the reaction tube following completion of the test samples.

4. Switch on the PCRun® Reader and note that it is adjusted to 60^{0}C. Once the PCRun® Reader has reached the target temperature, continue with the reaction.

5. 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. Eight 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.

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

7. Carefully open the lid of the reaction tubes, one at a time. Dispense 15 µl of PCRun® Buffer into the reaction tube. Close the lid and incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

8. Add 5 µl of DNA sample into the PCRun® reaction tube and mix thoroughly. Close the lid of the tube firmly and tap the tube on a surface to bring all the fluid to the bottom of the tube.

9. Place the reaction tube into the PCRun® Reader which has been pre heated to 60°C and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

ANALYSIS OF PCRUN REACTION

After one hour incubation, final results of each reaction will appear on the touch screen. Follow instructions found in the manual accompanying the PCRun® Reader.

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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