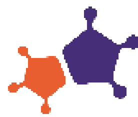

Canine Pathogenic Leptospira Molecular Detection Kit

Cat. No.30CLP116/30CLP148
For *in vitro* veterinarian diagnostic use only
User Manual

INTENDED USE

PCRun® Canine Pathogenic Leptospira Molecular Detection Kit is intended for the detection of pathogenic *Leptospira* in DNA isolated from canine whole blood, bone marrow and concentrated urine. The kit contains all the disposable components required for performing an easy and accurate test and should be employed at the early stages of infection.

PRINCIPLE

PCRun® is a molecular assay based on isothermal amplification of part of the *HapI* gene. It is intended for the qualitative detection of pathogenic species of Leptospires. 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

Leptospirosis is a bacterial zoonotic disease caused by pathogenic species of the genus *Leptospira*. Typical pathogenic serovars infecting dogs include *icterohaemorrhagiae, canicola, pomona, bratislava, grippotyphosa* and *autumnalis*(1). Leptospires, transmitted by direct contact with contaminated sources, can penetrate abraded skin or mucous membranes and disseminate within the host. In canines the motile spirochaetes can be found in the bloodstream in the first 7-10 days after exposure. The septicemic phase is followed by an immune phase which is characterized by the appearance of antibodies and the clearance of *Leptospires* from the bloodstream. The spirochaetes then colonize the renal tubules of chronically infected hosts. Leptospiruria begins approximately in the second week following the onset of clinical signs. Maintenance hosts such as dogs typically remain clinically asymptomatic and shed Leptospires into the environment via urine.

DIAGNOSIS

Leptospirosis can be suspected based on clinical signs and results of kidney and liver functions. A conclusive diagnosis is usually made by demonstrating the presence of the bacteria in the samples or by finding increasing levels of antibodies over time. Techniques available include histochemistry, culture, antigen/antibody reactions and molecular based hybridization or amplification. Culture isolation of causative organisms from clinical samples takes several weeks, microscopic analysis is highly subjective and antibodies are detectable in blood only 5-7 days after the onset of symptoms. Serological surveys report more than 20% of examined canine sera contain antibodies specific for pathogenic *Leptospira* serovars, however it is difficult to correlate serological titres with the prevalence of chronic infections (2).
Molecular amplification is employed for the rapid detection of pathogens involved in acute infections. Protocols can be applied to selectively amplify highly conserved DNA sequences which are present in pathogenic Leptospires, but absent in saprophytic species. Molecular amplification of DNA isolated from clinical samples can rapidly confirm the diagnosis in the early phase of the disease, before antibody titers are at detectable levels and urine samples can be used to identify chronic carriers of the disease (2).

KIT CONTENTS

Components	16 Test Kit	48 Test Kit
PCRun® strip of 8 lyophilized *Leptospira* single reaction tubes	2	6
1X PBS 1.5 ml	1	1
10X PBS 1.5 ml	1	1
PCRun® buffer to re-dissolve lyophilized reaction pellets	2 vials, 200 µl	6 vials, 200 µl
PCRun®lyophilized *Leptospira* 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 suitable 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
- For urine samples microcentrifuge which reaches 10,000 rpm

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from whole blood or the pellet derived from concentration of 2 ml of urine using most DNA extraction kits designed for use with PCR.

Blood samples
Whole blood 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.

Urine Sediment
Four drops (200 µl) of 10X PBS (provided) should be added to 2 ml of fresh urine. The sample should then be centrifuged at high speed (10,000 x rpm) for ten minutes. The supernatant is discarded and the solid material (pellet) found at the bottom of the test tube should be resuspended in one drop (50 µl) of 1X PBS (provided) prior to DNA extraction. Concentrated urine samples can be stored frozen.

For best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRun® Kit. It is recommended to process samples close to collection time. Blood samples and urine pellets must be maintained frozen at -20^{0}C if not processed immediately.
Transportation of samples should always be under cold conditions.

PROTOCOL - PCRUN® REACTION

1. Prepare a clean working area for the assay.
Working 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
√ 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.
Animals undergoing antibiotic treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

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

REFERENCES

1. Levett, P. N. 2001. Leptospirosis. Clin. Microbiol. Rev. 14:296–326.

2. Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, Mazel M, Matsunaga J, Levett PN, Bolin CA (2000) The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. Infect Immun 68(4):2276–2285

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