



# PCRRun™

## Parvovirus Molecular Detection Kit

Cat. No.30CFP148

For *in vitro* veterinarian diagnostic use only

User Manual

### INTENDED USE

PCRRun™ Parvovirus Molecular Detection Kit is intended for detection of canine and feline parvovirus (canine CPV-2a, 2b and 2c) in DNA isolated from whole blood and feces. The kit contains all the disposable components required for performing an easy and accurate test.

### PRINCIPLE

PCRRun™ is a molecular assay based on the isothermal amplification of part of the VP2 capsid protein gene. It is intended for the qualitative detection of canine and feline parvovirus. This kit is designed to be used with a compatible PCRRun™ Reader.

### STORAGE AND HANDLING

- Storage 2-25°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 PCRRun™ 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 PCRRun™ reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRRun™ reaction tubes to the original aluminum packet together with the desiccator. Seal with tape.**
- 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

Canine parvovirus (CPV) and feline panleukopenia virus (FPLV) are autonomous parvoviruses which are the causative agent of parvoviral infections in dogs and panleukopenia in cats (otherwise known as feline infectious enteritis, feline parvoviral enteritis and feline distemper). Although thought to be species-specific, new variants of CPV have developed the potential to cause disease also in cats<sup>1</sup>. Infections with CPV and FPLV result in highly contagious diseases with worldwide distribution in wild and domestic animals. Virus particles can be transmitted to a susceptible host by oronasal route. Settings such as animal

shelters, pet shops, kennels and research colonies are ideal for spread and transmission of the viruses. Canines and felines of all ages can be infected but severe disease is most common in unvaccinated or partially vaccinated puppies and kittens between 6 weeks and 4 months old. The period of greatest susceptibility to infection is when maternal antibodies are waning and vaccine-induced immunity is not fully developed. Both CPV and FPLV are associated with high morbidity and mortality, but infections can range from subclinical to severe depending on the immune status and the age of the animal. The virus initially infects and replicates in oropharyngeal lymphoid tissue. They have an affinity for rapidly dividing cells and for this reason, cells lining the intestinal tract and those making up the bone marrow are most affected. During the acute phase of the infection, FPLV and CPV particles may be shed in saliva, vomit, feces or urine<sup>2</sup>.

Parvovirus are ubiquitous, very stable and resistant to most disinfectants making them a source of infections for susceptible animals.

### DIAGNOSIS

Dogs and cats affected by acute infections with CPV or FPLV, display similar enteric diseases symptoms. The first clinical sign, pyrexia (3 - 5 days after virus infection), is followed by viral shedding in high titers in the feces. The virus primarily attacks the lining of the gastrointestinal tract leading to ulceration, shedding of the intestinal epithelium, diarrhea (less frequent in cats), vomiting, dehydration, malnutrition, anemia, and often death. Complete blood counts show decreased white blood cells. A tentative diagnosis of CPV or FPLV viral infection is based on the general history (exposure to contamination), vaccination history, clinical signs and supportive blood tests<sup>3,4</sup>. Serological tests can be used to determine disease status, but must be performed on paired sera to determine whether there is a rise in titer between the acute and convalescent sample. A definitive diagnosis can be made based on positive fecal ELISA or immunochromatography antigen tests. These tests are considered to be moderately sensitive, but false negatives often occur. Several molecular methods directed at the detection of CPV and FPLV in blood and fecal samples have reported greater sensitivity than conventional immunochromatography and are progressively replacing the antigen procedure as the standard procedure for virus surveillance and diagnosis.

### KIT CONTENTS

Components	Contents	Amount
Aluminum pouch Cat No. 03CPV100	PCRRun™ strip of 8 lyophilized Parvovirus single reaction tubes	6
PCRRun™ Buffer Cat No. 31000120	PCRRun™ buffer to re- dissolve lyophilized reaction pellets	6 Vials, 200 µl
PCRRun™ Positive Ctrl Cat. No. 03000007	PCRRun™ lyophilized Parvovirus positive control	1 Vial

#### EQUIPMENT TO BE SUPPLIED BY USER:

- DNA extraction kit compatible with PCRRun™ kit. Biogal can advise which kits are most suitable.
- If a rectal swab or stool sample are to be tested the user will require a kit designed specifically for this purpose. Biogal can recommend an appropriate kit.
- PCRRun™ Reader acquired from Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves
- Accurate laboratory pipettes with aerosol barrier tips.

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## SAMPLE COLLECTION, STORAGE AND TRANSPORT

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The kit is suitable for detecting nucleic acid extracted from whole blood or DNA purified from fecal swabs employing most DNA extraction kits designed for use with PCR. Blood samples can be collected in EDTA, heparin or sodium citrate. 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. If using rectal or fecal swabs it is recommended that high quality flocked swabs be employed.

For best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCR<sup>™</sup> kit.

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

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## PROTOCOL - PCR<sup>™</sup> REACTION

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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
- ✓ PCR<sup>™</sup> buffer
- ✓ Pipettor for dispensing 15 µl volume
- ✓ Pipettor for dispensing 5 µl volume
- ✓ PCR<sup>™</sup> Positive Control
- ✓ Fine tipped permanent marker
- ✓ PCR<sup>™</sup> Reader (Please refer to the PCR<sup>™</sup> 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 PCR<sup>™</sup> reactions.

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

a. Add 500 µl molecular grade water (MGW) 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 is the stock concentration.

b. Prepare a vial containing 45 µl molecular grade water.

c. Add 5 µl of the  $10^6$  copies/5µl concentration prepared in step a to give a concentration of  $10^5$  copies/5 µl and vortex well. Label clearly. This dilution will be employed as the positive control.

d. Use 5 ul of the positive control in place of the DNA sample for PCR<sup>™</sup> positive control reactions. The remainder of the stock solution should be aliquoted into small volumes and maintained at -20° 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 PCR<sup>™</sup> Reader and note that it is adjusted to 60°C. Once the PCR<sup>™</sup> Reader has reached the target temperature, continue with the reaction.

5. Remove the PCR<sup>™</sup> strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with tape 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 PCR<sup>™</sup> Buffer to each 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 PCR<sup>™</sup> 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 PCR<sup>™</sup> 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.

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## ANALYSIS OF PCR<sup>™</sup> REACTION

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After one hour incubation, final results of each reaction will appear on the touch screen. Follow instructions found in the manual accompanying the PCR<sup>™</sup> Reader.

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## LIMITATIONS

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A positive fecal or blood PCR alone is not sufficient for diagnosis of parvoviral infection as recently vaccinated animals may have positive PCR results. Diagnosis should be based on a combination of history, clinical signs, laboratory parameters, (leucopenia) and the PCR test results.

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## ANALYTICAL SENSITIVITY

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The PCR<sup>™</sup> reaction can detect  $10^3$  copies of the target gene in pure DNA.

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## REFERENCES

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1. Characterisation of canine parvovirus strains isolated from cats with feline panleukopenia (2010). Decaro O. et al. Research in Veterinary Science, Vol 89, Issue 2, pp 275-278
2. Infectious Diseases of the Dog and Cat. Fourth edition. Saunders – Elsevier
3. Feline parvovirus infection and associated diseases - Review. (2014) Stuetzer B, Hartmann K. Vet. J. Aug;201(2):150-5
4. An updated TaqMan real-time PCR for canine and feline parvoviruses. (2013). Andre Felipe Streke et al. Journal of Virological Methods. Vol 193. Issue 1 October, pp 6-8



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