



Ready To Use PCR Reagents

Canine *Dirofilaria repens*

Cat. No. 60CDR100

INSTRUCTION MANUAL

I. Intended Use

CDR Ready to Use PCR Reagents are intended for *Dirofilaria repens* amplifications. All reagents are ready to use for a successful amplification, from DNA extraction to obtaining PCR products suitable for loading onto Agarose gel.

II. General Information

Each package contains **Rapid One Step Extraction Buffer** (Tube A), which is intended for use with fresh or dry blood samples. The extraction step yields appropriate amount of crude DNA needed for a successful amplification of CDR via PCR. No purification is needed!

Tubes B, C and D are the components for subsequent use in PCR amplification. Tube B contains **CDR-PCR mix**, Tube C contains **CDR Activation Buffer** and Tube D contains the **Positive Control**. The **Extraction Buffer** (Tube A) also serves as **Negative Control**. Also included are **Tissue/Swab Extraction Buffer** (Tube E) and **Tissue/Swab Neutralization Buffer** (Tube F). Each PCR set up should include 3 reaction vials; each vial should be added with: **5µl CDR-PCR mix**, **10µl CDR Activation Buffer** and **5µl DNA product of the Extraction step / Positive Control / Negative Control**. Following the addition and mixing of all the above ingredients, the reaction vials are placed in thermal cycler for amplification according to the program detailed in the Step by Step chapter (see section VIII). At the end of the program the product should be visualized on 1.5% Agarose gel, yielding a **275bp** band.

III. Description Of The Disease

Dirofilaria repens is a filarial subdermal parasite of canids transmitted by mosquitoes. The adult worms, found mainly in the sub-cutaneous tissue of dogs, cats and foxes deposit microfilariae that circulate in the blood. Infections with *D. repens* in dogs have been reported in Africa, southern Europe and Asia. Infection is associated with skin swelling and hyperpigmentation, sub-cutaneous granulomas containing adult worms and local pruritus, or described as an incidental hematological finding. An increasing number of zoonotic infections with the filaria from different parts of the world have been reported. A few cases of *D. repens* were reported in cats as well during the recent years.

IV. Diagnosis Of The Disease

The mature *D. repens* live in the tissues and organs of vertebrates, while their immature stages prefer the blood and lymph vessels. A PCR test can identify the *D. repens* in all its stages from any organ. The test is very sensitive and specific and can be used for validation of diagnosis in post mortem samples and of living dogs and cats via blood samples.

V. Contents (Sufficient for 48 tests)

Tube A	Rapid One Step Blood Extraction Buffer
Tube B	CDR-PCR mix (Green cap)
Tube C	Specific CDR Activation Buffer (Blue cap)
Tube D	Specific CDR Positive Control (Red Cap)
Tube E	Tissue/Swab Extraction Buffer
Tube F	Tissue/Swab Neutralization Buffer
	CDR Instruction Manual

VI. Essentials Not Included

RNAase free PCR reaction vials.
 PCR Thermo-Cycler.
 5-10µ, 100µl Pipettes and filter tips.
 Micro-centrifuge.
 Heating bath or heating block.
 Agarose, DNA size marker.
 Microwave for Agarose casting.
 Horizontal Mini-Electrophoresis chamber, Comb and power pack.
 TBE /TAE Buffer and Ethidium Bromide (EB).
 UV Transilluminator (254nm for EB).
 A pair of sterile scissors.
 A cutter (for swab application).

VII. Storage and handling

- Store at 4°C for 6 months or at -20°C for two years.
- Use gloves and maintain clean working conditions.
- Avoid spillage and cross contamination of solutions.
- Change tips between reagents and between reaction vials.
- Disinfect scissors before and after each cutting of blood filters.
- Do not mix reagents from different batches.
- Always treat samples with precaution, and dispose as biological material.
- Remember that Ethidium Bromide is hazardous, and use the UV transilluminator carefully.
- It is recommended to incinerate the contents after use.

Developed by Karnieli Ltd.

VIII. Step By Step Protocol

Blood Extraction:

- (1) Into an empty clean vial, add **100µl of Rapid OneStep Blood Extraction Buffer (Tube A)** for **every 5µl** of fresh blood sample or approximately 3/5 mm² piece of Whatman/tissue paper soaked with blood. Make sure the piece of paper is submerged underneath the extraction buffer.
- (2) Incubate samples at **50°C** for **10 minutes** followed by a subsequent **95°C** for additional **10 minutes**.
- (3) Centrifuge sample at **>10,000 rpm** for **1 minute** to allow the paper and cell debris to pellet. The extracted DNA product is in the liquid phase, ready to be used for PCR.

Tissue/Swab Extraction:

- (1) Into a clean 1.5 ml vial add **300µl of Tissue/Swab Extraction Buffer (Tube E)**.
- (2) When using tissue sample, cut a 3 mm² from the fresh or frozen tissue and add it to the 1.5 ml vial containing **300µl of Tissue/Swab Extraction Buffer**.
- (3) Incubate the tissue within buffer **E** for **10 minutes at 95°C**.
- (4) Add **300µl of Tissue/Swab Neutralization Buffer (Tube F)** and the product will be ready for PCR use.

Extracted DNA product (of any source)* may be applied immediately for PCR or stored for a few days at 4°C / several months at -20°C. Please mark the vial properly for future identification.

* Note: **The reagents have been adjusted for use with crude DNA extraction to enable better sensitivity (with less handling).**

PCR Procedure:

- (1) Into a clean reaction vial add: **5µl CDR-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific CDR-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl CDR-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific CDR Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl CDR-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific CDR Activation Buffer (Tube C)**. Mark this vial as **Negative Control** reaction.
- (4) Gently mix each reaction vial (do not vortex!) and place in the thermal cycler for amplification.

PCR Program:

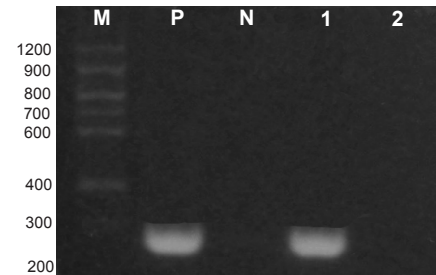
- 95°C for 2 minutes**
- 38 cycles of:**
- 94°C for 30 seconds**
 - 60°C for 30 seconds**
 - 72°C for 30 seconds**
- End cycles**
- 72°C for 2 minutes**
 - Stop at 8°C**

- (5) If not used immediately, store PCR products at 4°C until application on Agarose.

IX. Reading And Interpreting The Results

- Visualize PCR products on 1.5% Agarose gel, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial into the relevant wells.
- The Positive Control should yield a single band at **275bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **275bp**.

Fig. 1 - Visualization of the PCR product.



Lanes: M Size Marker, P Positive Control, N Negative Control
Lanes 1 - 2 are test samples of which 1 is positive for CDR.

X. Limitations And Troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false positive result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.

XI. References

- Baneth G et al. (2002) *Dirofilaria repens* infection in a dog: diagnosis and treatment with melarsomine and doramectin. *Vet Parasitol.* 30;105(2):173-8.
- Lee SE, Kim HC, Chong ST, Klein TA, Lee WJ. (2007) Molecular survey of *Dirofilaria immitis* and *Dirofilaria repens* by direct PCR for wild caught mosquitoes in the Republic of Korea. *Vet Parasitol.* Sep 1;148(2):149-55. Epub 2007 Jul 17.
- Magi M, Calderini P, Gabrielli S, Dell'Omodarme M, Macchioni F, Prati MC, Cancrini G. (2008) *Vulpes vulpes*: a possible wild reservoir for zoonotic filariae. *Vector Borne Zoonotic Dis.* Apr;8(2):249-52.
- Tarello W. (2003) Retrospective study on the presence and pathogenicity of *Dirofilaria repens* in 5 dogs and 1 cat from Aosta Vally. *Schweiz Arch Tierheilkd.* Oct;145(10):465-9.
- Vakalis N, Spanakos G, Patsoula E, Vamvakopoulos NC. (1999) Improved detection of *Dirofilaria repens* DNA by direct polymerase chain reaction. *Parasitol Int.* Aug;48(2):145-50.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.