



Ready To Use PCR Reagents

CANINE BABESIOSIS

Cat. No. 60CBC100

INSTRUCTION MANUAL

I. Intended Use

CB PCR Ready to Use PCR Reagents are intended for *Babesia canis* 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 Blood 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 **CB** via PCR. No purification is needed! Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **CB-PCR mix**, Tube **C** contains **CB 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 CB-PCR mix**, **10µl CB 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 **270bp** band.

III. Description Of The Disease

Canine Babesiosis (CB) is a tick borne infectious disease caused by protozoans of the genera *Babesia* that is characterized by fever, anemia and hemoglobinuria. The species *Babesia canis* and *Babesia gibsoni* are able to cause natural infections in dogs, with large geographic distribution. *B. canis* is grouped into three subspecies: *Babesia canis canis*, found in Europe; *B. canis vogeli*, found in North and South Africa, North America and Brazil; and *B. canis rossii*, found in South Africa. Another way of transmission is via infected blood transfusions.

IV. Diagnosis Of The Disease

The diagnosis of canine babesiosis is usually based on the presence of suggestive clinical signs (e.g., apathy, fever, anorexia, weight loss, pale mucous membranes, and jaundice) and patient history. The infection by *Babesia spp.* is confirmed by detection of the specific pathogen DNA in the blood using PCR. DNA within the blood stream may be detected, with high sensitivity, during all stages.

V. Contents (Sufficient for 48 tests)

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

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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) Carefully cut the agar-free swab close to its cotton edge and insert it into the vial. The swab should fit entirely inside the vial, must be covered with buffer and the cap should close easily.
- (3) Incubate the swab 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 CB-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific CB-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl CB-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific CB Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl CB-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific CB 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:

A. 95°C for 2 minutes

38 cycles of:

B. 94°C for 30 seconds

C. 60°C for 30 seconds

D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes

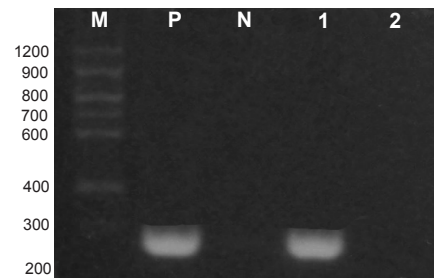
F. Stop at 8°C

- (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 **270bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **270bp**.

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 CB.

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. Avoid testing during 2-6 weeks post CBC vaccination.

XI. References

- Allsopp MT & Allsopp BA (2006) Molecular sequence evidence for the reclassification of some Babesia species. Ann N Y Acad Sci. 1081:509-17.
- Birkenheuer et al. (2004) Detection and molecular characterization of a novel large Babesia species in a dog. Vet. Parasitol. 124, 151-160.
- Jefferies et al. (2003) Two Species of Canine Babesia in Australia: detection and characterization by PCR. J. Parasitol. 89, 409-412.
- Zahler et al. (1998) Characteristic genotypes discriminate between *Babesia canis* isolates of differing vector specificity and pathogenicity to dogs. Parasitol. Res. 84, 544-548.

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.