



# Ready To Use PCR Reagents

## FELINE TOXOPLASMOSIS

Cat. No. 60FTG100  
INSTRUCTION MANUAL

### I. Intended Use

TG Ready to Use PCR Reagents are intended for *Toxoplasma gondii* amplifications. All reagents are ready to used 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 TG via PCR. No purification is needed! Tubes B, C and D are the components for subsequent use in PCR amplification. Tube B contains **TG-PCR mix**, Tube C contains **TG 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 TG-PCR mix**, **10µl TG 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 **190/300bp** band.

### III. Description Of The Disease

Toxoplasmosis is a worldwide zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular parasite that can infect the central nervous system of warm-blooded animals, including humans. The infection is mainly acquired by ingestion of oocysts or tissue cysts of *T. gondii*. In healthy people like in cats, infection will usually be asymptomatic. Clinical toxoplasmosis appears to occur in individuals who are immunocompromised, especially patients with acquired immunodeficiency syndrome (AIDS) or cancer. In women infected during pregnancy, transplacental transmission may occur. Cats play an important role in the spread of toxoplasmosis because they are the only animal that secrete resistant oocysts into the environment.

### IV. Diagnosis Of The Disease

The Diagnosis of *Toxoplasma* is possible by latex agglutination test (LAT), ELISA, indirect hemagglutination assay (IHA) and PCR for various target genes. The development of a highly sensitive and specific PCR protocol to identify *T. gondii* DNA can help in the early diagnosis of toxoplasmosis, providing proof of presence of the pathogen and preventing wide spread of infection. Furthermore, PCR can be used for monitoring the efficiency of treatment.

### V. Contents (Sufficient for 48 tests)

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

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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 mm<sup>2</sup> 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 TG-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific TG-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl TG-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific TG Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl TG-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific TG 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:

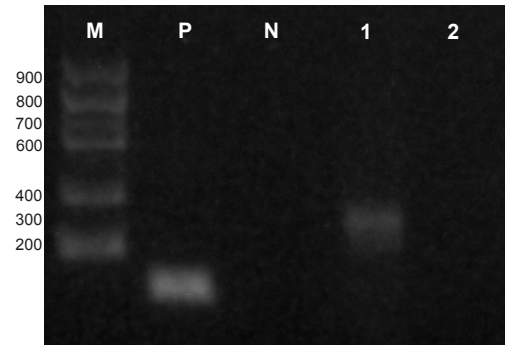
- 95°C for 2 minutes**
- 38 cycles of:**
- 94°C for 30 seconds**
  - 56°C for 30 seconds**
  - 72°C for 30 seconds**
- End cycles**
- 72°C for 2 minutes**
  - Stop at 8°C**

- (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 **190bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **190/300bp**.

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 TG.

## 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

- Jones CD et al. (2000) Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of *T. gondii* in aqueous humor. Invest Ophthalmol Vis Sci 41: 634-644.
- Kasper LH & Buzoni-Gatel D (1998) Some opportunistic parasitic infections in AIDS: candidiasis, pneumocystosis, cryptosporidiosis, toxoplasmosis. Parasitol Today 14: 150-156.
- Kim HY et al. (2008) Prevalence of *Toxoplasma gondii* in stray cats of Gyeonggi-do, Korea. Korean J Parasitol.46(3):199-201.
- Lee JY et al. (2008) Nested PCR-detection of *Toxoplasma gondii* in German shepherd dogs and stray cats in South Korea. Res Vet Sci 85: 125-127.
- Montoya JG & Liesenfeld O (2004) Toxoplasmosis. Lancet, 363: 1965-1976.
- Silva JC et al. (2001) Seroprevalence of *Toxoplasma gondii* in captive neotropical felids from Brazil. Vet Parasitol. 102: 217- 224.

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.