# Toxoplasma Detection Kit v1

# USER MANUAL

For *Research* Use

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**Contents**

## 1. PRODUCT DESCRIPTION

Bosphore® Toxoplasma Detection Kit v1 detects Toxoplasma DNA in human biological samples such as serum, plasma and tissue. The analytic sensitivity is 10 copies/ml. A region within the Toxoplasma genome B1 gene is amplified and fluorescence detection is accomplished using the FAM filter.

An internal control has been integrated into the kit in order to check PCR inhibition. The amplification data of the internal control is detected with the CY5/HEX/JOE filter. The internal control is added during PCR step.

## 2. CONTENT

Bosphore® Toxoplasma Detection Kit v1 is composed of Real-Time PCR reagents, an internal control and a positive control.

| Component | REAGENT | 100 Tests Reactions | 50 Tests Reactions | 25 Reactions |
|---|---|---|---|---|
| 1 | $dH_2O$ | (1000 µl) | (500 µl) | (500 µl) |
| 2 | PCR Mix | (1376 µl) | (688 µl) | (344 µl) |
| 3 | Detection Mix1 | (140 µl) | (70 µl) | (35 µl) |
| 4 | Detection Mix2 | (56 µl) | (28 µl) | (14µl) |
| 5 | Internal Control | (15µl) | (15 µl) | (15 µl) |
| 6 | Positive Control 1 | (88 µl) | (44 µl) | (22 µl) |

## 3. STORAGE

Bosphore® Toxoplasma Detection Kit v1 PCR reagents should be stored at -20°C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR; the components should not be exposed to room temperature for more than 10 min. and the detection mix components should not be exposed to light or air since the fluorophores may be susceptible to light and ozone. We recommend preparing the PCR on a cooling block, and keeping the detection mixes caps closed except during pipetting.

The components maintain their stability until the expiry dates on the labels, if they are stored at advised conditions.

## 4. REQUIRED MATERIALS AND DEVICES

- Montania® 483 or Montania® 4896 Real-Time PCR Instrument (Anatolia Geneworks), or another Real-Time PCR system with FAM and CY5/HEX/JOE filters (iCycler, iQ5, CFX–BioRad, LightCycler 2.0, 480-Roche, 7300, 7500 Real-Time PCR System, ABI StepOne or StepOne Plus systems -ABI, Stratagene Mx3005P, Mx3000P-Agilent, LineGeneK, LineGene 9600-Bioer, Rotorgene 2000, 3000, 6000, Q-Qiagen).
- 0.2 ml Thin-Wall PCR tubes or strips
- Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Bacterial DNA Extraction Kit/ Magnesia® Tissue Genomic DNA Extraction Kit (Anatolia Geneworks) or other high quality DNA extraction kits and systems
- Deep freezer (-20°C)
- Desktop centrifuge with rotor for 2 ml. microcentrifuge tubes
- Calibrated adjustable micropipettes
- DNAse, RNAse, pyrogen free micropipette tips with filters
- DNAse, RNAse, pyrogen free 1.5 or 2 ml. microcentrifuge tubes
- Disposable laboratory gloves

## 5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

**Important!:**

- The product should be delivered on dry ice. Check for presence of dry ice upon arrival.
- Check for the expiry dates on the box and tube labels, upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNAse, RNAse, pyrogen free micropipette tips with filters, and DNAse, RNAse, pyrogen free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds), and mixed well to ensure homogeneity prior to use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared, and they should be quickly returned to -20°C.
- PCR and nucleic acid isolation must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health related risks.
- Clinical samples should be handled with extreme caution: Physical contact with pathogens should be avoided by; wearing lab coats and gloves, no allowance for eating or drinking within the workspace, prevention of unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid isolation step; including the biological samples and material contacted with them, should be discarded into medical waste and disposed safely.

## 6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for research purpose.
- This product should be used in accordance with this user manual.
- This product is to be used by personnel specially trained to perform molecular genetics laboratory procedures.

## 7. PATHOGEN

### Causative Agents

Toxoplasma gondii is a parasitic protozoon which is creating the disease factors. The genome of the parasite can be found in different forms in different stages (oocyste, tachyzoite, bradyzoite). The size of the genome is 80 MB with 11 chromosomes. However it alters during the sexual reproduction period in the cats.

### Epidemiology

Toxoplasma gondii can cause infection in all mammalians and birds. This infection is affecting one third of world population which is a common infection worldwide. Age, life style( keeping a cat, eating flesh meat, etc), geographical factors determine the distribution of the infection across the globe ( France 80%, Turkey 39-75%, Austria 62%, UK 50%, USA 30-40%). Congenital infection frequency is 1 to 3 of each 1000 live births. The infection can result in serious consequences for the patients with immunodeficiency.

**Modes of Transmission**

Transmission of the infection is generally caused by infected cat stool, contaminated food and water, cooked or undercooked meat infected with cytes (bradyzoite), uncooked egg and dairy products, blood and organ transplantation, from mother to infant through placenta and rarely by inhalation.

## 8. METHOD

Bosphore® Toxoplasma Detection Kit v1 is based on the Real-Time PCR method. Polymerase chain reaction is a technique that is used for amplification of a DNA region. The reaction occurs by the repeating cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq polymerase enzyme, buffer solution and template. As a brief explanation, primers are small synthetic DNA those anneal to the specific regions of the template in order to start the synthesis. dNTPs are the building blocks of the amplified products. Taq polymerase amplifies the DNA template. Buffer solution provides the pH adjustment required for the reaction and template, as referred, is the target region for synthesis.

In Real Time PCR technique, in contrast to conventional PCR, PCR product can be monitored during the reaction. Therefore Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, whereby minimizing the risk of contamination. Dual labeled probes employed in the reaction in addition to the conventional PCR reagents, enable detection of the amplified target with increased sensitivity.

The assay utilizes the 5′ exonuclease activity of Taq Polymerase to cleave a dual-labeled fluorescent hybridization probe during the extension phase of PCR.

The probe is labeled at the 5′ end with a fluorescent 'reporter' molecule, and at the 3'end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are in close proximity, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, Taq Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above background level and becomes distinguishable, is called the threshold cycle ($C_T$). There is a linear relationship between the log of the starting amount of a template and its threshold cycle.

Bosphore Toxoplasma Detection Kit v1 employs multiplex PCR, and an internal control is incorporated into the system in order to check for possible PCR inhibition. Toxoplasma DNA and an internal control are co-amplified in a single reaction, using sequence-specific primers. The fluorescent signal generated by the Toxoplasma amplification is detected by a probe labeled at the 3′ end with FAM, through the FAM channel. The fluorescent signal generated by the internal control amplification, is detected by a second probe (labeled at the 5′ end with a different reporter molecule, CY5/HEX/JOE) through the CY5/HEX/JOE channel.

## 9. PROCEDURE

### 9.1. DNA Isolation

We recommend that the Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Bacterial DNA Extraction Kit/ Magnesia® Tissue Genomic DNA Extraction Kit (Anatolia Geneworks) isolation system or an alternative high quality DNA extraction system is used with Bosphore® Toxoplasma Detection Kit v1. The DNA isolation should be performed according to the manufacturers' instructions.

### 9.2. Kit Components

#### 9.2.1. PCR Mix

HotStarTaq DNA Polymerase: HotStarTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus,* cloned into E.Coli. The enzyme is provided in an inactive form. It is activated by a 15-minute 95 ºC incubation step. This prevents the formation of misprimed products and primer-dimers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate qualitative analysis. PCR Buffer: contains Tris-Cl, KCl, $(NH_4)_2SO_4$, $MgCl_2$. dNTP Mix: Contains ultrapure quality dATP, dGTP, dCTP ve dTTP/dUTP.

#### 9.4.2. Detection Mix 1

Detection Mix 1 contains Toxoplasma-specific forward and reverse primers and a dual-labeled probe.

#### 9.4.3. Detection Mix 2

Detection Mix 2 contains internal control-specific forward and reverse primers and a dual-labeled probe.

#### 9.4.4. Internal Control

An internal control is included in the kit to control PCR inhibition. The internal control is a synthetic DNA molecule derived from human genome. It is added directly into the PCR master mix to control the PCR inhibition exclusively. For this purpose, 0.1 µl of internal control should be added for each reaction into the master mix. Lack of internal control amplification in the FAM negative samples, may indicate a problem in PCR or PCR inhibition. In this case, PCR should be repeated. In samples that contain a high bacterial load, internal control can be suppressed and no increase of the signal is detected. Please use the table below for the interpretation of internal control data:

| Toxoplasma (FAM) | Internal Control (CY5/HEX/JOE) | Interpretation |
|---|---|---|
| + | + | Sample positive |
| - | + | Sample negative |
| + | - | Sample positive |
| - | - | Repeat the test! |

#### 9.4.5. Positive Control

The positive control contains Toxoplasma DNA. It can be included in the PCR to test the efficiency of the PCR exclusively. The threshold cycle for the positive control is given in the acceptance criteria table (Section 10. Analysis). Threshold cycles higher than the acceptance criteria may indicate an efficiency loss in the reaction.

### 9.3 Preparing the PCR

Positive control should be added into the PCR reaction together with the samples and the negative control (PCR-grade water) in order to deduce the positivity/negativity of the samples. Make sure that all the kit components are thawed before use. Refer to the table below for preparing the PCR. It is for only one reaction, multiply these values with the sample number to find the values required for the master mix. While preparing master mixes for more than 5 samples, an extra 10% should be added to the total sample number.

| | |
|---|---|
| **PCR Mix** | 12.5 µl |
| **Detection Mix 1** | 1.25 µl |
| **Detection Mix 2** | 0.5 µl |
| **Internal Control** | 0.1 µl |
| **dH2O** | 0.65 µl |
| **Sample DNA** | 10 µl |
| **Negative/Positive Control** | |
| **Total Volume** | 25 µl |

Pipette 15 µl of the master mix into the PCR tubes or strips, and add 10 µl of DNA (sample/positive or negative control). Close the tube cap. Make sure that the solution in each tube is at the bottom of the tube. Centrifuge if necessary.

### 9.4 Programming the Real-Time PCR Instrument

The thermal protocol for Bosphore® Toxoplasma Detection Kit v1 is composed of an initial denaturation for activation the HotStarTaq DNA Polymerase, a two-step amplification cycle and a terminal hold. The real-time data is collected at the second step of the amplification cycle.

| | | | |
|---|---|---|---|
| Initial denaturation | 95°C | 14:30 min. | |
| Denaturation | 97°C | 00:30 min. | } 50 cycles |
| Annealing and Synthesis (Data Collection) | 55°C | 01:30 min. | |
| Hold | 22°C | 05:00 | |

Before starting a Real-Time PCR reaction using the Bosphore® Kits, the following steps should be completed:

- Choose the filter pairs to be used (FAM and CY5/HEX/JOE).
- Identify unknown samples, positive and negative controls.
- Select the correct thermal protocol.

## 10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold.

Example of an amplification curve is given in Fig. 1.

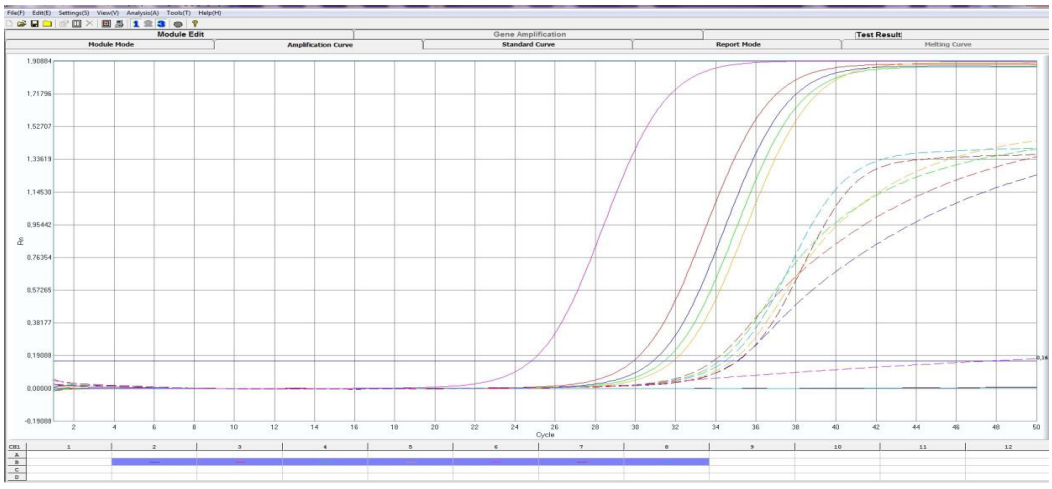


Fig. 1: Amplification Curve of a Bosphore® Toxoplasma v1 test

Analysis of the results should be performed by trained personnel who have received the required training for analysing Real-Time PCR data. We recommend that the test results must be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel, who have received the required training from manufacturer, consider it as necessary, if the system allows pulling down the threshold as much as possible in order to detect low positive samples, attention should be paid to keep the threshold line above

the background and to keep the correlation coefficient at the maximum possible value (and within its acceptance criteria.)

Positive control of Bosphore® Toxoplasma v1 is essential for accurate result analysis. The cycle threshold acceptance criteria for the positive control is 27±3 . Test results should not be reported unless the assay results meet the criteria stated above. Please contact the manufacturer if impairment in the product's performance is observed (See the last page for contact information).

The qualitative results of the test are displayed on the "Report Mode" screen. The samples that cross the threshold in FAM Channel are displayed as positive whereas samples that do not cut the threshold are displayed as "Negative" or "No Ct". These samples are regarded as negative or having a bacterial load below the detection limit of the assay. For these undetectable samples, the CY5/HEX/JOE data of the internal control should also be checked to avoid false negative results.

The following table shows the possible results and their interpretation:

| | | |
|---|---|---|
| Signal detected in FAM filter pair | The sample contains Toxoplasma DNA, the result is positive | No need to check the internal control since the sample is positive (high positive samples may suppress the signal from the internal control) |
| No signal in FAM, signal in CY5/HEX/JOE | The Toxoplasma DNA in the sample is not detectable | Signal from CY5/HEX/JOE filter pair rules out the possibility of PCR inhibition |
| No signal in FAM and CY5/HEX/JOE | The diagnosis is inconclusive | No signal in CY5/HEX/JOE points out to PCR inhibition or to a problem in DNA isolation |

## 11. REFERENCES


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4. Sibel AYDOĞAN, Funda DOĞRUMAN AL, Employing Real Time Polymerase Chain Reaction Method for Diagnosis of Toxoplasma Gondii, Turkish Journal of Parasitology, 29 (2): 80-84, 2005
5. B. Nagy et al, Detection of Toxoplasma gondii from amniotic fluid, a comparison of four different molecular biological methods, Clinica Chimica Acta 368 (2006) 131– 137

## 12. SYMBOLS

 Use by

 Lot/Batch

 Catalog number

 Temperature limitation

 Caution, consult accompanying documents

 Manufacturer

## 13. CONTACT INFORMATION


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