DNA-TECHNOLOGY

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**For research use only**

# Toxoplasma gondii

# REAL-TIME PCR Detection Kit

# INSTRUCTION FOR USE

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

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## 1. INTENDED USE

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Toxoplasma gondii REAL-TIME PCR Detection Kit** is designed for detection of *Toxoplasma gondii* DNA in human biological material (blood, cerebrospinal fluid, amniotic fluid, biopsy specimens or punctate from lesions of organs and tissues) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Toxoplasma gondii REAL-TIME PCR Detection Kit.**

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

## 2. METHOD

Method: real-time polymerase chain reaction; qualitative analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent completion of the polynucleotide chains from these primers with Taq-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a “hot” start is provided. For package S, “hot” start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The mixing of the layers and their transformation into a reaction mixture occurs only after the paraffin melts, which excludes nonspecific annealing of primers on the DNA target during the initial heating of the tube. “Hot” start for package U is provided by using polymerase which activity is blocked by antibodies. The activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific annealing of primers on the DNA target during the initial heating of the test tube.

DNA probes containing a fluorescent label and a fluorescence quencher are introduced into the PCR-mix. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

PCR-mix includes internal control (IC) designed to control the polymerase chain reaction.

The DNA probes for the detection of the DNA amplification product contain fluorescent dye Fam. The DNA for the detection of internal control amplification product contain fluorescent dye Hex.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

| Fam/Green | Hex/Yellow/Vic | Rox/Orange | Cy5/Red | Cy5.5/Crimson |
|---|---|---|---|---|
| *Toxoplasma gondii* | IC* | - | - | - |

* — internal control

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime real-time thermal cyclers for **Toxoplasma gondii REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** is also approved for use with

Rotor-Gene Q (Qiagen), CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

## 3. CONTENT

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** contain paraffin sealed PCR-mixes, Taq-polymerase solution, mineral oil and positive control. The detailed description of content is represented in Table 2.

Table 2. The **Toxoplasma gondii REAL-TIME PCR Detection Kit** content, package S, strips, for R1-P031-S3/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| Paraffin sealed PCR-mix | Colorless or pink transparent liquid under waxy white fraction | 1920 µL (20 µL in each) | tubes, 12 strips of 8 |
| Taq-polymerase solution | Colorless transparent liquid | 1 mL (500 µL in each tube) | 2 tubes |
| Mineral oil | Colorless transparent viscous oily liquid | 2.0 mL (1.0 mL in each tube) | 2 tubes |
| Positive control[1] | Colorless transparent liquid | 130 µL | 1 tube |
| Strip caps | 12 strips of 8 | | |

Table 3. The **Toxoplasma gondii REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-P031-23/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| Paraffin sealed PCR-mix | Colorless or pink transparent liquid under waxy white fraction | 1920 µL (20 µL in each) | 96 individual tubes |
| Taq-polymerase solution | Colorless transparent liquid | 1 mL (500 µL in each tube) | 2 tubes |
| Mineral oil | Colorless transparent viscous oily liquid | 2.0 mL (1.0 mL in each tube) | 2 tubes |
| Positive control[1] | Colorless transparent liquid | 130 µL | 1 tube |

[1] - can be marked as C+

Table 4. The **Toxoplasma gondii REAL-TIME PCR Detection Kit** content, package U, for R1-P031-UA/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| PCR-mix | Colorless or pink transparent liquid | 600 µL | 1 tube |
| TechnoTaq MAX polymerase | Colorless transparent viscous liquid | 30 µL | 1 tube |
| PCR-buffer | Colorless transparent liquid | 600 µL | 1 tube |
| Positive control[1] | Colorless transparent liquid | 130 µL | 1 tube |

All components are ready to use and do not require additional preparation for operation.

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** (package S) is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** (package U) is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

## 4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

### 4.1. Specimen collection

- Sterile containers to collect biomaterial;
- For blood collection: 2.0 - 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

### 4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator with freezer;
- Vortex mixer;
- High speed centrifuge for 1.5 mL tubes (RCF(g) no less than 12,000);
- Solid-state thermostat (temperature range 50-65 °C);
- RNase and DNase free 1.5 mL tubes with caps;
- Tube rack for 1.5 mL tubes;
- Electric laboratory aspirator with trap flask to remove supernatant;
- RNase and DNase free pipette tips for electric laboratory aspirator;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL; 1,000 µL);
- Pipette stand;
- Nucleic acid extraction kit ("DNA-Technology" made, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU, **PREP-MB RAPID** REF P-116-A/8EU, **PREP-RAPID** REF P-001/1EU, **PREP-MB MAX** REF P-103-N/4EU, P-103-A/8EU, **PREP-OPTIMA** REF P-016-1/2EU, REF P-016-N/2EU are recommended);
- Physiological saline solution 0.9% NaCl (sterile);

- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves
- Transport medium (if necessary);
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator with freezer;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips (in case of using package S, strips);
- tube rack for 0.2 mL tubes;
- PCR tube rack for 0.2 mL strips;
- tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL)
- RNase and DNase free tips for semi-automatic pipettes (volume 200 µL; 1,000 µL);
- Pipette stand;
- RNase and DNase free 1.5 mL microfuge tubes with caps;
- 0.2 mL PCR tubes or a 96-well microplate (only for manual dosing, not used for DTlite detecting thermal cycler);
- DTstream *M1 dosing device (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream *M1 dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- 384-well microplate (only for automated dosing in case of using package U);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Transport medium (if necessary, STOR-F manufactured by DNA-Technology is recommended);
- Sterile physiological saline solution (0.9% NaCl) (if necessary).

Post-amplification – amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

## 5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

### 5.1. Transport and storage conditions. Package S

- All components of the reagent kit shall be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- Paraffin sealed PCR-mix shall be kept away from light.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

### 5.2. Transport and storage conditions. Package U

- All components of the reagent kit, except for TechnoTaq MAX polymerase, shall be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- PCR-mix shall be kept away from light.
- TechnoTaq MAX polymerase shall be stored in a freezer at the temperature from minus 22 °C to minus 18 °C throughout the shelf life of the kit.
- Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.

**WARNING!** Reagent kits transported with violation of temperature conditions shall not be used.

### 5.3. Shelf-life of the kit following the first opening of the primary container

#### 5.3.1. Package S

- All components of the kit should be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C during the storage period
- Paraffin sealed PCR-mix should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period

#### 5.3.2. Package U

- All components of the kit, except for TechnoTaq MAX polymerase, should be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- TechnoTaq MAX polymerase should be stored in a freezer at temperatures from minus 22 °C to minus 18 °C during the storage period.

**WARNING!** The kits stored under undue regime should not be used.

An expired **Toxoplasma gondii REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Toxoplasma gondii REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

## 6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA**.** Avoid direct contact with the biological samples reagents and materials used to carry out the analysis. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Work surfaces, as well as rooms where PCR is performed, must

be irradiated with bactericidal irradiators for 30 minutes before and after the work. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

**Emergency actions**

**Inhalation:** Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

**Eye Contact:** If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

**Skin Contact:** If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

**Ingestion:** If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.
Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

## 7. SAMPLES

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, cerebrospinal fluid, amniotic fluid, biopsy specimens or punctate from lesions of organs and tissues.

### 7.1. Interfering substances

The presence of PCR inhibitors in a sample may cause doubtful (uncertain/unreliable) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product amplification.

PCR inhibitors include the following substances: hemoglobin and medicaments present in the DNA sample as a result of incomplete removal from the sample, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of wash solutions during sample preparation. Maximum concentrations of interfering substances at which amplification of laboratory and internal controls were not influenced: hemoglobin — 0.35 mg/mL of DNA sample, isopropyl alcohol — 100 µL/mL of DNA sample, methyl acetate — 100 µL/mL of DNA sample.
To assess possible interference of medicaments, those potentially residual in human biomaterial samples taken from the corresponding biotopes were selected (Miramistin®, chlorhexidine bigluconate).
For all the studied medicaments, the absence of their influence in concentration up to 10% in biomaterial sample was found.

### 7.2. General requirements

PCR analysis is a direct method of laboratory analysis, thus human biomaterial sample collection must be performed from the lesion of infection. The decision on studying a lesion shall be taken based on the collected information.

Quality of biomaterial sample collection, its transport, storage and pretreatment are crucial for obtaining correct results.

If biomaterial must be taken from several biotopes, repeat the procedure taking material into a new tube each time.

Incorrect biomaterial sampling may lead to unreliable results and, thus, to the necessity of repeated sampling.

At the biomaterial preparation stage use single-use RNase and DNase free filter tips.

To prevent contamination, only open the cap of the tube where biomaterial is introduced, and close it before proceeding to the next tube.

### 7.3. Sample collection

**WARNING!** Before DNA extraction, preparation of biological material samples may be needed.

**Peripheral blood**

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

**Method limitations:** intravenous heparin injections, infusions of parenteral nutrition less than 6 hours before the analysis.

**Cerebrospinal fluid, amniotic fluid, feces, meconium**

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

**Biopsy specimens/punctate**

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

**If using PREP-NA and PREP-GS reagent kits for DNA extraction:**

Collect samples into 1.5 mL tubes with transport medium designed for transport and storage of PCR biomaterial samples.

### 7.4. Transport and storage of the samples

Transport and storage conditions for biomaterial samples are determined by instructions for use to NA extraction reagent kits or to transport media used for transport and storage of samples.

It is allowed to store biomaterial samples at temperature from 2 °C to 8 °C for up to 24 hours. If it is not possible to deliver material to the laboratory within 24 hours, a one-time freezing of material is allowed. It is allowed to store frozen material at temperature from minus 22 °C to minus 18 °C for one month (if it aligns with the requirements for the used NA extraction reagent kits or transport media).

**WARNING!** Avoid repeated freezing and thawing of samples.

### 7.5. Sample preparation for DNA extraction

#### 7.5.1. Peripheral blood, cerebrospinal fluid, amniotic fluid

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

#### 7.5.2. Biopsy specimens/punctate

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

**If using PREP-NA and PREP-GS reagent kits for DNA extraction:**

7.5.3.1. Vortex the tubes with samples for 3-5 seconds, then spin on vortex for 3-5 seconds.

7.5.3.2. Remove supernatant.

The sample is ready for DNA extraction.

## 8. PROCEDURE

**DNA extraction from biological material**

It is recommended to use extraction reagent kits designed for the corresponding types of biomaterial for further PCR, for example, PREP-NA, PREP-GS, PREP-MB RAPID, PREP-RAPID, PREP-OPTIMA, PREP-MB MAX (see Table 5).

Table 5. Reagent kits recommended for DNA extraction and further analysis using Toxoplasma gondii reagent kit

| Reagent kit | Biomaterial | Minimum eluate volume, µL |
|---|---|---|
| PREP-NA | Cerebrospinal fluid, biopsy specimens, punctate | 50 |
| PREP-GS | Cerebrospinal fluid, biopsy specimens, punctate | 100 |
| PREP-MB RAPID | Cerebrospinal fluid, amniotic fluid | 100 |
| PREP-RAPID | Cerebrospinal fluid | 500 |
| PREP-OPTIMA | Whole peripheral blood[2] | 100 |
| | Cerebrospinal fluid, amniotic fluid, biopsy specimens, punctate | 400 |
| PREP-MB MAX | Whole peripheral blood | 50 |

DNA extraction is performed in accordance with instruction for use to the reagent kit.

**WARNING!**

1. Simultaneously with DNA extraction from biological material a negative control must be prepared and carried through all the stages of sample preparation. Physiological saline solution can be used as a negative control in volumes as indicated in the instructions for use of extraction kits or negative control that is included in the corresponding extraction kit.
2. Perform DNA extraction from biopsy specimens/punctate using PREP-NA and PREP-GS reagent kits according to **this instruction**.

### 8.1. DNA extraction from biopsy specimens/punctate using PREP-NA and PREP-GS reagent kits

#### 8.1.1. General requirements

8.1.1.1. Use RNase and DNase free filter tips. Change the tips after each solution removal from the tube. When working with aspirator, use RNase and DNase free tips without filter.

8.1.1.2. When adding solution into the tube containing biomaterial, add the solution carefully without touching the walls of the tube. If touching occurs, change the tip.

8.1.1.3. To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube. It is prohibited to work with several tubes with open caps simultaneously.

[2] - only for PREP-OPTIMA MAX extraction kit

8.1.1.4. Test samples and negative control (C-) must be treated in the same manner simultaneously according to this instruction.

**8.1.2. DNA extraction from biopsy specimens/punctate using PREP-NA reagent kit**

**WARNING!**

1. Before starting work:
   - turn on the thermostat and heat it to 65 °C
   - take the nucleic acid extraction kit out of the refrigerator and check if there is precipitate in lysis solution. If there is precipitate, heat the lysis solution at 65 °C on the preheated thermostat until complete dissolution of the precipitate, then stir by turning the vial upside down 5-10 times. Avoid foaming. Before use cool the solution down to room temperature (18-25 °C). The precipitate may also dissolve at room temperature (18-25 °C) in the course of approximately 12 hours.
2. Cap opening may occur during heating! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp lid (e.g. solid-state thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

8.1.2.1. Mark one 1.5 mL plastic tube for each test sample and for negative control (C-).

8.1.2.2. Add 300 µL of lysis solution into the tubes with prepared biopsy specimens/punctate samples and into the tube with C-. Avoid touching the walls of the tubes.

8.1.2.3. Add 100 µL of negative control into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.1.2.4. Heat the tubes on thermostat at 65 °C for 30 minutes and spin on vortex for 3-5 seconds.

8.1.2.5. Transfer supernatant into the corresponding marked tubes with test samples. Do not transfer supernatant from the C- tube.

8.1.2.6. Add 400 µL of precipitation reagent into each tube. Avoid touching the walls of the tubes. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.1.2.7. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 15 minutes.

8.1.2.8. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.9. Add 500 µL of washout solution №1 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and turn the tubes upside down carefully 3-5 times.

8.1.2.10. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.1.2.11. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.12. Add 300 µL of washout solution №2 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and turn the tubes upside down carefully 3-5 times.

8.1.2.13. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 5 minutes.

8.1.2.14. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.1.2.15. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

8.1.2.16. Add 50 µL of dilution buffer to the precipitate. Close the tubes.

8.1.2.17. Spin down the drops on vortex for 1-3 seconds.

8.1.2.18. Heat the tubes on thermostat at 65 °C for 10 minutes and shake on vortex for 3-5 seconds

8.1.2.19. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 30 seconds.

DNA preparation is ready for introduction into the PCR reaction mixture.

DNA preparation may be stored at temperature from minus 22 °C to minus 18 °C for up to one month and at temperature from minus 72 °C to minus 68 °C for up to one year.

Before using DNA preparation for PCR thaw the tubes with DNA preparation at room temperature (18 °C — 25 °C) or at temperature from 2 °C to 8 °C, shake on vortex for 3-5 seconds and spin for 1-3 seconds.

**WARNING!** Only one thawing of DNA preparation is allowed!

DNA preparation is ready for introduction into the PCR reaction mixture.

### 8.2. DNA extraction from biopsy specimens/punctate using PREP-GS reagent kits

**WARNING!** Before starting work turn on the thermostat and heat it to 50 °C.

8.2.1.1. Mark one 1.5 mL plastic tube for each test sample and for negative control (C-).

8.2.1.2. Add 150 µL of lysis solution into the tubes with prepared biopsy specimens/punctate samples and into the tube with C-. Avoid touching the walls of the tubes.

8.2.1.3. Add 50 µL of sterile physiological saline solution or transport medium into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

8.2.1.4. Heat the tubes on thermostat at 50 °C for 30 minutes and spin on vortex for 3-5 seconds.

8.2.1.5. Transfer supernatant into the corresponding marked tubes with test samples. Do not transfer supernatant from the C- tube.

8.2.1.6. Resuspend the sorbent thoroughly on vortex. Turn the tube upside down to make sure that the sorbent does not stick to the bottom of the tube.

8.2.1.7. Add 20 µL of previously resuspended sorbent into each tube (including C-). Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.1.8. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.1.9. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.1.10. Add 200 µL of washout solution №1 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.1.11. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.1.12. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.1.13. Add 200 µL of washout solution №2 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.1.14. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.1.15. Add 200 µL of washout solution №3 to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3-5 seconds.

8.2.1.16. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

8.2.1.17. Using a new tip for each tube, remove supernatant completely. Do not touch the precipitate.

8.2.1.18. Open the tubes and dry the precipitate at 50 °C for 5 minutes.

8.2.1.19. Add 100 µL of elution solution to the precipitate. Avoid touching the walls of the tubes. Close the tubes and shake on vortex for 5-10 seconds.

8.2.1.20. Heat the tubes at 50 °C for 5 minutes.

8.2.1.21. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

Supernatant containing the extracted DNA is ready for introduction into the PCR reaction mixture. If the sample will be stored for longer than 7 days, transfer supernatant into a separate tube.

The obtained DNA preparation may be stored for up to 7 days at temperature from 2 °C to 8 °C. Before using DNA preparation for PCR repeat 8.2.1.20 — 8.2.1.21.

If you plan to store the sample for more than 7 days, transfer the supernatant to a separate tube and store it at temperature from minus 22 °C to minus 18 °C.

DNA preparation may be stored at temperature from minus 22 °C to minus 18 °C for up to 6 months.

If after extraction supernatant containing the extracted DNA was transferred into new tubes, before using DNA preparation for PCR thaw the tubes with DNA preparation and negative control at room temperature (18 °C — 25 °C) or at temperature from 2 °C to 8 °C, shake on vortex for 3-5 seconds and spin for 1-3 seconds.

**WARNING!** Only one thawing of DNA preparation is allowed!

DNA preparation is ready for introduction into the PCR reaction mixture.

### 8.3. Preparing PCR for package S

**WARNING!**

1. The reagents and tubes should be kept away from direct sun light.
2. Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.3.1. Mark one tube/stripped tube with the paraffin sealed PCR-mix for each test sample, negative control (C-), positive control (C+).

**WARNING!** The volume of reagents is calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

**Example**: To test 4 test samples, mark 4 tubes for test samples, one negative control tube "C-" and one positive control tube "C+". Total number of tubes is 6.

8.3.2. Shake the tubes with Taq-polymerase solution on vortex mixer for 3-5 seconds and spin in a vortex mixer for 1-3 seconds.

8.3.3. Add 10 µL of Taq-polymerase solution to each tube. Avoid paraffin layer break.

**WARNING!** If using Rotor-Gene Q detecting thermal cycler, do not add mineral oil!

8.3.4. Add one drop (~20 µL) of mineral oil into each strip tube (if necessary). Loosely cover the tubes/strips with caps.

8.3.5. Shake the tube with positive control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.

**WARNING!**

1. Before introducing DNA preparation and negative control into tubes with PCR-mix for DNA preparation and negative control, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kits, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

8.3.6. Add 5.0 µL of DNA sample into the corresponding tubes. Do not add DNA into the tubes "C-" and "C+". Avoid paraffin layer break.

8.3.7. Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube. Avoid paraffin layer break.

8.3.8. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.3.9. Spin the tubes/strips for 3-5 seconds in a vortex mixer (if using Rotor-Gene Q, spinning is not necessary).

8.3.10. Set the strips into the real-time thermal cycler.

8.3.11. Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.9) and run PCR. See Table 6.

8.3.12. For Rotor-Gene Q, CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 7, 8, 9.

Table 6. The PCR program for DTlite and DTprime thermal cyclers

| **Step** | **Temperature, °C** | **Min.** | **Sec.** | **Number of cycles** | **Optical measurement** | **Type of the step** |
|---|---|---|---|---|---|---|
| 1 | 80 | 0 | 30 | 1 | | Cycle |
| | 94 | 1 | 30 | | | |
| | | | | | | |
| 2 | 94 | 0 | 30 | 5 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 3 | 94 | 0 | 10 | 45 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 4 | 94 | 0 | 5 | 1 | | Cycle |
| | | | | | | |
| 5 | 25[5] | | … | Holding | | Holding |
| √ - optical measurement | | | | | | |

Table 7. Amplification program for Rotor-Gene Q detecting thermal cycler (package S, tubes)

| Cycling | Temperature, °C | Hold Time, s | Cycle Repeats |
|---|---|---|---|
| Cycling | 80 deg | 60 | 1 time |
| | 94 deg | 90 | |
| Cycling 2 | 94 deg | 30 | 5 times |
| | 57 deg √ | 15 | |
| Cycling 3 | 94 deg | 10 | 45 times |
| | 57 deg √ | 15 | |
| √- optical measurements, set up fluorescence measurement (Acquiring) on Green (Fam) and Yellow (Hex) detection channels at 57 °C. | | | |

Table 8. The PCR program for CFX96 thermal cyclers (packages S, U)

| Step | Temperature, °C | Time min: sec | Number of cycles (repeats) |
|---|---|---|---|
| 1 | 80 | 01:00 | 1 |
| 2 | 94 | 01:30 | 1 |
| 3 | 94 | 0:15 | 50 |
| 4 | 64 **√** | 0:20 | |

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

[5] Holding at 10 °C is allowed

| |
|---|
| √- optical measurements (Plate Read), set the fluorescence measurement on the Fam and Hex channels at 64 °C |

Table 9. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

| Stage | Step | Temperature, °C | Time min: sec | Number of cycles (repeats) |
|---|---|---|---|---|
| Holding | 1 | 80 | 01:00 | 1 |
| | 2 | 94 | 01:30 | 1 |
| PCR | 1 | 94 | 0:20 | 50 |
| | 2 | 64 **√** | 0:20 | |
| √- data collection for fluorophores (Fam, Vic (Hex)) is on | | | | |

### 8.4. Preparing PCR for package U, manual dosing

**WARNING!** For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[6], sealed hermetically with thermal film. It is not recommended to use strips due to increased risk of postamplification contamination.

**WARNING!** The reagents and tubes should be kept away from direct sunlight.

8.4.1 Mark the required number of 0.2 mL tubes or a 96-well PCR microplate for each test sample, negative control (C-) and positive control (C+).

Note: It is recommended to test at least 5 samples per analysis (3 test samples, negative control and positive control).

**Example:** to test 4 samples, mark 4 tubes/reserve 4 wells for samples, 1 tube/well for "C-" and 1 tube/well for "C+". The resulting number of tubes/wells is 6.

8.4.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.4.3 Add 6.0 µL of PCR-mix to each tube/well (including "C-" and "C+").

8.4.4 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

**WARNING!** Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.4.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:

6.0 x (N+1) µL of PCR-buffer,

0.3 x (N+1) µL of TechnoTaq MAX polymerase,

where N is the number of marked tubes/microplate wells considering "C-", "C+".

**Example:** to test 4 samples, "C-" and "C+" in one PCR run, prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes/wells, i.e. 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.4.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin on vortex for 1-3 seconds to collect the drops.

**WARNING!** Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.4.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube/well with PCR-mix. Loosely cover the tubes.

[6] - 96-well plates are not used with DTlite detecting thermal cycler

**WARNING!** Follow the steps 8.4.8 – 8.4.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.4.8 Vortex the tube with positive control "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

**WARNING!**

1. Before introducing DNA preparation and negative control into tubes/wells with PCR-mix for DNA preparation and negative control, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kits, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. Close the tubes/strips tightly. Use filter tips.

8.4.9 Add 6.0 µL of DNA sample into corresponding tubes/wells. Do not add DNA into the "C-", "C+" tubes/wells.

8.4.10 Add 6.0 µL of negative control ("C-") which passed whole DNA extraction procedure into the corresponding tube/well.

8.4.11 Add 6.0 µL of positive control sample ("C+") into the corresponding tube/well.

8.4.12 **In case of using 96-well PCR microplates:**

8.4.12.1. Place the PCR microplate carefully, without shaking into the DTpack sealing device.

8.4.12.2. Seal the PCR microplate with polymer thermal film according to the DTpack operation manual.

8.4.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.4.13 **In case of using tubes:**
Spin the tubes for 3-5 seconds on vortex mixer (in case of using Rotor-Gene Q, spinning is not necessary).

8.4.14 Set the tubes into the real-time thermal cycler.

8.4.15 Launch the operating software for DT instrument[7]. Add corresponding test[8], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 10.

8.4.16 For Rotor-Gene Q, CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Tables 7, 8, 11.

[7] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.
[8] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 10. The PCR program for DTlite and DTprime thermal cyclers for package U

| Step | Temperature, °C | Min | Sec | Number of cycles | Optical measurement | Type of the step |
|---|---|---|---|---|---|---|
| 1 | 80 | 0 | 05 | 15 | | Cycle |
| | 94 | 0 | 05 | | | |
| | | | | | | |
| 2 | 94 | 5 | 00 | 1 | | Cycle |
| | | | | | | |
| 3 | 94 | 0 | 30 | 5 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 4 | 94 | 0 | 10 | 45 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 5 | 94 | 0 | 5 | 1 | | Cycle |
| | | | | | | |
| 6 | 25* | | | Holding | | Holding |
| √ - optical measurements<br>* - holding at 10 °C is allowed | | | | | | |

Table 11. Amplification program for Rotor-Gene Q detecting thermal cycler (package U)

| № /Cycling | Temperature, °C | Hold Time, s | Cycle Repeats |
|---|---|---|---|
| Cycling | 80 deg | 60 | 1 time |
| | 94 deg | 300 | |
| Cycling 2 | 94 deg | 30 | 5 times |
| | 57 deg √ | 15 | |
| Cycling 3 | 94 deg | 10 | 45 times |
| | 57 deg √ | 15 | |
| √- optical measurements, set up fluorescence measurement (Acquiring) on Green (Fam) and Yellow (Hex) detection channels at 57 °C. | | | |

### 8.5. Preparing PCR for package U, using DTstream

**WARNING!** The reagents and tubes should be kept away from direct sun light.

**WARNING!** For amplification use 384-well microplates hermetically sealed with thermal film.

Note – It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.5.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.5.2 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

**WARNING!** TechnoTaq MAX polymerase should be taken out from the freezer immediately prior to use.

8.5.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.5.4 Vortex the tube with the mixture PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds,

then spin for 1-3 seconds to collect the drops.

8.5.5 Vortex the tubes with positive control for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

**WARNING!**

1. Before dosing of DNA preparation and negative control, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using PREP-NA, PREP-RAPID and PREP-GS extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using PREP-MB RAPID and PREP-MB MAX DNA extraction kits, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

8.5.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microplate on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.5.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the working table of DTpack sealing device.

8.5.8 Run the process of sealing of PCR microplate according to the user manual of DTpack sealing device.

8.5.9 Centrifuge the microplate at RCF(g) 100 for 30 seconds.

8.5.10 Set the PCR microplate into the real-time thermal cycler.

8.5.11 Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and ID's of the samples, positive and negative controls. Specify position of the tubes in thermal unit and run PCR. The volume of reaction mixture is 18 µL. See Table 10.

## 9. CONTROLS

The **Toxoplasma gondii REAL-TIME PCR Detection Kit** contains positive control ("C+").

Positive control is a cloned part of the bacterial genome. It is produced with genetic engineering techniques and characterized by automatic sequencing.

To reveal possible contamination a negative control is required.

**WARNING!** A negative control should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

## 10. DATA ANALYSIS

[9] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[10] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

**10.1.** Registration of fluorescence signal on DT, Rotor-Gene Q, CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers is performed automatically during amplification.

**10.2.** Recording of amplification results is carried out automatically during amplification by the software provided with detecting thermocycler.

**10.3.** When using CFX96 (Bio-Rad) detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the "Baseline Subtraction" tab select "Baseline Subtraction Curve Fit".

**10.4.** Result interpretation is carried out according to Table 12. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 12. PCR results interpretation

| **Fam/Green (sample DNA)**, Cp/Cq/Ct | **Hex/Yellow/Vic (internal control)**, Cp/Cq/Ct | **Result interpretation** |
|---|---|---|
| **Test samples** | | |
| **Specified** | Not considered | ***Toxoplasma gondii* DNA detected** |
| Not specified | **Specified** | *Toxoplasma gondii* not detected |
| Not specified | Not specified | Unreliable result |
| **Negative control** | | |
| Not specified | **Specified** | **Negative result** Run results are valid |
| **Positive control** | | |
| **Specified** | Not considered | **Positive result** Run results are valid |

**10.5.** Unreliable result may be due to the presence of inhibitors in the DNA preparation obtained from biomaterial; wrong performance of analysis protocol; noncompliance with amplification temperature mode etc. In this case a repeated analysis with the same DNA preparation, or repeated DNA extraction and amplification, or resampling (performed sequentially) shall be performed.

**10.6.** If a positive result is obtained for a negative control, the results of the whole run are considered invalid. In this case, special measures are necessary to identify and eliminate possible contamination.

**10.7.** If a negative result is obtained for a positive control, the results of the whole run are considered invalid. In this case, a repeated amplification run of the whole batch of samples is necessary.

## 11. SPECIFICATIONS

### a. Analytical specificity

In the human biomaterial samples containing DNA of *Toxoplasma gondii*, the PCR detector software registers positive amplification result for specific product (*Toxoplasma gondii* genome fragment) on Fam/Green detection channel.

In the human biomaterial samples not containing DNA of *Toxoplasma gondii*, the PCR detector software or the thermal cycler software registers negative amplification result for specific product (*Toxoplasma gondii* genome fragment) on Fam/Green detection channel and positive amplification result for internal control on Hex/Yellow/Vic detection channel.

Non-specific positive amplification results are absent if the following microorganisms are present in the DNA sample: *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Ureaplasma parvum*, *Neisseria gonorrhoeae*, *Candida albicans* and human DNA in concentration up to $1.0\times10^8$ copies/mL of sample.

**b. Analytical sensitivity**

5 copies of *Toxoplasma gondii* DNA per amplification tube.

Analytical sensitivity, or LOD (limit of detection) is determined by the analysis of serial dilutions of the laboratory controls (LC).

LOD corresponds to the following DNA concentration values for the assigned DNA extraction reagent kits and the final elution volume of the extracted DNA:

| Biomaterial | Name of DNA extraction kit | Preparation volume, µL | LOD, copies/sample |
|---|---|---|---|
| Cerebrospinal fluid (extraction from 500 µL of sample) | PREP-NA | 50 | 50 |
| | PREP-GS | 100 | 100 |
| | PREP-MB RAPID | 100 | 100 |
| | PREP-RAPID | 500 | 500 |
| Amniotic fluid (extraction from 500 µL of sample) | PREP-MB RAPID | 100 | 100 |
| Cerebrospinal fluid, amniotic fluid (extraction from 1.0 mL of sample) | PREP-OPTIMA | 400 | 400 |
| Biopsy specimens/punctate | PREP-NA | 50 | 50 |
| | PREP-GS | 100 | 100 |
| | PREP-OPTIMA | 400 | 400 |
| Whole peripheral blood (500 µL[11]) | PREP-OPTIMA MAX | 100 | 100 |
| Whole peripheral blood (100 µL) | PREP-MB MAX | 50 | 50 |

**c. Diagnostic characteristics**

| **Biomaterial** | **Diagnostic sensitivity** | | **Diagnostic specificity** | |
|---|---|---|---|---|
| | **Samples** | **Value (95 % CI)** | **Samples** | **Value (95 % CI)** |
| Blood | 25 | 100 % (86.28 % – 100 %) | 25 | 100 % (86.28 % – 100 %) |
| Biopsy specimens/punctate from the lesions of organs and tissues | 30 | 100 % (88.43 % – 100 %) | 50 | 100 % (92.89 % – 100 %) |
| Cerebrospinal fluid | 25 | 100 % (86.28 % – 100 %) | 25 | 100 % (86.28 % – 100 %) |
| Amniotic fluid | 25 | 100 % (86.28 % – 100 %) | 25 | 100 % (86.28 % – 100 %) |
| **Total** | 105 | 100 % (96.55 % – 100 %) | 125 | 100 % (97.09 % – 100 %) |

[11] - if adding 100 µL of lysis solution

### d. Precision and repeatability

Precision amounts to 100 %

Repeatability amounts to 100 %.

## 12. TROUBLESHOOTING

Table 13. Troubleshooting

| | Result | Possible cause | Solution |
|---|---|---|---|
| C+ | - | Operation error<br>PCR inhibition<br>Violation of storage and handling requirements | Repeat whole test<br><br>Dispose current batch |
| C- | + | Contamination | Dispose current batch<br>Perform decontamination procedures |
| IC | Invalid | PCR inhibition | Repeat whole test<br>Resample |

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

## 13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **Toxoplasma gondii REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

**Manufacturer:** "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

**Seller:** "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

## 14. KEY TO SYMBOLS

| | | | |
|---|---|---|---|
| RUO | For research use only |  | Date of manufacture |
|  | Temperature limit |  | Consult instructions for use |
|  | Contains sufficient for <n> tests | REF | Catalogue number |
|  | Use-by date |  | Manufacturer |
| LOT | Batch code |  | Keep away from sunlight |
|  | Non-sterile | VER | Version |

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