



For *in Vitro* Diagnostic Use

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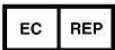
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AmpliSens® *Toxoplasma gondii*-FRT

PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens[®] Toxoplasma gondii-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Toxoplasma gondii* DNA in the clinical material (peripheral blood, umbilical cord blood, white cells of peripheral or umbilical cord blood, biopsy and autopsy material, cerebrospinal fluid, amniotic fluid) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION

Toxoplasma gondii detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region by using special primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens[®] Toxoplasma gondii-FRT** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF) that activates by heating at 95°C for 15 min.

Toxoplasma gondii DNA detection in clinical samples includes:

- a) Total DNA isolation from white blood cells of peripheral and umbilical cord blood, biopsy and autopsy material, cerebrospinal fluid, and amniotic fluid simultaneously with the exogenous Internal Control.
- b) Multiplex real-time PCR of DNA fragment of the non-structural repetitive gene (529 bp length) encoding *Toxoplasma gondii* protein and cloned λ -phage used as the noncompetitive exogenous Internal Control.

Toxoplasma gondii DNA amplification is detected in the **JOE/Yellow/HEX** channel, noncompetitive exogenous **Internal Control** amplification is detected in the **FAM/Green** channel.

The exogenous Internal Control allows controlling of the main steps of the PCR analysis (DNA extraction, amplification). The main advantage of the noncompetitive exogenous Internal Control is extension of linear detection range and therefore analytical sensitivity of the test.

3. CONTENT

AmpliSens[®] Toxoplasma gondii-FRT PCR kit is produced in 1 form:

AmpliSens[®] *Toxoplasma gondii-FRT* PCR kit variant FRT-50 F (for use with RG, iQ, Mx) **REF** R-P1(RG,iQ,Mx)-E.

AmpliSens[®] Toxoplasma gondii-FRT PCR kit variant FRT-50 F includes:

| Reagent | Description | Volume (ml) | Quantity |
|--|-------------------------|-------------|----------|
| PCR-mix-1-FRT <i>Toxoplasma gondii</i> | colorless, clear liquid | 0.6 | 1 tube |
| PCR-mix-2-FRT | colorless, clear liquid | 0.3 | tube |
| Polymerase (TaqF) | colorless, clear liquid | 0.03 | 2 tubes |
| Positive Control DNA <i>Toxoplasma gondii</i> and STI (C+) | colorless, clear liquid | 0.1 | 1 tube |
| DNA-buffer | colorless, clear liquid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless, clear liquid | 1.2 | 1 tube |
| Internal Control STI-87 (IC)** | colorless, clear liquid | 1.0 | 1 tube |

* must be used in the isolation procedure as Negative Control of Extraction.

** add 10 μ l of Internal Control STI-87 during the DNA isolation procedure directly to the sample/lysis mixture (see “RIBO-prep”, **REF** K1-9-Et-50-CE, “DNA-sorb-C” **REF** K1-6-50-CE protocols).

AmpliSens[®] *Toxoplasma gondii-FRT* PCR kit is intended for 60 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (up to 200 μ l)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ iCycler (BioRad, USA) Instrument; Mx3000P/Mx3005P (Stratagene, USA) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for 2–8 °C
- Deep-freezer with temperature below minus 16°C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING



Obtaining of biological material samples for PCR-analysis, transportation, and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Toxoplasma gondii*-FRT PCR kit is intended to analyze DNA extracted with DNA isolation kits from:

- *Whole peripheral and umbilical cord blood*
- *White cells of peripheral or umbilical cord blood*
- *Biopsy and autopsy material*

- *Cerebrospinal fluid*
- *Amniotic fluid*

6.1. *Whole peripheral and umbilical blood*. Blood should be collected in a tube with 6% EDTA solution at a ratio 20:1 (20 portions of blood per 1 portion of EDTA) after overnight fasting. Umbilical cord blood is obtained by cordocentesis. Invert the tube several times to ensure proper mixing.



Do not freeze the whole blood samples!

6.2. *White blood cells* taken from peripheral and/or umbilical cord blood are to be treated with "Hemolytic" REF 137. To do this, add 1.0 ml of "Hemolytic" and 0.25 ml of whole blood in a 1.5 ml tube. Vortex. Centrifuge (2 min, 8,000 rpm). Remove the supernatant, leaving 100 µl of liquid over the sediment. Cells sediment should be white after washing. Presence of little pink deposit is acceptable.



Add 300 µl of Solution for Lysis to the tube with the obtained leukocyte sample (for RIBO-prep protocol).

6.3 *Biopsy and autopsy material* is obtained from the expected location of the pathogen, from the damaged tissue or from the area adjoining with the damaged tissue. Collect the samples in a 2 ml tube with 0.3 ml of transport medium.

Transfer the sample to the porcelain mortar; add equal volume of saline solution or PBS-buffer. Thoroughly homogenize the specimen by porcelain pestle. Remove 100 µl aliquot and transfer to the sterile tube for DNA extraction.

6.4. *Cerebrospinal fluid* should be obtained in accordance with standard procedure and collected in a sterile tube like Eppendorf.

6.5. *Amniotic fluid* should be obtained during amniocentesis in accordance with standard procedure and collected in a sterile tube like Eppendorf. Thoroughly resuspend obtained sample and transfer 1 ml of it in a new sterile tube. Centrifuge the tube at 8,000-9,000 g for 10 min. Remove the supernatant leaving 200 µl of the fluid over the pellet. Use the tips with aerosol barrier. Resuspend the pellet.

7. PROTOCOL

7.1. DNA Isolation

It's recommended that the following nucleic acid extraction kits are used:

| Extraction kit | REF | Clinical material for DNA extraction |
|----------------|---------------|---|
| RIBO-prep | K2-9-Et-50-CE | Whole peripheral and umbilical cord blood White cells of peripheral or umbilical cord blood Cerebrospinal fluid Amniotic fluid |
| DNA-sorb-C | K1-6-50-CE | Biopsy and autopsy material |

7.2. Preparing the PCR

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

7.2.1 Preparing tubes for PCR

1. Prepare the **reaction mix**. Per **one** reaction mix:

- **10 µl PCR-mix-1-FRT *Toxoplasma gondii***
- **5.0 µl PCR-mix-2-FRT**
- **0.5 µl polymerase (TaqF)**

Refer to Appendix 1 for calculation of reaction volumes. Take into account that the analysis should include two control points: Positive and Negative Controls of Amplification (C+, NCA, respectively).

2. Prepare required number of tubes or stripes for amplification of DNA of clinical and control samples.
3. Add **15 µl** of prepared reaction mix into each tube.
4. Using tips with aerosol barrier **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.
5. Carry out **control amplification reactions**:

NCA - Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

C+ Add 10 µl of **Positive Control DNA *Toxoplasma gondii* and STI** to the tube for Positive Control of Amplification (C+).

7.2.2. Amplification

Program the Real-time instrument according to the manual provided by the manufacturer.

AmpliSens-1 amplification program for rotor-type instruments¹

| Step | Temperature, °C | Time | Fluorescence detection | Repeats |
|----------|-----------------|--------|------------------------|---------|
| Hold | 95 | 15 min | – | 1 |
| Cycling | 95 | 5 sec | – | 5 |
| | 60 | 20 sec | – | |
| | 72 | 15 sec | – | |
| Cycling2 | 95 | 5 sec | – | 40 |
| | 60 | 20 sec | FAM/Green, JOE/Yellow | |
| | 72 | 15 sec | – | |

See Appendix 2 for the settings.

AmpliSens-1 amplification program for plate-type instruments²

| Step | Temperature, °C | Time | Fluorescence detection | Repeats |
|------|-----------------|--------|------------------------|---------|
| 1 | 95 °C | 15 min | – | 1 |
| 2 | 95 °C | 5 sec | – | 5 |
| | 60 °C | 20 sec | – | |
| | 72 °C | 15 sec | – | |
| 3 | 95 °C | 5 sec | – | 40 |
| | 60 °C | 30 sec | FAM, HEX | |
| | 72 °C | 15 sec | – | |

See Appendices 3, 4 for the settings.

8. DATA ANALYSIS

Accumulation of ***Toxoplasma gondii* DNA** amplification product is detected in the **JOE/Yellow/HEX** channel, Internal Control amplification product is detected in the **FAM/Green** channel.

The results are interpreted by the crossing (or not) of the fluorescence curve with the threshold line.

See **Appendices 2, 3, 4** for data analysis settings.

The analysis results are considered valid, only if the control samples results comply with the following:

Results for controls

| Control | Stage for control | Ct in channel | | Interpretation |
|---------|-------------------|---------------|----------------|----------------|
| | | FAM/Green | JOE/Yellow/HEX | |
| C- | DNA isolation | ≤ X | Neg | OK |
| NCA | Amplification | Neg | Neg | OK |
| C+ | Amplification | ≤ Y | ≤ Z | OK |

*For X, Y, Z values see Appendices 2, 3, 4.

¹ For example, Rotor-Gene 3000/6000 (Corbett Research, Australia)

² For example, iQCyler, iQ5 (BioRad, USA); Mx3000P (Stratagene, USA)

1. The sample is considered positive if its Ct value detected in the FAM channel is less than **A** while Ct detected in the JOE/Yellow/HEX channel is less than **B**. Moreover, the fluorescence curve should represent typical sigmoid curve and once cross the threshold line at the region of reliable gain of fluorescence.
2. The sample is considered negative if its fluorescence curve does not cross the threshold line (Ct is absent) and does not represent the typical curve.

For **A, **B**, values see Appendices 2, 3, 4.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

9. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If any Ct value appears for the Negative Control of amplification (C-) in the JOE/Yellow and/or JOE/Yellow/HEX channel, it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
2. If Ct value is absent for Positive Control of amplification (C+) on JOE/Yellow/HEX channel and/or on FAM/Green the result is invalid for all samples. PCR should be repeated for all samples.
3. If Ct values detecting on FAM/Green channel (IC) are absent in clinical samples it indicates a failure of the extraction stage. For these samples the analysis should be repeated starting from the DNA extraction. If for clinical samples Ct value detecting on FAM/Green channel (IC) exceeds **A**** while Ct value detecting on JOE/Yellow/HEX channel (*Toxoplasma gondii*) exceeds **B**** the analysis should be repeated starting from the DNA extraction. High Ct values may occur due to the loss of DNA during extraction or because of inhibitors.
4. If Ct value of a clinical sample obtained on JOE/Yellow/HEX channel exceeds **B**** then the result is considered equivocal. It is necessary to repeat the analysis twice. In case of the reproducible positive Ct value, the sample is positive.

10. STABILITY AND STORAGE

All components of the **AmpliSens® Toxoplasma gondii-FRT** PCR kit (except for Polymerase(TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *Toxoplasma gondii* are to be stored between 2 and 8 °C. All components of the **AmpliSens® Toxoplasma gondii-FRT** PCR kit are to be stable until the expiry date stated on the label.



Polymerase (TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *Toxoplasma gondii* are to be stored at not more than minus 16 °C.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical sensitivity of **AmpliSens® Toxoplasma gondii-FRT** PCR kit is 400 *Toxoplasma gondii* DNA copies/ml.



The claimed analytical features of **AmpliSens® Toxoplasma gondii-FRT** PCR kit are guaranteed only when additional reagent kit (RIBO-prep or DNA-sorb-C) is used.

11.2. Specificity

Specificity of **AmpliSens® Toxoplasma gondii-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Toxoplasma gondii-FRT** PCR kit was confirmed in laboratory clinical tests.

12. REFERENCES

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Toxoplasma gondii-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number

IC

Internal Control



Contains sufficient
for <n> tests



Authorised representative
in the European
Community.



Consult instructions for use



Caution, consult
accompanying documents

RG

For working with
Rotor-Gene™ 3000/6000
(Corbett Research)

iQ

For working with
iQ5, iQiCycler
(Bio-Rad)

Mx

For working with Mx3000P or
Mx3005P (Stratagene)

NCA

Negative Control of
amplification