CE 1023

IVD

For Professional Use Only

AmpliSens® *Toxoplasma gondii*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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, and amniotic fluid) by means of real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

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

Toxoplasma gondii DNA detection in clinical samples includes:

(a) Total DNA extraction 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 a DNA fragment of a nonstructural repeated gene (529 bp long) encoding *Toxoplasma gondii* protein and an artificial DNA fragment cloned into phage λ, which is used as a noncompetitive exogenous Internal Control.

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

The exogenous Internal Control allows monitoring the main steps of PCR analysis (DNA extraction and amplification). The main advantage of a noncompetitive exogenous Internal Control is the extension of the linear detection range and, therefore, an increase in the 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)-CE.

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *Toxoplasma gondii*	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control DNA *Toxoplasma gondii* and STI (C+$_{T.gondii \text{ and STI}}$)	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 extraction procedure as Negative Control of Extraction.

** add 10 µl of Internal Control STI-87 during the DNA extraction 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 extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocycler (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia), iCycler iQ or iQ5 (BioRad, USA), Mx3000P/Mx3005P (Stratagene, USA)).

- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA)**.**
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 another suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucous membranes. If skin, eyes, or mucous membranes 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 DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. 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 extraction 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 to 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 to a 1.5-ml tube, vortex, and centrifuge (8,000 rpm, 2 min). Remove the supernatant leaving 100 µl of fluid over the sediment. Cell pellet should be white after washing. The presence of a small amount of a pinkish film-like pellet above the major part of cell pellet is allowed.

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 to a 2-ml tube with 0.3 ml of transport medium.

Transfer the sample to a porcelain mortar; add an equal volume of saline or PBS. Thoroughly homogenize the specimen with a porcelain pestle. Take a 100-µl aliquot and transfer to a sterile tube for DNA extraction.

6.4. *Cerebrospinal fluid* should be obtained by the standard procedure and collected to a sterile Eppendorf tube.

6.5. *Amniotic fluid* should be obtained during amniocentesis by the standard procedure and collected to a sterile Eppendorf tube. Thoroughly resuspend the obtained sample and transfer 1 ml of it to 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 tips with aerosol barrier. Resuspend the pellet.

7. WORKING CONDITIONS

AmpliSens® *Toxoplasma gondii*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is 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*

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the **reaction mixture.** Per **one** reaction mixture:
   - **10 µl** of **PCR-mix-1-FRT *Toxoplasma gondii***
   - **5.0 µl** of **PCR-mix-2-FRT**
   - **0.5 µl** of **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 the required number of tubes or strips for amplification of DNA from clinical and control samples.
3. Add **15 µl** of the prepared reaction mixture to each tube.
4. Using tips with aerosol barrier, **add 10 µl of DNA samples** obtained from clinical or control samples at the DNA extraction stage.
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+).

8.2.2. Amplification

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

AmpliSens-1 amplification program for rotor-type instruments[1]

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

For settings, see Guidelines.

AmpliSens-1 amplification program for plate-type instruments[2]

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

For settings, see Guidelines.

9. DATA ANALYSIS

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 software of the PCR instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

See **Guidelines** and ***Important Product Information Bulletin*** for data analysis settings.

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

[1] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia)
[2] For example, Cycler iQ, iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)

Results for controls

Control	Stage for control	Ct in channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
C-	DNA extraction	≤ boundary value	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	≤ boundary value	≤boundary value	OK

*For boundary Ct values, see ***Important Product Information Bulletin***.

1. The sample is considered **positive** if Ct values detected in the FAM/Green and JOE/Yellow/HEX channel are less than the boundary Ct values for these channels. The fluorescence curve should have a typical sigmoid shape and cross the threshold line in the region of significant fluorescence increase only once.
2. The sample is considered **negative** if its fluorescence curve does not cross the threshold line (Ct value is absent) and does not have the typical shape.

The results of analysis are considered reliable only if the results obtained for Positive and Negative Controls of Amplification as well as for the Negative Control of Extraction are correct.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If any Ct value appears for the Negative Control of amplification (C–) in the JOE/Yellow/HEX and/or FAM/Green channel, this indicates the contamination of reagents or samples. In this case, the results of analysis of all samples are considered **invalid**. It is necessary to repeat the analysis of all tests and to take measures to detect and eliminate the source of contamination.
2. If the Ct value is absent for the Positive Control of amplification (C+) in the JOE/Yellow/HEX and/or FAM/Green channel, the results are invalid for all samples. PCR should be repeated for all samples.
3. If Ct values in the FAM/Green channel (IC) are absent in clinical samples, this indicates improper DNA extraction. For these samples, analysis should be repeated starting from the DNA extraction stage. If, for clinical samples, the Ct value detected in the FAM/Green channel exceeds the specified boundary Ct value for IC and the Ct value detected in the JOE/Yellow/HEX channel (*Toxoplasma gondii*) exceeds the specified boundary Ct value, analysis should be repeated starting from the DNA extraction stage. High Ct values may be due to the loss of DNA during extraction or because of inhibitors.

4. If the Ct value of a clinical sample detected in the JOE/Yellow/HEX channel exceeds the specified boundary Ct value, the result is considered **equivocal**. It is necessary to repeat the analysis twice. If a positive Ct value is detected twice, the sample is considered as **positive**.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Toxoplasma gondii*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. 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 at 2–8 °C. All components of the **AmpliSens® *Toxoplasma gondii*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *Toxoplasma gondii* are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FRT *Toxoplasma gondii* is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The 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.

13.2. Specificity

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

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for 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.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**IC**	Internal control
Mx	For working with Mx3000P or Mx3005P (Stratagene)	**iQ**	For work with iCycler iQ and iQ5 (Bio-Rad)
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”