AmpliSens® *Toxoplasma gondii*-FRT
PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
	Caution		

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 (white blood cells of whole peripheral blood, autopsy material, cerebrospinal fluid, amniotic fluid) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Toxoplasma gondii DNA detection in clinical samples includes:

(a) Total DNA extraction from white blood cells of whole peripheral blood, autopsy material, cerebrospinal fluid, and amniotic fluid simultaneously with the exogenous Internal Control.

(b) Simultaneous amplification (multiplex PCR) of the 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. 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 measurement range and, therefore, an increase in the analytical sensitivity of the test.

Toxoplasma gondii detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Toxoplasma gondii* primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of 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 the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF), which is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control STI-87-rec (IC)	*Toxoplasma gondii*
Target gene	genetically engineered construction	rep529

3. CONTENT

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

variant FRT-50 F REF R-P1(RG,iQ,Mx)-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *Toxoplasma gondii*	clear liquid from colorless to light lilac colour	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* 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-rec (IC)** 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).

Variant FRT-50 F 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 filters (up to 100 and 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ or iCycler iQ5 (Bio-Rad, USA); Mx3000P or Mx3005P (Stratagene, USA).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
  b) 0.2-ml (for 36-well rotor) or 0.1-ml (for 72-well rotor) PCR tubes (flat caps, nonstriped) if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir 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 all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 compliance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, 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

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage is 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 for analysis of the DNA extracted with DNA extraction kits from the clinical material (white cells of whole peripheral blood, autopsy material, cerebrospinal fluid, and amniotic fluid).

6.1. *White blood cells* are obtained from whole peripheral blood. Blood should be collected into a tube with 6% EDTA solution at a ratio 20:1 (20 portions of blood per 1 portion of EDTA) after overnight fasting. Invert the tube several times to ensure proper mixing. Whole peripheral blood can be stored at 20-25 °C for 12 hour and at 2-8 °C for 1 day. Do not freeze the whole blood samples!

To obtain white blood cells, add 1.0 ml of **Hemolytic** REF 137-CE and 0.25 ml of whole blood to a 1.5-ml tube. Vortex carefully. Centrifuge at 8,000 rpm for 2 min. Remove the supernatant using vacuum aspirator and leaving 100 µl of liquid over the pellet. 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.

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

Lyzed leukocyte pellet can be stored at 2-8 °C for 1 day, and at the temperature from minus 24 to minus 16 °C if necessary to store it more than 1 day.

6.2 *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 into a 2-ml tube with 0.3 ml of transport medium.

The samples can be stored at room temperature for 6 hour, at 2-8 °C for 3 days, and at the temperature from minus 24 to minus 16 °C if necessary to store it more than 3 days.

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. The suspension can be stored at the temperature from minus 24 to minus 16 °C.

6.3. *Cerebrospinal fluid* should be obtained by the standard procedure and collected to a sterile Eppendorf tube. The cerebrospinal fluid can be stored at room temperature for 6 hour, at 2-8 °C for 1 day, at the temperature from minus 24 to minus 16 °C for a month, and at the temperature ≤68 °C for a long time.

6.4. *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 the material by a pipette with a filter tip into a new sterile tube. Centrifuge the tube at 8,000–9,000 g for 10 min. Carefully remove the supernatant using a filter tip and leaving 200 µl of the liquid over the pellet. Then, resuspend the pellet on vortex.

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 to use the following nucleic acid extraction kits:

- **RIBO-prep**, REF K2-9-Et-50-CE – for white cells of whole peripheral blood, cerebrospinal fluid, amniotic fluid.
- **DNA-sorb-C**, REF K1-6-50-CE – for autopsy material.

The DNA extraction of each test sample is carried out in the presence of **Internal Control STI-87 (IC).**

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

NOTE: Extract the DNA according to the manufacturer's protocol.

NOTE: In case of extracting with the **RIBO-prep** reagent kit, use 200-µl tips for removing supernatant after each washing. The volume of elution is **50 µl**.

8.2. Preparing the 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.** All components of the reaction mixture should be mixed immediately before use. Mix the reagents per **one** reaction:
   - **10 µl** of **PCR-mix-1-FRT *Toxoplasma gondii***
   - **5.0 µl** of **PCR-mix-2-FRT**
   - **0.5 µl** of **polymerase (TaqF)**

Calculate the reagents volumes for the required number of reactions, including test and control samples, according to the Table 2. Take into account that it is necessary to carry out two control reactions (Positive Control of Amplification (C+) and Negative Control of Amplification (NCA)) even for one test sample.

Table 2

Scheme of reaction mixture preparation

Total reaction volume - 25 µl Volume of reagents per 1 reaction - 15 µl Volume of DNA sample - 10 µl			
Number of test clinical samples including controls[1]	**PCR-mix-1-FRT *Toxoplasma gondii*, µl**	**PCR-mix-2-FRT, µl**	**Polymerase (TaqF), µl**
1	**40**	**20**	**2.0**
2	**50**	**25**	**2.5**
3	**60**	**30**	**3.0**
4	**70**	**35**	**3.5**
5	**80**	**40**	**4.0**
6	**90**	**45**	**4.5**
7	**100**	**50**	**5.0**
8	**110**	**55**	**5.5**
9	**120**	**60**	**6.0**
10	**130**	**65**	**6.5**
11	**140**	**70**	**7.0**
12	**150**	**75**	**7.5**
13	**160**	**80**	**8.0**
14	**170**	**85**	**8.5**
15	**180**	**90**	**9.0**
16	**190**	**95**	**9.5**
17	**200**	**100**	**10.0**
18	**210**	**105**	**10.5**
19	**220**	**110**	**11.0**
20	**230**	**115**	**11.5**
21	**240**	**120**	**12.0**
22	**250**	**125**	**12.5**
23	**260**	**130**	**13.0**
24	**270**	**135**	**13.5**
25	**280**	**140**	**14.0**
26	**290**	**145**	**14.5**
27	**300**	**150**	**15.0**
28	**310**	**155**	**15.5**
29	**320**	**160**	**16.0**
30	**330**	**165**	**16.5**

2. Prepare the required number of tubes for amplification of DNA from test and control samples. Select the type of tubes, strips or plates depending on the instrument used.
3. Add **15 µl** of the prepared reaction mixture to each tube.
4. **Add 10 µl of DNA samples** obtained at the DNA extraction stage.
5. Carry out the control amplification reactions:

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

C+ – Add **10 µl** of **Positive Control DNA *Toxoplasma gondii* and STI ($C+_{T.gondii\ and\ STI}$)** to the tube labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 3

AmpliSens-1 amplification program

	Rotor-type instruments[2]			Plate-type instruments[3]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**5 s**	**5**	**95**	**5 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**5 s**	**40**	**95**	**5 s**	**40**
	60	**20 s Fluorescence acquiring**		**60**	**30 s Fluorescence acquiring**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores**.**

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection
5. Analyze results after the amplification program is completed

[1] Given volumes include 2 control points (positive and negative control of amplification) and 1 extra reaction.
[2] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia).
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

– The signal of the IC DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the ***Toxoplasma gondii*** DNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– ***Toxoplasma gondii*** DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the boundary *Ct* value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***Toxoplasma gondii*** DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value.
– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore or greater than the specified boundary *Ct* value, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary *Ct* value.
– The result is **equivocal** if the *Ct* value determined in the channel for the JOE fluorophore is greater than the boundary *Ct* value, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is 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 (see Table 4).

Table 4

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	DNA extraction	≤ boundary value	Absent
NCA	PCR	Absent	Absent
C+	PCR	≤ boundary value	≤boundary value

10. TROUBLESHOOTING

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

1. If any *Ct* value is determined for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE fluorophores, it indicates the contamination of reagents or samples. In this case, the results of analysis for all samples are invalid. The analysis for all samples should be repeated and measures for detecting and elimination of contamination source must be taken.
2. If the *Ct* value is absent for the Positive Control of amplification (C+) in the channels for the JOE and FAM fluorophores, the results of analysis for all samples are invalid. PCR should be repeated for all samples.
3. If *Ct* values in the channel for the FAM fluorophore (IC) are absent in clinical samples, it indicates improper DNA extraction. For these samples, analysis should be repeated starting from the DNA extraction stage. If the *Ct* values for clinical samples determined in the channels for the FAM (IC) and JOE (*Toxoplasma gondii*) fluorophore exceeds the values specified in the bulletin, analysis should be repeated starting from the DNA extraction stage. High *Ct* values may be obtained due to the loss of DNA during extraction or presence of inhibitors.
4. If the *Ct* value of a clinical sample determined in the channel for the JOE fluorophore exceeds the value specified in the bulletin, the result is considered **equivocal**. It is necessary to repeat the analysis twice. If a reproducible positive *Ct* value is determined twice, the sample is considered **positive**. If irreproducible values are obtained in two repeats, the result is considered equivocal.

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 are to be stored at 2–8 °C when not in use (except for polymerase (TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *Toxoplasma gondii*). All components of the **AmpliSens® *Toxoplasma gondii*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated

NOTE: Polymerase (TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *Toxoplasma gondii* are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: 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 4 tachyzoites/ml (400 *Toxoplasma gondii* DNA copies/ml).

NOTE: 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 the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been 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, 2010.
2. Guidelines to the **AmpliSens® *Toxoplasma gondii*-FRT** PCR kit for qualitative detection *of Toxoplasma gondii* DNA in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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 the **AmpliSens® *Toxoplasma gondii*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

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”
31.08.15 ME	Through the text	Corrections according to the template. Grammar corrections
	1. Intended use	The types of clinical material were corrected
	2. Principle of PCR detection	The section was rewritten
	6. Sampling and handling	Information about storage conditions of clinical samples was added
	8.1. RNA Extraction	Information about controls of extraction and additions in PIBO-prep protocol were added
	8.2.1 Preparing tubes for PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
	13.1. Sensitivity	“4 tachyzoites/ml” was added for analytical sensitivity
	14. References	The reference to Guidelines was added
28.12.15 PM	Through the text	The clinical material umbilical cord blood, biopsy material was deleted
20.12.17 PM	3. Content	The color of the reagent was specified
05.12.18 PM	2. Principle of PCR detection	The table with targets and the information about the enzyme UDG were added
	Through the text	The text formatting was changed
27.02.20 PM	Footer	The phrase “Not for use in the Russian Federation” was added
01.03.21 KK	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

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