AmpliSens® *Rubella virus*-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
RUO	Research Use Only		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
	Caution	IC	Internal control
		PCE	Positive control of extraction

1. INTENDED USE

AmpliSens® *Rubella virus*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Rubella virus* RNA in the biological material (peripheral and umbilical cord blood plasma, saliva, oropharyngeal swabs, and amniotic fluid) using real-time hybridization-fluorescence detection of amplified products.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Rubella virus detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region by using specific 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 ® *Rubella virus*-FRT PCR kit is a qualitative test that contains the Internal Control **(Internal Control STI-87-rec (IC))**. It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Rubella virus*-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 a chemically modified polymerase (TaqF), which is activated by heating at 95 °C for 15 min.

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

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	Internal Control STI-87-rec (IC) cDNA	*Rubella virus* cDNA
Target gene	genetically engineered construction	*p150 R.virus* gene

3. CONTENT

AmpliSens® *Rubella virus*-FRT PCR kit is produced in 2 forms:

variant FRT-50 F REF R-V24-S(RG,iQ,Mx)-CE;

variant FRT-50 F in bulk[1] REF R-V24-S(RG,iQ,Mx)-CE-B.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FRT *Rubella virus*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *Rubella virus* / STI (C+$_{Rubella\ virus\ /\ STI}$)	colorless clear liquid	0.1	1 tube
RNA-buffer	colorless clear liquid	0.6	1 tube
Negative Control (C–)*	straw-colored clear liquid	0.5	2 tubes
Positive Control *Rubella virus*-rec**	colorless clear liquid	0.1	2 tubes
Internal Control STI-87-rec (IC)***	colorless clear liquid	0.5	1 tube

* must be used in the extraction procedure as Negative Control of Extraction.
** must be used in the extraction procedure as Positive Control of Extraction.
*** add **10 µl** of **Internal Control STI-87-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep**, **RIBO-sorb** protocols).

Variant FRT-50 F is intended for 60 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl, 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 optical transparent domed or flat 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.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

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® *Rubella virus*-FRT PCR kit is intended for analysis of the RNA extracted with DNA/RNA extraction kits from the biological material (peripheral and umbilical cord blood plasma, saliva, throat swabs, amniotic fluid):

6.1. P*eripheral and umbilical cord blood plasma.*
Collect blood to a Vacuette® tubes (lavender cap, with 6% EDTA) from the ulnar veins using disposable needle (0.8-1.1 mm diameter) after overnight fasting or at least 3 h after the patient had a meal. Invert the tube several times to ensure proper mixing of blood with the anticoagulant. Insert the tubes in the rack.
Centrifuge the tubes at 800–1600 g at room temperature for 20 min to separate the blood. Take the blood plasma (no less than 1.0 ml) using individual tips with filters and transfer it to sterile 2.0-ml Eppendorf tubes.
Blood plasma should be stored at 2-8 °C for 5 days (quantitative DNA/RNA detection) or 1 day (quantitative detection of pathogen DNA/RNA), at the temperature from minus 24 to minus 16 °C for a year, and at the temperature ≤-68 °C for a long time.
It is necessary to aliquote the sample (to 0.2-0.5 ml aliquotes) in case of long-term storage because of partial virus destruction and the degradation of viral RNA at every thawing.

6.2. *Saliva.* Collect saliva (0.2–1.0 ml) to sterile 1.5-ml Eppendorf tube. Have the patient to rinse his mouth with water 3 times before sampling saliva.
Saliva should be stored at 2-8 °C for 1 day and at the temperature ≤-68 °C for a year.

6.3. *Oropharyngeal swabs* are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water. When material is obtained, insert the swab into a sterile disposable tube with 500 µl of transport medium. Break off the end of shaft to allow tight closing of tube cap. Close the tube with the solution and the swab.
The oropharyngeal swabs are to be stored 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 year.

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 it using tip with filter 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 by vortexing.
Amniotic fluid and the pretreated material should be stored 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 year

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA Extraction

It is recommended that the following nucleic acid extraction kits are used:
- **RIBO-prep**;
- **RIBO-sorb**;
- NucliSENS easyMAG automated system (for details see Guidelines [2]).

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

PCE – Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control *Rubella virus*-rec** to the tube labeled **PCE** (Positive Control of Extraction)**.**

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

If using the RIBO-sorb kit extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:
- The volume of the **Internal Control STI-87-rec (IC)** reagent added to each tube is **10 µl**.

NOTE:
- Vortex the tubes after adding of **Lysis Solution, Internal Control** and **sample** and then incubate the tubes at 60 °C for 10 min. Make sure that there are not suspended particles in the tubes before adding the sorbent. Otherwise, centrifuge the tubes at 10,000 rpm for 1 min and then transfer the supernatant to new tubes.

8.2. Preparing the PCR

8.2.1 Preparing tubes for PCR

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

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to Table 2. Note that even for analysis of one test or control RNA sample, it is necessary to carry out all controls of the RT-PCR stage. It is recommended to mix the reagents for an even reaction number to ensure more exact dosage

1. Take the required number of tubes for amplification for the test and control samples. The type of tubes depends on the PCR instrument used for analysis.
2. Prepare the reaction mixture (see Table 2).

Table 2

Scheme of reaction mixture preparation

Volume per one reaction (µl)		10.00	5.00	0.25	0.50	0.25
Number of samples[2]	Number of reactions[3]	RT-PCR-mix-1-FRT *Rubella virus*	RT-PCR-mix-2-FEP/FRT	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMlv)
3	6	60	30	1.5	3.0	1.5
5	8	80	40	2.0	4.0	2.0
7	10	100	50	2.5	5.0	2.5
9	12	120	60	3.0	6.0	3.0
11	14	140	70	3.5	7.0	3.5
13	16	160	80	4.0	8.0	4.0
15	18	180	90	4.5	9.0	4.5
17	20	200	100	5.0	10.0	5.0
19	22	220	110	5.5	11.0	5.5
21	24	240	120	6.0	12.0	6.0
23	26	260	130	6.5	13.0	6.5
25	28	280	140	7.0	14.0	7.0
27	30	300	150	7.5	15.0	7.5
29	32	320	160	8.0	16.0	8.0
31	34	340	170	8.5	17.0	8.5
33	36	360	180	9.0	18.0	9.0
35	38	380	190	9.5	19.0	9.5
37	40	400	200	10.0	20.0	10.0
39	42	420	210	10.5	21.0	10.5
41	44	440	220	11.0	22.0	11.0
43	46	460	230	11.5	23.0	11.5
45	48	480	240	12.0	24.0	12.0
47	50	500	250	12.5	25.0	12.5
49	52	520	260	13.0	26.0	13.0
51	54	540	270	13.5	27.0	13.5
53	56	560	280	14.0	28.0	14.0
55	58	580	290	14.5	29.0	14.5
57	60	600	300	15.0	30.0	15.0

3. Add **15 µl** of the prepared reaction mixture to each tube.
4. Add **10 µl of RNA samples** obtained at the RNA extraction stage.
5. Carry out the control amplification reactions:

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

C+ – Add **10 µl** of **Positive Control cDNA *Rubella virus* / STI (C+$_{Rubella\ virus\ /\ STI}$)** to the tube labeled C+$_{Rubella\ virus\ /\ STI}$ (Positive Control of Amplification)

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

PCE – Add **10 µl** of **the sample extracted from the Positive Control *Rubella virus*-rec reagent** to the tube labeled PCE (Positive Control of Extraction).

8.2.2. Amplification

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

Table 3a

AmpliSens-2 amplification program for rotor-type instruments[4]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	50	15 min	–	1
Hold 2	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, ROX/Orange, Cy5/Red	
	72	15 s	–	

Table 3b

AmpliSens-1 amplification program for plate-type instruments[5]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4	95	5 s	–	40
	60	30 s	FAM, HEX, ROX, Cy5	
	72	15 s	–	

NOTE: The **ROX** and **Cy5** channels are enabled when required if the "multiprime" format tests are performed

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.

[2] Include test samples and controls of RNA extraction (N+2).
[3] Include test samples, controls of RNA extraction, and controls of RT-PCR (N+2+3).
[4] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia).
[5] For example, iCycler iQ, iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA).

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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** cDNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the ***Rubella virus*** cDNA 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 cDNA sample in the corresponding column of the results grid.

– ***Rubella virus* cDNA** 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 specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***Rubella virus* cDNA** is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.

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-	RNA extraction	≤ boundary value	Absent
PCE	RNA extraction	≤ boundary value	≤ boundary value
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 the *Ct* value is absent for Positive Control of Extraction (PCE) it indicates improper RNA extraction. RNA extraction should be repeated.
2. If the *Ct* value is absent for the Positive Control of Amplification (C+), this indicates errors in amplification procedure (for example, incorrectly chosen amplification program). PCR should be repeated for all samples.
3. If the *Ct* value of a test sample determined in the channel for the JOE fluorophore is absent or greater than the boundary *Ct* value specified in the *Important Product Information Bulletin,* whereas the *Ct* value determined in the channel for the FAM fluorophore is greater than the *Ct* value specified for the Internal Control, it indicates improper treatment of biological material, that resulted RNA loss or the presence of RT-PCR inhibitors. The PCR analysis (beginning with the RNA extraction stage) should be repeated for such samples.
4. If any *Ct* value is determined for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE fluorophores and for the Negative Control of Extraction (C–) in the channel for JOE fluorophore, 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.
5. If the *Ct* value of a test sample determined in the channel for the JOE fluorophore exceeds the value specified in the *Important Product Information Bulletin*, the result is considered **equivocal**. It is necessary to repeat the analysis twice. If a reproducible positive *Ct* value is detected, the sample is considered to be **positive**. If irreproducible values are obtained in two repeats, the result is considered equivocal.

11. TRANSPORTATION

AmpliSens® *Rubella virus*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Rubella virus*-FRT** PCR kit (except for RT-G-mix-2, RT-PCR-mix-1-FRT *Rubella virus,* RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMlv)) are to be stored at 2–8 ºC. All components of the **AmpliSens® *Rubella virus*-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

NOTE: RT-G-mix-2, RT-PCR-mix-1-FRT *Rubella virus*, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMlv) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

NOTE: RT-PCR-mix-1-FRT *Rubella virus* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *Rubella virus*-FRT** PCR kit is 400 copies/ml.

NOTE: The claimed analytical features of **AmpliSens® *Rubella virus*-FRT** PCR kit are guaranteed only when an additional reagent kit (**RIBO-prep** or **RIBO-sorb**) or the NucliSENS easyMAG automated system is used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Rubella virus*-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.

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.
2. Guidelines to the **AmpliSens® *Rubella virus*-FRT** PCR kit qualitative detection of *Rubella virus* RNA in the biological material by 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® *Rubella virus*-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"
05.03.13 PE	Content	"C+" was changed to "C+$_{Rubella\ virus\ /\ STI}$"
	Front page, key to symbols used	Symbol IVD was changed to RUO
26.07.16 ME	Through the text	Corrections according to the template. Grammar corrections
	6. Sampling and handling	The chapters were completed
	8.1. RNA Extraction	
	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	
	14. References	The reference to the Guidelines was added
20.12.17 PM	3. Content	The color of the reagent was specified
31.05.21 VA	6. Sampling and handling	In the procedure of oropharyngeal swabs sampling the probe with cotton swab was changed to rayon swabs with plastic shafts for oropharyngeal swabs
	2. Principle of PCR detection	The table with targets was added
	Through the text	The text formatting was changed
	Footer	The phrase "For research use only. Not for diagnostic procedures" was added
22.06.23 EM	3. Content Footer	REF R-V24-S(RG,iQ,Mx)-CE was added

AmpliSens®

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