CE
1023

IVD

For Professional Use Only

AmpliSens® *Rubella virus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

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 clinical material (peripheral and umbilical cord blood plasma, saliva, oropharyngeal swabs, 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

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 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 of the reaction tubes after the PCR run. **AmpliSens ® *Rubella virus*-FRT** PCR kit is a qualitative test that contains the Internal Control (IC), which 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.

3. CONTENT

AmpliSens® *Rubella virus*-FRT PCR kit is produced in 1 form:

AmpliSens® ***Rubella virus*-**FRT PCR kit variant FRT-50 F (for use with RG, iQ, Mx)

REF R-V24-S(RG,iQ,Mx)-CE

AmpliSens® *Rubella virus*-FRT PCR kit 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*	colorless clear liquid	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 during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, **REF** K1-9-Et-50-CE, RIBO-sorb **REF** K2-1-Et-50-CE protocols).

AmpliSens® *Rubella virus*-FRT PCR kit 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 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 (Bio-Rad, USA), Mx3000P/Mx3005P (Stratagene, USA)).
- Disposable polypropylene microtubes for PCR (0.5- 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 authorities' 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® *Rubella virus*-FRT PCR kit is intended to analyze RNA extracted with DNA/RNA extraction kits from:

- *Plasma of peripheral and umbilical cord blood*
- *Saliva*
- *Throat swabs*
- *Amniotic fluid*

6.1. P*eripheral and umbilical cord blood plasma*. Collect blood to a Vacuett tube (lavender cap, 6% EDTA) 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. Centrifuge the tube with blood at 800–1600 g at room temperature for 20 min. Take 1.0 ml of plasma and transfer it to a sterile 2.0-ml Eppendorf tube.

6.2. *Saliva.* Collect 0.2–1.0 ml of saliva to a 1.5-ml Eppendorf tube. Have the patient to rinse his mouth with water 3 times before sampling saliva.

6.3. *Oropharyngeal swabs* are obtained with a dry cotton probe from the tonsillar area, palatine arches, and posterior oropharyngeal surface. Have a patient to rinse his mouth with water before swabbing.

After sampling, the cotton end of the probe should be placed into a sterile tube containing 500 µl of transport medium. Then the probe should be broken off at the score mark and the tube should be tightly closed.

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 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® *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, **REF** K1-9-Et-50-CE;
- RIBO-sorb, **REF** K2-1-Et-50-CE;
- NucliSENS easyMAG automated system.

Extract RNA according to the manufacturer's protocol.
During extraction, use the following controls:

- Positive Control *Rubella virus*-rec (Positive Control of Extraction, PCE);
- Negative Control (C–);
- Internal Control STI-87-rec (IC).

If the NucliSENS easyMAG automated system is used:

- set the sample volume from 0.1 to 1.0 ml;
- set the eluate volume as 55 µl;
- both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the **reaction mixture.** 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+ and NCA, respectively).
2. Prepare the required number of tubes or strips for reverse transcription and amplification of RNA and cDNA of 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 RNA samples** obtained from clinical or control samples at the RNA extraction stage.
5. Carry out **control amplification reactions:**

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

C+ Add 10 µl of **Positive Control cDNA *Rubella virus* / STI** to the tube for Positive Control of Amplification (C+).

8.2.2. Amplification

Program the real-time instrument according to manufacturer's manual.

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

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Repeats***
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	
	72	15 s		

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green and JOE/Yellow fluorescence channels.

For settings, see Guidelines.

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

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

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

Fluorescence is detected at stage 4 (60 °C) in FAM and HEX fluorescence channels.

For settings, see Guidelines.

9. DATA ANALYSIS

Accumulation of ***Rubella virus*** **cDNA** 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 used by the crossing (or not crossing) of the fluorescence curve with the threshold line.

See **Guidelines** for data analysis settings.

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.

Results for controls

Control	**Stage for control**	**Ct in channel**		**Interpretation**
		FAM/Green	**JOE/Yellow/HEX**	
C–	RNA extraction	≤ boundary value*	Neg	OK
PCE	RNA extraction	≤ boundary value*	≤ boundary value)	OK
NCA	RT-PCR	Neg	Neg	OK
C+	RT-PCR	≤ boundary value*	≤ boundary value*	OK

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

1. The sample is considered **positive** if its Ct value detected in the JOE/Yellow/HEX channel does not exceed the boundary Ct value defined in the *Important Product Information Bulletin* and the Ct value detected in the FAM/Green channel does not exceed the value specified for the Internal Control. The fluorescence curve should have a typical sigmoid shape and cross the threshold line once in the region of significant fluorescence increase.

[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)

2. The sample is considered **negative** if its Ct in the JOE/Yellow/HEX channel is not detected (the fluorescence curve does not cross the threshold line) and the Ct value detected in the FAM/Green channel does not exceed the boundary Ct value specified for the Internal Control.

10. TROUBLESHOOTING

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

1. If the Ct value of a clinical sample detected in the JOE/Yellow/HEX channel exceeds the boundary Ct 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**.
2. If any Ct value is detected for the Negative Control of Amplification (NCA) in both channels or the Ct value is detected for Negative Control of Extraction (C–) in the JOE/Yellow/HEX 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.
3. If the Ct value is absent for the Positive Control of Extraction (PCE), this indicates improper extraction procedure. RNA extraction should be repeated for all samples.
4. If the Ct value is absent for the Positive Control of RT-PCR (C+), this indicates errors in carrying out PCR or an incorrect amplification program. RT-PCR should be repeated for all samples.
5. If the Ct value of a clinical sample is absent or greater than the boundary Ct value specified in the *Important product information bulletin* for the JOE/Yellow/HEX channel and the Ct value in the FAM/Green channel is greater than the Ct values specified for the Internal Control, the result is **invalid**. Analysis of such samples should be repeated starting from the RNA extraction stage.

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

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.

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.

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.

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 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® *Rubella virus*-FRT** PCR kit was confirmed in laboratory clinical tests.

14. REFERENCES

1. “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Instituteof 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 Instituteof Science “Central Research Institute of Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Rubella virus*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

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

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 Institutewas changed to Federal Budget Instituteof Science “Central Research Institute for Epidemiology”