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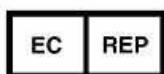
For *in Vitro* Diagnostic Use

AmpliSens[®] Rubella virus-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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TABLE OF CONTENTS

1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION	3
3. CONTENT	3
4. ADDITIONAL REQUIREMENTS	4
5. GENERAL PRECAUTIONS.....	4
6. SAMPLING AND HANDLING.....	5
7. PROTOCOL.....	6
8. DATA ANALYSIS	7
9. TROUBLESHOOTING	8
10. STABILITY AND STORAGE	9
11. SPECIFICATIONS.....	9
12. REFERENCES	9
13. QUALITY CONTROL	9
14. EXPLANATION OF SYMBOLS.....	10

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 clinical material (plasma of peripheral and umbilical cord blood, saliva, throat swabs, amniotic fluid) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION

Rubella virus 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[®] Rubella virus-FRT** PCR kit is a qualitative test and contains the Internal Control (IC) which must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens[®] Rubella virus-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.

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 <i>Rubella virus</i>	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 (MMIv)	colorless, clear liquid	0.015	1 tube
Positive Control cDNA <i>Rubella virus</i> and STI (C+)	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 <i>Rubella virus-rec</i>**	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 isolation procedure as Negative Control of Extraction.

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

***add 10 µl of Internal Control STI-87-rec during the RNA isolation 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 isolation 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 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[®] *Rubella virus*-FRT PCR kit is intended to analyze RNA extracted with RNA isolation kits from:

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

6.1. *Plasma of peripheral and umbilical cord blood*. Collect blood in a "Vacuett" tube (lavender top – 6% EDTA solution) after overnight fasting or at least 3 hours after the patient had a meal. Invert the tube several times to ensure proper mixing of blood with the anticoagulant.

Centrifuge the tube with the blood at 800-1600 g for 20 min at room temperature. Remove 1.0 ml of a plasma sample and transfer in a sterile 2.0 ml tube (like Eppendorf).

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

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

After the sampling the cotton end of the probe should be placed into the sterile tube with 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 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. RNA Isolation

It's 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.

Please carry out the RNA isolation according to the manufacturer protocol.



During extraction stage apply 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 are possible.

7.2. Preparing the PCR

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

7.2.1 Preparing tubes for PCR

1. Prepare the **reaction mix**. 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 reverse transcription and amplification of RNA and cDNA 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 RNA samples**, obtained from clinical or control samples at the stage of RNA extraction.
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* and STI** to the tube for Positive Control of Amplification (C+).

7.2.2. Amplification

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

AmpliSens-2 amplification program for rotor-type instruments¹

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	50	15 min	–	1
Hold 2	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	50 °C	15 min	–	1
2	95 °C	15 min	–	1
3	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
4	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 *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 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 on channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
C-	RNA isolation	≤ Ct*	Neg	OK
PCE	RNA isolation	≤ Ct*	≤ Ct*	OK
NCA	RT-PCR	Neg	Neg	OK
C+	RT-PCR	≤ Ct*	≤ Ct*	OK

*For Ct values see **Important product information bulletin** enclosed to AmpliSens[®] *Rubella virus*-FRT PCR kit.

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 JOE/Yellow/HEX channel does not exceed the value defined in the *Important product information bulletin* while Ct value obtained in the FAM/Green channel does not exceed the value specified for the Internal Control.

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 Ct in the JOE/Yellow/HEX channel is not detected (fluorescence curve does not cross the threshold line) while Ct value obtained in the FAM/Green channel does not exceed the value specified for the Internal Control.

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

9. TROUBLESHOOTING

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

1. If Ct value of a clinical sample obtained in the JOE/Yellow/HEX channel exceeds the value defined in the *Important product information bulletin*, then the result is considered equivocal. It is necessary to repeat the analysis twice. In case the reproducible positive Ct value is obtained, the sample considered positive.
2. If any Ct value appears for Negative Control of amplification (NCA) in both channels or Ct value is detected for Negative Control of extraction (C-) in the 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.
3. If Ct value is absent for Positive Control of extraction (PCE) it indicates failures of the extraction procedure. RNA extraction should be repeated for all samples.
4. If Ct value is absent for Positive Control of RT-PCR (C+) it indicates errors in PCR conducting or amplification program false. RT-PCR should be repeated for all samples.
5. If Ct value of a clinical sample is absent or exceeds the value specified in the *Important product information bulletin* for the JOE/Yellow/HEX channel, while its Ct value in the FAM/Green channel is more than Ct specified for the Internal Control, the result is invalid and should be repeated from the extraction.

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

10. 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 between 2 and 8 °C. All components of the **AmpliSens® Rubella virus-FRT** PCR kit are to be stable until the expiry date stated on the label.



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 or below minus 16 °C.

11. SPECIFICATIONS

11.1. 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 additional reagent kit (RIBO-prep or RIBO-sorb) is used or NucliSENS® easyMAG® automated system is applied.

11.2. Specificity

Specificity of **AmpliSens® Rubella virus-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® Rubella virus-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® Rubella virus-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 <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Consult instructions for use
	Contains sufficient for <n> tests		Caution, consult accompanying documents
NCA	Negative Control of Amplification	IC	Internal Control
RG	For working with Rotor-Gene™ 3000/6000 (Corbett Research)	PCE	Positive Control of Extractor
Mx	For working with Mx3000P or Mx3005P (Stratagene)	iQ	For working with iQ5, iQiCycler (Bio-Rad)
	Authorised representative in the European Community.		