


IVD For *in Vitro* Diagnostic Use

For Professional Use Only

Rubella Real-TM Qual

Handbook

Real-Time PCR test for qualitative detection of
Rubella Virus

 50

REF V24-50FRT

NAME

RUBELLA Real-TM Qual

INTENDED USE

RUBELLA Real-TM Qual is a Real-Time test for the qualitative detection of Rubella (Rosolia) Virus RNA in the peripheral and umbilical cord blood plasma, saliva, oropharyngeal swabs, and amniotic fluid. RUBELLA RNA is extracted from specimens, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for Rubella or Rubella IC.

PRINCIPLE OF ASSAY

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. **RUBELLA Real-TM Qual** 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. **RUBELLA Real-TM Qual** 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.

MATERIALS PROVIDED

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FRT <i>Rubella</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</i> / 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</i> -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 isolation procedure as Negative Control of Extraction.

** must be used in the isolation procedure as Positive Control of Extraction (add 10 µl of Positive Control *Rubella* -rec and 90 µl of Negative Control (C-) to the tube labeled PCE).

*** add 10 µl of Internal Control STI-87-rec (IC) to each sample during the RNA purification procedure directly to the sample/lysis mixture.

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers.
- Disposable polypropylene 1,5/2,0 ml tubes.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes.
- PCR Workstation.
- Real Time Thermal cycler.
- Disposable polypropylene microtubes for PCR.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD *In Vitro* Diagnostic Medical Device

For *In Vitro* Diagnostic Use Only

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
9. Material Safety Data Sheets (MSDS) are available on request.
10. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
11. PCR reactions are sensitive to contamination. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practice.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.



Sampling of biological materials for PCR-analysis, transportation, and storage are described in details in the handbook of the manufacturer. It is recommended that this handbook is read before beginning of the work.

STORAGE INSTRUCTIONS

The components of the **RUBELLA Real-TM Qual** PCR kit must be stored at 2–8 °C excepting **RT-PCR-mix-1-FRT** *Rubella*, **RT-G-mix-2**, **RT-PCR-mix-2-FEP/FRT**, **Polymerase (TaqF)**, and **TM-Revertase (MMIv)** that must be stored at -16°C or below. The kit can be shipped at 2-8°C no longer than 5 days but should be stored at 2-8°C and -16°C or below immediately on receipt.

STABILITY

RUBELLA Real-TM Qual is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

The shelf life of reagents before and after the first use is the same, unless otherwise stated.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

RUBELLA Real-TM Qual can analyze RNA extracted from:

- *Peripheral 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.
- *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.
- *Oropharyngeal swabs:* 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.

- *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.

Specimens can be stored at +2-8°C for no longer than 12 hours, or frozen at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇒ **Ribo-Sorb** (Sacace, REF K-2-1/100)
- ⇒ **DNA/RNA-Prep** (Sacace, REF K-2-9);

Please carry out the RNA extraction according to the manufacturer’s instructions.

During extraction, use the following controls:

- Positive Control of Extraction: add 10 µl of Positive Control Rubella -rec and 90 µl of Negative Control (C-) to the tube labeled PCE.
- Negative Control of extraction: *add 100 µl of Negative Control (C-) to the tube labeled C-.*
- Internal Control: *add 10 µl of Internal Control STI-87-rec (IC) during the RNA isolation procedure directly to the sample/lysis mixture.*

PROTOCOL (Reaction volume 25 µl):

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



Unfreeze RT-PCR-mix-1-FRT Rubella, RT-G-mix-2, RT-PCR-mix-2-FEP/FRT before mixing.

1. Prepare the required number of the tubes for amplification of DNA from clinical and control samples.
2. Prepare in a new sterile tube the **Reaction Mix**. For each sample mix **10 µl** of **RT-PCR-mix-1-FRT Rubella**, **5 µl** of **RT-PCR-mix-2-FEP/FRT**, **0,25 µl** of **RT-G-mix-2**, **0,50 µl** of **Polymerase (TaqF)** and **0,25 µl** of **TM-Revertase (MMIv)**. Vortex thoroughly and centrifuge for 5 sec. This mix must be used immediately. Don't store the prepared mix!

Reagents volume x 1 reaction (µl)		10,0	5,00	0,25	0,50	0,25
N RNA samples ¹	N reactions ²	RT-PCR- mix-1-FRT Rubella	RT-PCR- mix-2- FEP/FRT	RT-G- mix-2	Polymerase (TaqF)	TM-Revertase (MMIv)
4	6	60	30	1,5	3,0	1,5
6	8	80	40	2,0	4,0	2,0
8	10	100	50	2,5	5,0	2,5
10	12	120	60	3,0	6,0	3,0
12	14	140	70	3,5	7,0	3,5
...58	60	600	300	15,0	30,0	15,0

¹ specimens plus 2 extraction controls (N+2)

² specimens plus extraction and amplification controls (N+2+2)

3. Add **15 µl** of **Reaction Mix** into each tube.
4. Add **10 µl** of **extracted RNA** sample to appropriate tubes with Reaction Mix and mix well by pipetting.
(Re-centrifuge all the tubes with extracted RNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction!).
5. Prepare for each panel 2 controls:
 - NCA: add **10 µl** of **RNA-buffer** to the tube labeled Negative Control;
 - C+: add **10 µl** of **Positive Control cDNA Rubella / STI (C+)** to the tube labeled Positive Control;

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.

Rubella cDNA is detected on the JOE(Yellow)/HEX/Cy3 channel, IC DNA on the FAM (Green) channel

Amplification

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

Amplification program for rotor-type instruments¹

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	50 °C	15 min	–	1
Hold 2	95 °C	15 min	–	1
Cycling	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
Cycling2	95 °C	5 sec	–	40
	60 °C	20 sec	FAM/Green, JOE/Yellow	
	72 °C	15 sec		

¹ For example Rotor-Gene™ 3000/6000 (Qiagen)

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

Amplification program for plate-and modular 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/Cy3/Joe	
	72 °C	15 sec		

² For example, iQ™/iQ5™ (BioRad); Mx3000P™ (Agilent) or equivalent.

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

Rotor-type instruments

Channel	Calibrate/Gain Optimisation...	Threshold	More Settings/ Outlier Removal	Slope Correct	Dynamic tube
FAM/Green	from 3 FI to 8 FI	0.03	10 %	On	On
JOE/Yellow	from 3 FI to 8 FI	0.03	10 %	On	On

Plate- or modular type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

DATA ANALYSIS

- **Rubella virus cDNA** amplification product is detected in the **JOE/Yellow/HEX** channel,
- **Internal Control** amplification product is detected in the **FAM/Green** channel.

RESULTS INTERPRETATION

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.

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 and samples

Boundary Ct values for rotor-type instruments (Rotor-Gene 3000/6000, Rotor-Gene Q, etc.)

Control	Stage for control	Ct in channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
NCE	RNA extraction	≤ 28	Neg	OK
PCE	RNA extraction	≤ 28	≤ 24	OK
C+	RT-PCR	≤ 28	≤ 27	OK
Samples	RT-PCR	≤ 28	≤ 35	OK

Boundary Ct values for for plate-type instruments (iCycler iQ, iCycler iQ5, Mx3000P, etc)

Control	Stage for control	Ct in channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
NCE	RNA extraction	≤ 31	Neg	OK
PCE	RNA extraction	≤ 31	≤ 27	OK
C+	RT-PCR	≤ 31	≤ 30	OK
Samples	RT-PCR	≤ 31	≤ 40	OK

1. The sample is considered **positive** if its Ct value detected in the JOE/Yellow/HEX channel does not exceed the boundary Ct value 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. 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.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of **RUBELLA Real-TM Qual** 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 **RUBELLA Real-TM Qual** PCR kit was confirmed in laboratory clinical tests.

Analytical sensitivity

The kit **RUBELLA Real-TM Qual** allows to detect *RUBELLA* RNA in 100% of the tests with a sensitivity of not less than 400 copies/ml.



The claimed analytical features of **RUBELLA Real-TM Qual** PCR kit are guaranteed only when an additional reagent kit (DNA/RNA-prep or RIBO-sorb) is used.

Target region

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

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), positive control of extraction (PCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

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 (>37), 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 (>40) 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 (>35), the result is **invalid**. Analysis of such samples should be repeated starting from the RNA extraction stage.

REFERENCES

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KEY TO SYMBOLS USED

	List Number		Caution!
	Lot Number		Contains sufficient for <n> tests
	For <i>in Vitro</i> Diagnostic Use		Version
	Store at	NCA	Negative Control of Amplification
	Manufacturer	C-	Negative control of Extraction
	Consult instructions for use	C+	Positive Control of Amplification
	Expiration Date	IC	Internal Control

* iCycler iQ5™ is a registered trademark of Bio-Rad Laboratories

* Rotor-Gene™ is a registered trademark of Qiagen

* MX3000P™ is a registered trademark of Agilent Technologies



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