IVD For *in Vitro* Diagnostic Use

CE

Streptococcus pyogenes Real-TM Quant

Handbook

Real Time PCR Kit for quantitative detection of Streptococcus pyogenes

REF B82-100FRT

REF TB82-100FRT

Σ **100**

NAME

Streptococcus pyogenes Real-TM Quant

INTRODUCTION

Streptococcus pyogenes is a spherical, Gram-positive bacterium that is the cause of group A streptococcal infections. *S. pyogenes* displays streptococcal group A antigen on its cell wall. S. pyogenes typically produces large zones of beta-hemolysis when cultured on blood agar plates, and are therefore also called Group A (beta-hemolytic) Streptococcus (abbreviated GABHS).

INTENDED USE

Streptococcus pyogenes Real-TM Quant kit is a Real-Time test for the qualitative and quantitative detection of *S. pyogenes* DNA in the clinical materials (swabs, plasma, CSF) by using real-time hybridization-fluorescence detection.

PRINCIPLE OF ASSAY

Streptococcus pyogenes Real-TM Quant kit is a Real-Time test for the Qualitative and Quantitative detection of *S. pyogenes* in the biological materials. DNA is extracted from samples, amplified and detected using fluorescent reporter dye probes specific for *S. pyogenes* DNA and Internal Control IC.

Internal Control (IC), added during the sample preparation from plasma, liquor, amniotic liquid and other cell free or low in DNA content materials, serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

S. pyogenes DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel and exogenous Internal Control IC is detected on FAM (Green) channel.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B82-100FRT)

Part N° 2 –“ **Streptococcus pyogenes Real-TM Quant”:** Real Time amplification

- **PCR-mix-1**, 1,2 ml;
- **PCR-buffer FRT**, 0,6 ml;
- **TE-buffer**, 0,2 ml;
- **Negative Control C-***, 1,2 ml;
- **Pos C+ *(S. pyogenes* DNA&IC Glob)****, 0,1 ml;
- **Internal Control IC*****, 1,0 ml;
- **Standard *S. pyogenes* DNA/IC**:
  - **QSG1**, 0,2 ml;
  - **QSG2**, 0,2 ml.

Contains reagents for 100 tests

* must be used during the sample preparation procedure: add 100 µl of C– (Negative Control) to labeled Cneg;
** add 90 µl of C– (Negative Control) and 10 µl of Pos C+ to the tube labeled Cpos. Pos C+ is the control with note concentration of S. pyogenes DNA (value is specific for each lot and reported in the Quant Data Card provided in the kit)
***add 10 µl of Internal Control to all samples during the DNA isolation procedure directly to the sample/lysis mixture

Module No.2: Complete Real Time PCR test with DNA purification kit (TB82-100FRT)

Part N° 1 – **"DNA/RNA Prep":** Sample preparation

- **Lysis Sol**, 2 x 15 ml;
- **Prec Sol**, 2 x 20 ml;
- **Washing Sol 3**, 2 x 25,0 ml;
- **Washing Sol 4**, 2 x 10,0 ml;
- **RE-buffer**, 8 x 1,2 ml;

Contains reagents for 100 extractions

Part N° 2 –" **Streptococcus pyogenes Real-TM Quant":** Real Time amplification

- **PCR-mix-1**, 1,2 ml;
- **PCR-buffer FRT**, 0,6 ml;
- **TE-buffer**, 0,2 ml;
- **Negative Control C-***, 1,2 ml;
- **Pos C+ *(S. pyogenes* DNA&IC)****, 0,1 ml;
- **Internal Control IC*****, 1,0 ml;
- **Standard *S. pyogenes* DNA/IC**:
  - **QSG1**, 0,2 ml;
  - **QSG2**, 0,2 ml.

Contains reagents for 100 tests

* must be used during the sample preparation procedure: add 100 µl of C– (Negative Control) to labeled Cneg;
** add 90 µl of C– (Negative Control) and 10 µl of Pos C+ to the tube labeled Cpos. Pos C+ is the control with note concentration of S. pyogenes DNA (value is specific for each lot and reported in the Quant Data Card provided in the kit)
***add 10 µl of Internal Control to all samples during the DNA isolation procedure directly to the sample/lysis mixture

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- DNA extraction kit (Module No. 1)
- Biological cabinet
- Vortex
- 65°C ± 2°C dry heat block
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Tube racks
- Microcentrifuge tubes, 1,5 - 2,0 ml
- Pipettes with sterile, RNase-free filters tips
- Biohazard waste container
- Disposable gloves, powderless
- Refrigerator, Freezer

Zone 2: Real Time amplification:

- Real Time Thermalcycler
- Tubes or PCR plate
- Workstation
- Pipettes with sterile, RNase-free filters tips
- Tube racks

STORAGE INSTRUCTIONS

Streptococcus pyogenes Real-TM Quant must be stored at -20°C. The **DNA/RNA Prep** must be stored at 4-8°C. The kits can be shipped at 2-8°C but should be immediately stored at 2-8°C and -20°C on receipt.

STABILITY

Streptococcus pyogenes Real-TM Quant Test 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.

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

The user should always pay attention to the following:

- ⚠ Lysis Solution contains guanidine thiocyanate*. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- ⚠ Component Prec Sol contains 2-propanol: flammable. Irritant. (R10-36-67, S7-16-24/25-26). Avoid contact with skin and eyes, S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
- 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 other 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 the techniques of DNA amplification.
- 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.

**** Only for Module No.2***

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.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Streptococcus pyogenes Real-TM Quant can analyze DNA extracted from:

- *swabs;*
- *plasma;*
- *CSF;*

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

DNA 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 kit:

- ⇨ **DNA/RNA-Prep** (Sacace, REF K-2-9);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006).

Please carry out DNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

SPECIMEN AND REAGENT PREPARATION*

1. Prepare required number of 1.5 ml disposable polypropylene micro centrifuge tubes including one tube for Negative Control of Extraction (**Negative Control**, **C-**) and one tube for Positive Control of Extraction (**Positive Control** DNA).
2. Add to each tube **10 µl** of **Internal Control** and **300 µl** of **Lysis Sol**
3. Add **100 µl** of samples to the appropriate tubes using pipette tips with aerosol barriers.
4. Prepare Controls as follows:
    - add **100 µl** of **C–** (**Neg Control** provided with the amplification kit) to the tube labeled C*neg*
    - add **90 µl** of **Negative Control** (provided with the amplification kit) and **10 µl** of **Positive Control** to the tube labeled C*pos.*
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 7-10 sec.
6. Add **400 µl** of **Prec Sol** and mix by vortex.Centrifuge all tubes at 13,000 r/min for 5 min and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
7. Add **500 µl of Wash Sol 3** into each tube. Vortex vigorously to ensure pellet washing. Centrifuge all tubes at 13,000 r/min for 60 sec and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
8. Add **200 µl of Wash Sol 4** into each tube. Vortex vigorously to ensure pellet washing. Centrifuge all tubes at 13,000 r/min for 60 sec and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
9. Incubate all tubes with open caps at **65 °C for 5 min**.
10. Resuspend the pellet in **50 µl of RE-buffer** (elution volume can be increased up to 90 µl). Incubate for 5 min at 65°C and vortex periodically.
11. Centrifuge the tubes at 13000g for 60 sec.

The supernatant contains DNA ready for amplification. If amplification is not performed the same day of extraction, the processed samples can be stored at 2-8°C for at maximum period of 5 days or frozen at - 20°/-80°C.

only for TB82-100FRT

PROTOCOL

1. Prepare required quantity of tubes or PCR plate.
2. Prepare for each sample in the new sterile tube **10*N µl** of **PCR-mix-1**, **5*N µl** of **PCR-buffer FRT.**
3. Add **15 µl** of **Reaction Mix** into each tube.
4. Add **10 µl** of **extracted DNA** sample to appropriate tube with Reaction Mix.
5. Prepare for qualitative run 1 positive control and 1 negative control:
   - add **10 µl** of **QSG2** to the tube labeled C*pos*;
   - add **10 µl** of **TE-buffer** to the tube labeled C*neg*;
6. For quantitative analysis prepare 4 tubes and perform QSG1 and QSG2* standards twice.
   **QSG1 and QSG2 values are specific for each lot and are reported in the Quant Data Card provided in the kit.*

Close tubes and transfer them into the instrument in this order: samples, negative controls, positive control, Standards.

Create a temperature profile on your Real-time instrument as follows:

Cycle	Temp, °C	Time	Fluorescence detection	Cycle repeats
1	95	15 min	-	1
2	95	10 s	-	45
	60	30 s	FAM(Green), JOE(Yellow)	

For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen), *SaCycler-96™ (Sacace), CFX96™*/iQ5™/iQ iCycler™ (BioRad); Mx3000P/Mx3005P™ (Stratagene), Applied Biosystems® 7300/7500 Real Time PCR (Applera), SmartCycler® (Cepheid)

INSTRUMENT SETTINGS

Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)

Channel	Calibration/Gain Optimization	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	from 5FI to 10FI	0.03	10 %	on
JOE/Yellow	from 5FI to 10FI	0.03	10 %	on

Plate- or modular type instruments

For result analysis, set the threshold line at a level corresponding to 10–20% of the maximum fluorescence signal obtained for Pos C+ sample during the last amplification cycle.

RESULTS INTERPRETATION

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line. To set threshold put the line at such level where curves of fluorescence are linear.

- *S. pyogenes* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel;
- Internal Control IC is detected on FAM (Green) channel.

Qualitative analysis

Results are accepted as relevant if positive and negative controls of amplification and extraction are passed.

Results for controls

Control	Stage for control	Ct FAM (Green)	Ct JOE(Yellow)/ HEX/Cy3	Interpretation
NCE	DNA isolation	Pos (<32)	–	OK
Pos C+	DNA isolation, PCR	Pos (<32)	Pos (<30)	OK
NCA	PCR	–	–	OK
QS2	PCR	Pos (<31)	Pos (<31)	OK

- The sample is considered to be positive for *S. pyogenes* if in the channel JOE(Yellow)/HEX/Cy3 the value of **Ct** is different from zero (Ct<40);
- The sample is considered to be uncertain for *S. pyogenes* if its Ct value is more than 40 on JOE(Yellow)/HEX/Cy3 channel. Additional double study of this sample should be conducted;
- Specimens with Ct < 32 in the channel FAM (Green) and absent fluorescence signal in the channel JOE(Yellow)/HEX/Cy3 are interpreted as negative.
- Specimens with absent signal in the FAM (Green) channel are interpreted as invalid.

Quantitative analysis

For each control and patient specimen, calculate the concentration of *S. pyogenes* DNA in 1 ml of sample using following formula:

S. pyogenes **reaction x 100*= copies *S. pyogenes*DNA/ml**

**value using DNA extraction from 100 µl of sample.*

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *S. pyogenes* primers and probes. The specificity of the kit **Streptococcus pyogenes Real-TM Quant** was 100%. The potential cross-reactivity of the kit **Streptococcus pyogenes Real-TM Quant** was tested against the group control (*Streptococcus pyogenes, Staphylococcus aureus, Neisseria meningitides, Haemophilus parainfluenza, Klebsiella pneumonium, Listeria monocytogenes* and other ones). It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **Streptococcus pyogenes Real – TM Quant** allows to detect *S. pyogenes* DNA in 100% of the tests with a sensitivity of not less than 300 copies/ml.

TROUBLESHOOTING

1. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
2. JOE(Yellow)/HEX/Cy3 signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        ⇒ Repeat the DNA extraction with the new set of reagents.
3. Any signal with Negative Control of PCR (TE-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        ⇒ Repeat the PCR preparation with the new set of reagents.

KEY TO SYMBOLS USED

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

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