



**IVD**

For *in Vitro* Diagnostic Use




# Strep B Real-TM Quant

## Handbook

Real Time PCR Kit for quantitative  
detection of  
*Streptococcus agalactiae*

**REF** B77-100FRT

 **100**

## NAME

### Strep B Real-TM Quant

## INTRODUCTION

***Streptococcus agalactiae*** (also known as **Group B streptococcus** or **GBS**) is a beta-hemolytic Gram-positive streptococcus. *S. agalactiae* is a member of the gastrointestinal normal flora in some humans and can spread to secondary sites - including the vagina in 10-30% of women. *S. agalactiae* can be transferred to a neonate passing through the birth canal and can cause serious group B streptococcal infection. In the western world, *S. agalactiae* is the major cause of bacterial septicemia of the newborn, which can lead to death or long-term sequelae. *S. agalactiae* invades via alveolar and pulmonary epithelial cells; newborns are especially susceptible to infection because they lack alveolar macrophages to prevent invasion. Newborn GBS disease is separated into early-onset disease occurring on living days 0-7 and late-onset disease which starts on days 7-90. Early-onset septicemia is more prone to be accompanied by pneumonia, while late-onset septicemia is more often accompanied by meningitis. *S. agalactiae* neonatal meningitis does often not present with the hallmark sign of adult meningitis, a stiff neck; rather, it presents with nonspecific symptoms, such as fever, vomiting and irritability, and can consequently lead to late diagnosis. Infection with GBS is the cause of some instances of stillbirth.

*S. agalactiae* is present in up to one-third of women of childbearing age, and 1.8 cases per 1000 live births will be affected by group B streptococcal infection. In the elderly or persons with compromised immune systems, septicemia or other serious infections are seen. This can also occur during pregnancy or maternity-

In the United States all pregnant women are screened for *S. agalactiae* and prophylactic antibiotics are given to all women testing positive.

## INTENDED USE

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

## PRINCIPLE OF ASSAY

**Strep B Real-TM Quant** kit is a Real-Time test for the Qualitative and Quantitative detection of *S. agalactiae* in the biological materials. DNA is extracted from samples, amplified and detected using fluorescent reporter dye probes specific for *S. agalactiae* DNA, Internal Control IC and endogenous IC glob ( $\beta$ -globine gene).

$\beta$ -globin gene DNA is a part of human genome DNA and it should be present in an adequate amount in DNA sample, obtained from the cells. There must be no less than 20 000 genomes per sample (DNA from 10 000 cells). 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, while endogenous IC ( $\beta$ -globin gene), present in all samples obtained from cells (swabs) allows not only to control analysis steps, but also to estimate sample handling and storage. *S. agalactiae* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel, the IC glob ( $\beta$ -globin gene) DNA amplification is detected on FAM (Green) channel and exogenous Internal Control IC is detected on Rox (Orange)/TexasRed channel.

## MATERIALS PROVIDED

### Real Time PCR kit (B77-100FRT)

“Strep B Real-TM Quant”: Real Time amplification

- **PCR-mix-1**, 1,2 ml;
- **PCR-buffer FRT**, 0,6 ml;
- **Hot Start DNA Polymerase**, 0,06 ml;
- **TE-buffer**, 0,5 ml;
- **Negative Control C-\***, 1,2 ml;
- **Pos C+ (*S. agalactiae* DNA&IC Glob)\*\***, 0,1 ml;
- **Internal Control IC\*\*\***, 2 x 0,6 ml;
- **Standard *S. agalactiae* DNA/IC glob:**
  - **QSG1**, 0,2 ml;
  - **QSG2**, 0,2 ml.

Contains reagents for 100 tests

\* must be used during the sample preparation procedure: add 100  $\mu$ l of Negative Control C– to labeled Cneg;

\*\* add 90  $\mu$ l of Negative Control C– and 10  $\mu$ l of Pos C+ to the tube labeled Cpos. Pos C+ is the control with note concentration of *S. agalactiae* DNA (value is specific for each lot and reported in the Quant Data Card provided in the kit)

\*\*\*add 10  $\mu$ l 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
- 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

**Strep B Real-TM Quant** must be stored at -20°C. The **Strep B Real-TM Quant** kit can be shipped at 2-8°C but should be immediately stored at -20°C on receipt.

## STABILITY

**Strep B 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



### ***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

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 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.

## 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

**Strep B 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 STD DNA Extraction kit\*** (Sacace, REF SM007);

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.

\* *Before performing the extraction, incubate the cellular pellet with 200 µl of Lysozyme solution 20 mg/ml for 30 min. Add 200 µl of sterile PBS, mix and use for DNA extraction according to the user manual.*

## PROTOCOL

1. Prepare required quantity of tubes or PCR plate.
2. Prepare the **Reaction Mix**. For each sample in the new sterile tube add **10\*N µl of PCR-mix-1**, **5\*N µl of PCR-buffer FRT** and **0,5\*N of Hot Start DNA Polymerase**.
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 *Cpos*;
  - add **10 µl of TE-buffer** to the tube labeled *Cneg*;
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 and standards.

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

	Rotor type instruments <sup>1</sup>				Plate type or modular instruments <sup>2</sup>			
Stage	Temp, °C	Time	Fluorescence detection	Cycle repeats	Temp, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1	95	15 min	–	1
Cycling	95	5 s	–	5	95	5 s	–	5
	60	20 s	–		60	20 s	–	
	72	15 s	–		72	15 s	–	
Cycling 2	95	5 s	–	40	95	10 s	–	40
	60	20 s	FAM(Green), JOE(Yellow), Rox (Orange)		60	40 s	FAM, JOE/HEX/Cy3, Rox/TexasRed	
	72	15 s	–		72	15 s	–	

<sup>1</sup> For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)

<sup>2</sup> For example, SaCycler-96™ (Sacace), iQ5™/iQ iCycler™ (BioRad); Mx3000P/Mx3005P™ (Stratagene), Applied Biosystems® 7300/7500 Real Time PCR (Applied), SmartCycler® (Cepheid)

## INSTRUMENT SETTINGS

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

Channel	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	0.03	10 %	on
JOE/Yellow	0.03	10 %	on
Rox/Orange	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. agalactiae* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel;
- IC glob (β-globin gene) DNA amplification is detected on FAM (Green) channel (only for the total DNA extraction from cell suspension (swabs))
- Exogenous Internal Control IC is detected on Rox (Orange)/TexasRed 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	Ct Rox (Orange)/TexasRed	Interpretation
NCE	DNA isolation	–	–	Pos (<25)	OK
Pos C+	DNA isolation, PCR	Pos (<30)	Pos (<30)	Pos (<25)	OK
NCA	PCR	–	–	–	OK
QS2	PCR	Pos (<31)	Pos (<31)	Pos (<31)	OK

- The sample is considered to be positive for *S. agalactiae* if in the channel JOE(Yellow)/HEX/Cy3 the value of **Ct** is different from zero (Ct<35);
- The sample is considered to be uncertain for *S. agalactiae* if its Ct value is more than 35 on JOE(Yellow)/HEX/Cy3 channel. Additional double study of this sample should be conducted;
- Specimens with Ct < 27 in the channel FAM (Green) (only for cell suspension), Ct < 25 in the channel Rox (Orange)/TexasRed and absent fluorescence signal in the channel JOE(Yellow)/HEX/Cy3 are interpreted as negative.
- Specimens with absent signal in the FAM (Green) (only for cell suspension) and Rox (Orange)/TexasRed are interpreted as invalid.

### Quantitative analysis

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

***S. agalactiae* DNA copies/reaction x 100\*= copies *S. agalactiae* 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. agalactiae* primers and probes. The specificity of the kit **Strep B Real-TM Quant** was 100%. The potential cross-reactivity of the kit **Strep B Real-TM Quant** was tested against the group control (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Neisseria meningitides*, *Haemophilus parainfluenza*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and other ones). It was not observed any cross-reactivity with other pathogens.











### Analytical sensitivity

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

## TROUBLESHOOTING

1. Weak or no signal of the IC (Rox (Orange)/TexasRed channel).
  - The PCR was inhibited.
    - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
  - The reagents storage conditions didn't comply with the instructions.
    - ⇒ Check the storage conditions
  - The PCR conditions didn't comply with the instructions.
    - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
2. 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.
3. JOE(Yellow)/HEX/Cy3 or Fam(Green) 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.
4. Any signal with Negative Control of PCR (DNA-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

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

\* SaCycler™ is a registered trademark of Sacace Biotechnologies  
\* Cycler™ and iQ5™ are trademarks of Bio-Rad Laboratories  
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