

INSTRUCTIONS FOR USE

PCG® I-GBS REAL-TIME PCR KIT

IVD

1. KIT CONTENT

REF	PI2600050
Package	50 tests
PCR Master Mix	1 x 1040 µL
Taq DNA Polymerase	1 x 8 µL
Positive Control	1 x 200 µL

2. STORAGE AND TRANSPORTATION CONDITIONS

- The kit is stable until the expiry date printed on the package when stored at -20 °C (±5 °C).
- The components are stable for up to 3 freeze-thaw cycles.
- Store PCR Master Mix protected from light.
- Keep DNA Taq Polymerase on a cooling block during the working steps.

3. TECHNICAL SPECIFICATIONS

Regulatory Status	IVD
Function	Multiplex Detection Real-time PCR
What is Detected/ Target	Group B <i>Streptococcus</i> (<i>Streptococcus agalactiae</i>)
Target sequence	<i>cfb</i> gene
Type of Analysis	Qualitative
Validated Specimen	Vaginal - rectal swab
Sensitivity (LoD with 95% probability)	100 CFU/mL
Extraction/ Inhibition Control	Proper sampling, DNA extraction efficiency and PCR inhibition control by Internal Control (<i>beta-Actin</i> gene)
Applied Instruments	QuantStudio 5 Real-time PCR System
	QuantStudio 7 Real-time PCR System
	CFX96 Real-time PCR Detection System
Detection Channels	FAM (GBS), HEX/VIC (Internal Control)

4. INDICATION AND METHOD PRINCIPLE

Indication	The test kit is recommended to be used to detect Group B <i>Streptococcus</i> (GBS) in vaginal-rectal swab. Regardless of the planned mode of birth, all pregnant women should undergo antepartum screening for GBS at 36 0/7 – 37 6/7 weeks of gestation (ACOG 2019)
Principle	The GBS detection is based on the amplification of a 99 bp DNA sequence of <i>cfb</i> gene and in measurement of fluorescence increase. The detection kit takes advantage of the “hot start” technology, minimizing non-specific reactions and assuring the maximum sensitivity. PCR Master Mix contains primers and probes for <i>cfb</i> gene and for Internal Control (<i>β-actin</i> gene) eliminating possible inhibition of the PCR and the DNA extraction process quality. The GBS presence is indicated by the FAM fluorophore. <i>β-actin</i> amplification is detected in the HEX channel.

5. PROCEDURES

SAMPLING AND SAMPLE STORAGE

- Use sterile swabs free of DNase and RNase to collect vaginal–rectal swab specimens according to CDC guidelines, and transport them immediately to the laboratory.
- According to the ASM 2020 recommendation, specimens should be incubated in selective enrichment broth for 18–24 hours at 35–37°C before proceeding with subsequent testing steps.

NUCLEIC ACID EXTRACTION

DNA extraction from clinical samples should be performed according to the correct procedure and recommended using:

- ▶ PCG® MagBead DNA Extraction Kit (Phacogen)

PCR SETUP

- Before use, thaw all reagents, DNA samples, Positive Control and Negative Control, invert and place them in a cooling block.
- Taq DNA Polymerase must always be stored at -20°C (±5°C) and only placed in a cooling block when in use.
- To limit the risk of contamination, the steps involving the Positive Control and DNA samples are kept separate from those involving the PCR Master mix and Taq DNA Polymerase.
- Dispense 1040 µL of PCR Master Mix into the Taq DNA Polymerase tube. Mix the contents thoroughly by pipetting, invert the tube 5–10 times, and then briefly spindown briefly.
- Each 20 µL of the mixture of PCR Master Mix and Taq DNA Polymerase is divided into Real-time PCR tubes.
Note: These real-time PCR tubes must be stored at -20°C (±5°C), away from light for up to 1 month. Repeated thawing (> 2x) should be avoided.
- Add 10 µL of specimen DNA or control.
- Seal each well completely with sealing film or cap, spindown briefly.

AMPLIFICATION PROFILE

Follow the thermocycler manufacturer's manual for setting the instrument and for analysis:

Step	Temperature	Duration	Cycles
Hold	95°C	15 min	1
PCR	94°C	30 sec	45
	58°C	30 sec (FAM + HEX/VIC)	
	72°C	30 sec	

6. DATA ANALYSIS & RESULTS INTERPRETATION

Confirm "Positive result" for Ct ≤ 40.0 (+) , "Negative result" for Ct > 40.0 (-)

Control Samples

Control Samples	Channel FAM (GBS)	Channel HEX/VIC (IC)
Negative Control	-	-
Positive Control	+	+

Clinical samples

No.	Result		Interpretation
	Channel FAM (GBS)	Channel HEX/VIC (IC)	
1	+	+/-	Positive
2	-	+	Negative
3	-	-	Invalid*

*: Require repeated extraction and followed by Real-time PCR

7. MATERIAL AND EQUIPMENT REQUIRED NOT PROVIDED WITH THE KIT

Devices	Consumable Material
- Real-time PCR instrument: CFX96 Real-time PCR System (Bio-Rad), QuantStudio 5/7 Real-time PCR System (Thermo Fisher Scientific) - Vortex mixer - Spindown centrifuge - Pipettes - Cooling block (1.5 mL, 0.2 mL) - PCR cabinet	- PCR tubes or plates - Pipette tips with filters - Personal Protective Equipment

8. WARNINGS, PRECAUTIONS AND PROCEDURE LIMITATIONS

- The optimal volume for each reaction is 30 µL. Any change in this volume may lead to incorrect results.
- The workflow in the laboratory should be proceed in unidirectional manner from the PCR mix room, the nucleic acid extraction room, and the amplification room, do not go opposite directions. Each area has its own devices and materials.
- Specimens and related items should be handled as a source of infection.
- Always wear protective disposable powder-free gloves when handling. Change gloves if there is any suspicion of cross-contamination or as needed.
- Before and after work, it is necessary to clean working area with PCG® DNAzap (Phacogen, Cat.No PR6000500) or equivalent.
- The presence of PCR inhibitors may cause false negative or invalid results.
- The kit must be performed by a trained person.
- Analytical steps and precautions must be strictly followed to obtain accurate results.
- Specimens are collected, transported, and stored according to proper procedures and under appropriate conditions. Improper collection, transportation, or storage of samples can give incorrect test results.
- Do not mix components from kits with different lot numbers.
- Use always sterile pipette tips with filters to avoid cross-contamination.

9. EXPLANATION OF SYMBOLS

Symbols	Explanation
	Manufacturer
	Instructions for use
	Contains sufficient amount for n-tests
	Catalogue number
	Lot number
	Expiry date
	Temperature limitation

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