Instruction for Use

# diarellaGBS

## real time PCR Kit

For qualitative *in vitro* detection of *Streptococcus agalactiae* (Group B *Streptococcus* (GBS)) DNA in clinical specimens.

| | | |
|---|---|---|
| REF | G01101-32 | G01101-96 |
| Σ | 32 | 96 |

- 18°C

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# 1 Intended Use

The diarellaGBS real time PCR is an assay for the detection of the DNA of GBS in clinical specimens.

# 2 Pathogen Information

Streptococci mainly colonise mucous membranes in humans. Beta-hemolyzing streptococci of serogroup B (GBS) in the maternal genital tract can be transmitted to the child during birth. The Transmission may lead to early onset illness within the first hours up to 3 days after birth with serious systemic infection and pneumonia. Aetiopathology with shock symptoms and neurologic long-term damages may occur. Late onset illness can be cuased by transmission by the mother or e.g. nursing staff. Early onset infection occurs within app. 1 of 2.000 newborns. The affected majority (app. 80 %) are mature newborns. App. 4 % of the diseased children die. The following factors promote the transmission of GBS:

- Presence of GBS in high titers in the maternal genital tract at birth.
- Period of more than 18 h between rupture of membranes and confinement.
- Maternal fever during birth.
- Preterm birth before week 37 of pregnancy.
- GBS present in maternal urine during pregnancy.
- Previous birth of a child with GBS infection.

In order to minimize the risk of GBS-transmission to newborns, pregnant women should be examined between week 35 and 37 of pregnancy. In case of a GBS-positive result, antibiotics treatment of the mother during birth can be done. In case of a negative result within 5 weeks before birth, antibiotics treatment of the mother can be avoided.

# 3 Principle of the Test

The diarellaGBS real time PCR contains specific primers and dual-labeled probes for the amplification and detection of GBS DNA in clinical specimens.
The presence of nucleic acid is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the pathogen-specific probes is measured in the FAM channel.

Furthermore, diarellaGBS real time PCR contains a Control DNA, which is added during DNA extraction and detected in the same reaction by a differently labeled probe.
The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acid was isolated from the clinical specimen. The fluorescence of the Control DNA is measured in the VIC®/HEX/JOE™/TET channel.

# 4 Package Contents

The reagents supplied are sufficient for 32 or 96 reactions respectively.

Table 1: Components of the diarellaGBS real time PCR Kit

| Label | Lid Colour | Content | |
|---|---|---|---|
| | | 32 | 96 |
| Reaction Mix | yellow | 1 x 512 µl | 2 x 768 µl |
| Positive Control | red | 1 x 50 µl | 1 x 100 µl |
| Negative Control | green | 1 x 50 µl | 1 x 100 µl |
| Control DNA | colourless | 1 x 160 µl | 2 x 240 µl |

# 5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Pure RNA/DNA, gerbion Cat. No. G05004 or NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation
- Optional: BLP-DNA (Bacterium-Like Particles, please look at chapter ‚Control DNA' for details.)

# 6 Transport, Storage and Stability

The diarellaGBS real time PCR Kit is shipped on dry ice or cool packs. All components must be stored at -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package.
Up to 20 freeze and thaw cycles are possible.
For convenience, opened reagents can be stored at +2-8°C for up to 6 months.
Protect kit components from direct sunlight during the complete test run.

# 7 Important Notes

- The diarellaGBS real time PCR must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Clinical samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

# 8 General Precautions

- Stick to the protocol described in the Instruction for Use.
- Set up different laboratory areas for the preparation of samples and for the set up of the PCR in order to avoid contaminations.
- Pipettes, tubes and other materials must not circulate between those different laboratory areas.
- Always use filter tips.
- Regulary decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine diarellaGBS real time PCR components of different lot numbers.

# 9 Sample Material

Starting material for the diarellaGBS real time PCR is DNA isolated or released from clinical specimens (e.g. vaginal swabs).

# 10 Sample Preparation

The diarellaGBS real time PCR is suitable for the detection of GBS DNA isolated from clinical specimens with appropriate isolation methods.
Commercial kits for DNA isolation such as the following are recommended:

- NukEx Pure RNA/DNA, gerbion Cat. No. G05004
- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

**Important:**
In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.
Comparing the amplification of the Control DNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

**Please note the chapter ‚Control DNA'.**

If the real time PCR is not performed immediately, store extracted DNA according to the instructions given by the manufacturer.

# 11 Control DNA

A Control DNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.
The Bacterium-Like Particles (BLP-DNA) is not supplied.

**DNA isolation from clinical specimens**

a) Control DNA or BLP-DNA used as Extraction Control:

diarellaGBS real time PCR Control DNA or BLP-DNA is added to the DNA extraction.
Add 5 µl Control DNA or BLP-DNA per extraction (5 µl x (N+1)). Mix well. Perform the DNA isolation according to the manufacturer's instructions. Please follow protocol A.

**The Control DNA must be added to the Lysis Buffer of the extraction kit.**

b) Control DNA used as Internal Control of the real time PCR:

If only inhibition will be checked please follow protocol B.

# 12 Real time PCR

## 12.1 Important Points Before Starting:

- Please pay attention to the chapter 7 ‚Important Notes'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed, and centrifuged very briefly.

## 12.2 Procedure

If the Control DNA or BLP-DNA is used to control both, the real time PCR and the DNA isolation procedure, please follow protocol A. If the Control DNA is solely used to detect possible inhibition of the real time PCR, please follow protocol B.

Protocol A

**The Control DNA or BLP-DNA was added during DNA extraction (chapter 11 ‚Control DNA'). In this case, prepare the Master Mix according to Table 2.**

The Master Mix contains all of the components needed for PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2:Preparation of the Master Mix (Control DNA was added during DNA extraction)

| Volume per Reaction | Volume Master Mix |
|---|---|
| 16.0 µl Reaction Mix | 16.0 µl x (N+1) |

## Protocol B

**The Control DNA is used for the control of the real time PCR only (see chapter 11 ‚Control DNA'). In this case, prepare the Master Mix according to Table 3.**

The Master Mix contains all of the components needed for real time PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 3: Preparation of the Master Mix (Control DNA is added directly to the Master Mix)

| Volume per Reaction | Volume Master Mix |
|---|---|
| 16.0 µl Reaction Mix | 16.0 µl x (N+1) |
| 0.5 µl Control DNA* | 0.5 µl x (N+1)* |

*The increase in volume caused by adding the Control DNA is not taken into account when preparing the PCR assay.

## Protocol A and B: real time PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument.
- Pipet **16 µl** of the Master Mix into each optical PCR reaction tube.
- Add **4 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube (Table 4).
- Close the optical PCR reaction tubes immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time PCR

| Component | Volume |
|---|---|
| Master Mix | 16.0 µl |
| Sample | 4.0 µl |
| Total Volume | 20.0 µl |

## 12.3 Instrument Settings

For the real time PCR use the thermal profile shown in Table 5.

Table 5: real time PCR thermal profile

| Description | Time | Temperature | Number of Cycles |
|---|---|---|---|
| *Reverse Transcription* | 10 min | 45°C | 1 |
| *Initial Denaturation* | 5 min | 95°C | 1 |
| *Amplification of DNA* | | | |
| Denaturation | 10 sec | 95°C | 45 |
| Annealing and Extension | 40 sec<br>Aquisition at the end of this step | 60°C | |

Dependent on the real time PCR instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the diarellaGBS real time PCR.

| Real time PCR Instrument | Parameter | Detection Channel | Notes |
|---|---|---|---|
| LightCycler 480II | GBS | FAM (465-510) | pre-installed universal Color Compensation FAM (510) – VIC (580) |
| | Control DNA | HEX (533-580) | |
| Stratagene Mx3000P / Mx3005P | GBS | FAM | Gain 8 Reference Dye: None |
| | Control DNA | HEX | Gain 1 |
| ABI 7500 | GBS | FAM | Option Reference Dye ROX: NO |
| | Control DNA | JOE | |
| Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000 | GBS | Green | Gain 5 |
| | Control DNA | Yellow | Gain 5 |

# 13 Data Analysis

The GBS specific amplification is measured in the FAM channel. The amplification of the Control DNA is measured in the VIC®/HEX/JOE™/TET channel.

Following results can occur:

- A signal in the FAM channel is detected:
  The result is positive, the sample contains GBS DNA.
  In this case, detection of a signal of the Control DNA in the VIC®/HEX/JOE™/TET channel is inessential, as high concentrations of bacterial DNA may reduce or completely inhibit amplification of the Control DNA.

- No signal in the FAM channel, but a signal in the VIC®/HEX/JOE™/TET channel is detected:
  The result is negative, the sample does not contain GBS DNA.
  The signal of the Control DNA excludes the possibilities of DNA isolation failure (in case the Control DNA is being used as an Extraction Control) and/or real time PCR inhibition. If the $C_T$ value of a sample differs significantly from the $C_T$ value of the water control, a partial inhibition occured, which can lead to negative results in weak positive samples (see chapter ‚Troubleshooting').

- Neither in the FAM nor in the VIC®/HEX/JOE™/TET channel a signal is detected:
  A diagnostic statement cannot be made.
  The DNA isolation was not successful or an inhibition of the PCR has occurred. In case the Control DNA was added during DNA isolation and not directly to the PCR Master Mix, the Negative Control is negative in both channels.

**Figure 1** and **Figure 2** show examples for positive and negative real time PCR results.

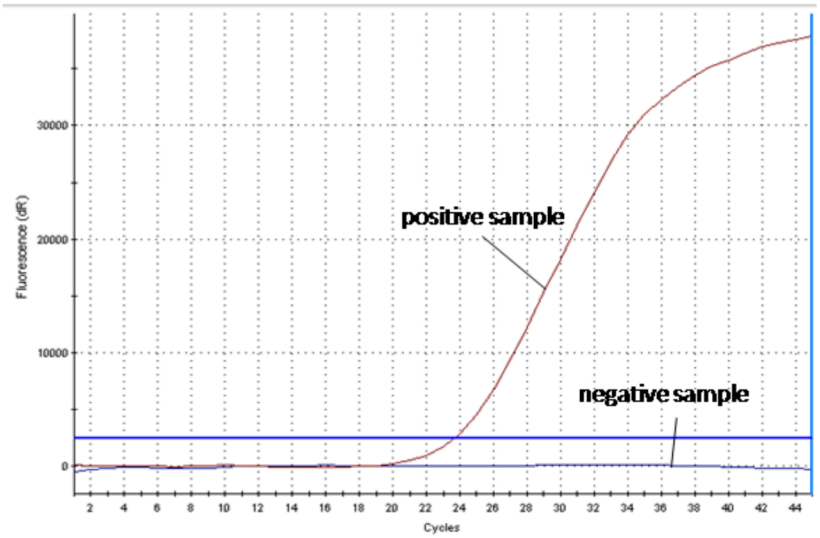


Figure 1: The positive sample shows bacteria specific amplification in the FAM channel, whereas no fluorescence signal is detected in the negative sample.

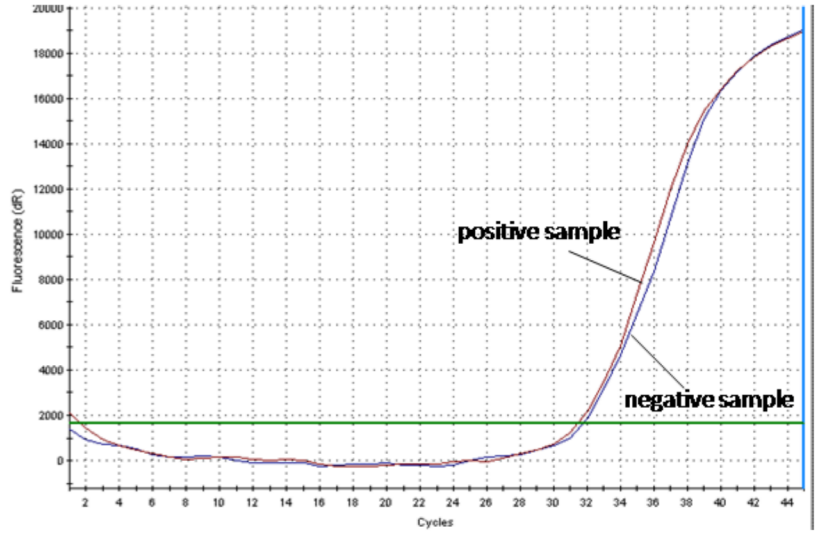


Figure 2: The positive sample as well as the negative sample show a signal in the Control DNA specific VIC®/HEX/JOE™/TET channel. The amplification signal of the Control DNA in the negative sample shows, that the missing signal in the bacteria specific FAM channel is not due to PCR inhibition or failure of DNA isolation, but that the sample is a true negative.

# 14 Assay Validation

Set a threshold as follows:

### Negative Controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high $C_T$ – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

### Positive Controls

All the positive controls must show a positive (i.e. exponential) amplification curve. The positive controls must fall below a $C_T$ of 30.

### Internal Controls

All internal controls must show a positive (i.e. exponential) amplification curve. The internal control must fall below a $C_T$ of 33. If the internal control is above $C_T$ 34, this points to a purification problem or a strong positive sample that can inhibit the IC. In the latter case, the assay is valid. If a water control run is performed, the IC must fall below a $C_T$ of 33.

# 15 Limitations of the Method

The results must always be considered in relation to the clinical symptoms. Therapeutical consequences should be made in consideration of clinical data.
A negative test result does not exclude a GBS infection.

# 16 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

| No fluorescence signal in the FAM channel of the Positive Control | |
|---|---|
| The selected channel for analysis does not comply with the protocol | Select the FAM channel for analysis of the bacteria specific amplification and the VIC®/HEX/JOE™/TET channel for the amplification of the Control DNA. |
| Incorrect configuration of the real time PCR | Check your work steps and compare with ‚Procedure' on page 7. |
| The programming of the thermal profile is incorrect | Compare the thermal profile with the protocol (Table 5, page 9). |

| | |
|---|---|
| Incorrect storage conditions for one or more kit components or kit expired | Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability', page 5. |
| **Weak or no signal of the Control DNA and simultaneous absence of a signal in the bacteria specific FAM channel.** | |
| real time PCR conditions do not comply with the protocol | Check the real time PCR conditions (page 7). |
| real time PCR inhibited | Make sure that you use an appropriate isolation method (see chapter ‚Sample Preparation') and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed. An additional centrifugation step at high speed is recommended before elution of the DNA. |
| DNA loss during isolation process | In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol. |
| Incorrect storage conditions for one or more components or kit expired | Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability'. |
| **Detection of a fluorescence signal in the FAM channel of the Negative Control** | |
| Contamination during preparation of the PCR | Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR. |

# 17 Kit Performance

## 17.1 Diagnostic Sensitivity and Specificity

During the validation study of the diarellaGBS real time PCR 7 positive and 41 negative samples were tested. The diagnostic sensitivity was found to be 100% and the diagnostic specificity 100 %

Table 7: Overview of the amount of samples tested and the resulting positive and negative predictive values

| | positive samples | negative samples |
|---|---|---|
| diarellaGBS positive | 7 | 0 |
| diarellaGBS negative | 0 | 41 |

| | |
|---|---|
| **Sensitivity** | 100 % |
| **Specificity** | 100 % |

## 17.2 Analytical Sensitivity

The limit of detection (LoD) of diarellaGBS real time PCR was determined using serial dilutions of synthetic target DNA-sequences in a Stratagene Mx3000 real time PCR instrument. The LoD of diarellaGBS real time PCR for GBS is at least 10 target copies per reaction each.

## 17.3 Analytical Specificity

The specificity of the diarellaGBS real time PCR was evaluated additionally with different other relevant viruses and bacteria found in clinical samples.

Results:

The diarellaGBS real time PCR showed a positive result for the sample containing GBS, whereas samples containing other pathogens were reliably tested negative. The results are shown in Table 8.

Table 8: Bacterial and viral pathogens tested for the determination of the analytical specifity of the diarellaGBS real time PCR Kit.

| **Strain** | **Expected Result** | **Result** |
|---|---|---|
| *Streptococcus agalactiae* | positive | positive |
| *Adenovirus Adenoid 6* | negative | negative |
| *Campylobacter jejuni* (DSZM 4688) | negative | negative |
| *Chlamydophila pneumoniae (*ATCC 15531) | negative | negative |
| *Citrobacter freundii* (ATCC 8090) | negative | negative |
| *Coxsackievirus A9 Strain P.B.* | negative | negative |
| *Enterococcus faecalis* (ATCC 29212) | negative | negative |
| *Enterovirus 68* | negative | negative |
| *Escherichia coli* (ATCC 15597) | negative | negative |
| *Herpes simplex Virus (HSV) Type 2 Str. G* | negative | negative |
| MRSA N315 | negative | negative |
| *Pseudomona aeruginosa* (DSMZ 50071/ ATCC 10145) | negative | negative |
| Varizella-Zoster-Virus (VZV) ATCC-VR-1367 | negative | negative |

# 18 Abbreviations and Symbols

| | | | |
|---|---|---|---|
| DNA | Deoxyribonucleid Acid |  | Upper limit of temperature |
| PCR | Polymerase Chain Reaction |  | Manufacturer |
| GBS | Group B *Streptococcus* |  | Use by YYYY-MM |
|  | Reaction Mix |  | Batch code |
|  | Positive Control |  | Content |
|  | Negative Control |  | Consult Instructions for use |
|  | Control DNA |  | *In vitro* diagnostic medical device |
|  | Catalog number |  | European Conformity |
|  | Contains sufficient for <n> test | | |

# 19 Literature

[1] Larsen JW, Sever JL. Group B Streptococcus and pregnancy: A review. Am J Obstet Gynecol 2008; 198:440-50.

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[3] Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Heath PT. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet 2012; 379:547-56.

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[5] Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease - revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59:1 -36.