

artus[®] GBS QS-RGQ Kit Handbook



Version 1



Qualitative in vitro diagnostics

For use with QIA Symphony[®] SP/AS and Rotor-Gene[®] Q instruments



4576366



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Intended Use

The *artus* GBS QS-RGQ kit is a real-time PCR DNA amplification assay performed on the QIAasymphony SP/AS and Rotor-Gene Q instruments for the direct qualitative detection of Group B Streptococcus (GBS) from enriched Lim broth cultures (grown for 18–24 hours) obtained from vaginal/rectal swab specimens from antepartum women.

The *artus* GBS QS-RGQ kit is intended for use as an aid in detecting GBS colonization in antepartum women. Culture isolates are required for performing susceptibility testing as recommended for penicillin-allergic women.

Summary and Explanation

The *artus* GBS QS-RGQ Kit constitutes a ready-to-use system for the detection of Group B Streptococcus DNA using polymerase chain reaction (PCR) on Rotor-Gene Q instruments with sample preparation and assay setup using the QIAasymphony SP and AS instruments.

Pathogen information

Group B Streptococci (GBS), including *Streptococcus agalactiae*, are Gram-positive, beta-hemolytic, chain-forming cocci that are the leading cause of life-threatening sepsis and meningitis in newborn infants, leading to a high rate of morbidity and mortality (1). Approximately 25% of pregnant women are colonized with GBS, and can transmit the bacteria to newborns *in utero* or during vaginal birth. The Centers for Disease Control and Prevention (CDC) guidelines recommend screening of antepartum women during weeks 35–37 of her pregnancy in order to prevent transmission to newborns (2). Nucleic acid amplification tests have been proven to be more sensitive than traditional culture methods and may help identify a larger population of GBS-colonized mothers (3).

Principle of the Procedure

The GBS Master A and GBS Master B contain reagents and enzymes for the specific amplification of target regions within the GBS genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of Rotor-Gene Q instruments.

In addition, the *artus* GBS QS-RGQ Kit contains a second heterologous control system to identify potential failures during the entire assay process. This is detected as an internal control (IC) in fluorescence channel Cycling Red of Rotor-Gene Q instruments.

Materials Provided

The contents of the *artus* GBS QS-RGQ Kit are sufficient for 72 tests in one to three batches of 24 reactions on the QIA Symphony RGQ. The Rotor Gene Q instrument rotor holds up to 72 reaction tubes.

Kit contents

<i>artus</i> GBS QS-RGQ Kit			(72)
Catalog no.			4576366
Number of reactions			72
Blue	GBS Master A	MASTER A	3 x 330 µl
Violet	GBS Master B	MASTER B	3 x 600 µl
Green	GBS Internal Control	IC	3 x 540 µl
Red	GBS Positive Control	CONTROL +	3 x 330 µl
White	GBS Negative Control	CONTROL -	3 x 330 µl
<i>artus GBS QS-RGQ Kit Handbook</i> (English)			1

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Adapters for the QIA Symphony SP

- Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730) in combination with the QIA Symphony SP/AS Transfer Frame
- Tube Insert 3B (Insert, 2.0 ml v2, samplecarr. (24), Qsym, cat. no. 9242083)

Consumables and reagents for the QIASymphony SP

- QIASymphony DSP Virus/Pathogen Mini Kit (cat. no. 937036)
- Buffer ATL (4 x 50 ml) (cat. no. 939016)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Elution Microtubes CL (EMTR) (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Micro tubes 2.0 ml Type H, without skirted base (cat. nos. 72.693) or Micro tubes 2.0 ml Type I, with skirted base (Sarstedt®, cat. nos. 72.694, www.sarstedt.com) for use with samples and internal controls
- Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning®, cat. no. 352051; BD™ was the previous supplier of this tube; Corning, Inc. is the new supplier), for use with internal controls

Adapters and reagent holders for the QIASymphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
- RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

Consumables for the QIASymphony AS

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)
- Tubes, conical, 5 ml, Qsym AS (cat. no. 997104)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Tip disposal bags (cat. no. 9013395)

General laboratory equipment

- Pipets (adjustable)* and sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes

Equipment for sample preparation and assay setup

- QIA Symphony SP (cat. no. 9001297),* software version 4.0 or higher
- QIA Symphony AS (cat. no. 9001301),* software version 4.0 or higher

Equipment for PCR

- Rotor-Gene Q MDx 5plex HRM instrument*†
- Rotor-Gene AssayManager® version 1.0 or higher

Warnings and Precautions

For in vitro diagnostic use

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

For safety information for the QIA Symphony DSP Virus/Pathogen Mini Kit, see the *QIA Symphony DSP Virus/Pathogen Kit Instructions for Use (Handbook)* supplied with this kit. For safety information regarding the instruments, see the *QIA Symphony SP/AS User Manual — General Description*, *QIA Symphony SP/AS User Manual — Operating the QIA Symphony SP*, *QIA Symphony SP/AS User Manual — Operating the QIA Symphony AS*, *QIA Symphony Management Console User Manual*, *Rotor-Gene AssayManager Core Application User Manual*, *artus Basic Plug-in User Manual*, and the user manual supplied with the Rotor-Gene Q instrument.

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyyynn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "ynn" indicates the unique instrument identifier.

Discard sample and assay waste according to your local safety regulations.

The following hazard and precautionary statements apply to components of the *artus* GBS QS-RGQ Kit:

GBS Positive Control



Contains: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1). Warning! May cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection.

General precautions

Always pay attention to the following:

- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly. Ensure that no foam or bubbles are present in the reagent tubes.
- Do not mix components from kits with different lot numbers.
- Follow universal precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Make sure that the required adapters are precooled to 2–8°C.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between QIAasympy AS and the Rotor-Gene Q instrument).
- Check that maintenance has been performed and replaceable parts (e.g., tip guards) have been reinstalled.
- Check that the Application Process files and required Rotor-Gene AssayManager plug-in are installed.

Reagent Storage and Handling

The components of the *artus* GBS QS-RGQ Kit should be stored at –30 to –10°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>3 x) should be avoided, as this may reduce assay performance. All reagents that are loaded on QIAAsymphony AS are for use in that run only. Do not remove the residual components to use them for a second PCR.

Specimen Handling and Storage

Information about specimen handling and storage for LIM broth samples is given in Table 1.



All specimens must be treated as potentially infectious material.

Table 1. Specimen handling, storage, and preparation for LIM broth samples

Specimen collection	A vaginal-rectal swab is collected and transported to the laboratory using standard bacterial swab transport systems containing a non-nutritive transport medium (e.g., Amies or Stuart). In the lab, the swab is removed from the transport medium and placed into selective Lim Broth (Todd-Hewitt Broth supplemented with colistin [10 µg/ml] and nalidixic acid [15 µg/ml]). After incubation of inoculated Lim Broth culture for 18–24 hours at 35°C ±2°C in ambient air or 5% CO ₂ , an aliquot of the broth is run using the <i>artus</i> GBS QS-RGQ Kit.
Specimen transport	Shatterproof transport Shipment within 24 hours of collection Mail shipment according to legal instructions for the transport of pathogen material* Samples should be shipped cool (2 to 8°C)
Specimen storage (including time needed for transport)	2–8°C for up to 7 days –30 to –10°C for up to 30 days
Sample preparation	Place 350 µl of post-incubation Lim culture broth into a Sarstedt 2.0 ml Micro tube and load onto the QIA Symphony SP

*International Air Transport Association (IATA). Dangerous Goods Regulations.

Procedure

Table 2. General information

Kit	<i>artus</i> GBS QS-RGQ Kit, REF 4576366
Sample material	Enriched Lim broth cultures (grown for 18–24 hours at 35°C ± 2°C) obtained from vaginal/rectal swab specimens from antepartum women
Front-end purification	QIAasymphony DSP Virus/Pathogen Mini Kit (cat. no. 937036)
Sample volume (including excess volume)	350 µl
Assay Parameter Set	<i>artus</i> _GBS_broth200_V1
Default Assay Control Set	Complex200_V6_DSP_ <i>artus</i> _GBS
Elution volume	60 µl
Required QIAasymphony software version	Version 4.0 or higher
Required QIAasymphony SP/AS configuration profile	Default profile 1
Master mix volume	25 µl
Template volume	15 µl
Number of reactions	24–72* (including all controls to be loaded onto QIAasymphony SP and QIAasymphony AS)
Runtime on QIAasymphony SP/AS	For 24 reactions: approximately 90 minutes For 72 reactions: approximately 280 to 290 minutes
Runtime on Rotor-Gene Q instrument	Approximately 120 minutes

* Ensure that the limit of 72 reactions and 1 assay rack adapter is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay run and transfer to the Rotor-Gene Q instrument.

Controls

Positive control

The GBS Positive Control (supplied with the *artus* GBS QS-RGQ Kit) monitors the efficiency of sample preparation and the downstream assay. This positive control is loaded onto the QIAasymphony SP before DNA purification (see step 7, page 20 for further details on loading the positive control).

Negative control

The GBS Negative Control (supplied with the *artus* GBS QS-RGQ Kit) is loaded onto the QIAasymphony SP before DNA purification in place of a patient sample and assists in identifying contamination during sample preparation and/or the downstream assay (see step 7, page 20 for further details on loading the negative control).

Preparation of carrier RNA (CARRIER) and internal control (GBS Internal Control)

Using QIAasymphony DSP Virus/Pathogen Kits in combination with the *artus* GBS QS-RGQ Kit requires introduction of the internal control (GBS Internal Control), consisting of synthetic plasmid DNA in a buffered solution, into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

The internal control (GBS Internal Control), supplied with the *artus* GBS QS-RGQ Kit, must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture. The total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture is 120 μ l per sample.

To prepare the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, add 1350 μ l Buffer AVE (AVE), supplied with the QIAasymphony DSP Virus/Pathogen Mini Kit, to resuspend the lyophilized carrier RNA (CARRIER). Invert tube to mix.

For internal control (IC) calculation, the “IC Calculator” within the QIAasymphony Management Console (QMC) should be used.

Table 3 represents the addition of internal control to the sample at a ratio of 0.1 μ l per 1 μ l elution volume. We recommend preparing fresh mixtures for each run just before use.

Table 3. Preparation of carrier RNA (CARRIER) and internal control (GBS Internal Control)

Component	n = number of samples and controls	
	n ≤ 13 Volume (μl) (Sarstedt tubes)*	n > 13 Volume (μl) (BD™ tubes)†
Stock carrier RNA (CARRIER)	(n + 3) x 3	(n + 5) x 3
Internal control (GBS Internal Control)	(n + 3) x 9	(n + 5) x 9
Buffer AVE (AVE)	(n + 3) x 108	(n + 5) x 108
Final volume per sample (excluding dead volume)	120	120
Total volume for n samples	(n + 3) x 120	(n + 5) x 120

* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694). If preparing the internal control as a stock solution in a larger tube, multiply the total volume of each component by the number of internal control tubes used. Internal control mixture corresponding to 3 additional samples (i.e., 360 μl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples).

If using more than 13 reactions in Micro tubes 2.0 ml, set up the reactions in a larger tube and load in multiple tubes. Make sure that for each tube the required excess volume of 3 additional reactions is added.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning, cat. no. 352051; BD was the previous supplier of this tube; Corning Inc. is the new supplier). Internal control mixture corresponding to 5 additional samples (i.e., 600 μl) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples).

Calculation of mixture by “IC Calculator”

1. Open the QMC.
2. Select the IC Calculator icon.
3. Select “Complex200_V6_DSP_artus_GBS” from the ACS drop-down list.
4. Enter the required number of samples.
5. Select the labware used for the internal control.
6. Select an elution volume of 60 μl.
7. Select “Internal Control/Eluate” and “0.1 μl” for internal control mode.
8. Press “Calculate” to start calculation of internal control mixture.

The IC calculator displays the different volumes of reagents to be mixed for the internal control mixture and the tube type to be used on the right side of the screen.

Assay Control Sets and Assay Parameter Sets

Assay Control Sets are the combination of a protocol plus additional parameters, such as internal control, for sample purification on the QIAasymphony SP. A default Assay Control Set is preinstalled for each protocol.

Assay Parameter Sets are the combination of an assay definition with additional parameters defined, such as replicate count and number of assay standards, for assay setup on the QIAasymphony AS.

For the integrated run on the QIAasymphony SP/AS, the Assay Parameter Set, `artus_GBS_broth200_V1`, is directly linked to the upfront Assay Control Set, `Complex200_V6_DSP_artus_GBS`, specifying the associated sample purification process.

Protocol: DNA isolation and assay setup on the QIAasympphony SP/AS

Important points before starting

- Ensure that you are familiar with operating the QIAasympphony SP/AS instruments. Refer to the user manuals supplied with your instruments and the most current versions available online at www.qiagen.com/products/qiasymphonyrgq.aspx for operating instructions.
- Download the Application Package from “Protocol Files” on the “Resources” tab of the *artus* GBS QS-RGQ Kit web catalog page (www.qiagen.com/p/artus-GBS-QS-RGQ-Kit-CE).
- Before using a reagent cartridge (RC) for the first time, check that Buffers QSL2 and QSB1 in the reagent cartridge (RC) do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.* Allow the reagents to cool down to room temperature (15–25°C).
- Check that Buffer ATL (ATL) does not contain a precipitate. If a precipitate has formed, dissolve by heating the buffer at 70°C with gentle agitation in a water bath.* Aspirate bubbles from the surface, and let the buffer cool to room temperature (15–25°C).
- Try to avoid vigorous shaking of the reagent cartridge (RC) and Buffer ATL (ATL) bottle. Otherwise foam may be generated, which can lead to liquid-level detection problems.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- The reagent volumes are optimized for 3 batches of 24 reactions per kit per run.
- Make sure that eluates from the sample preparation and all components of the *artus* GBS QS-RGQ Kit remain on the instrument for no more than the normal time required for sample purification and assay setup of 72 assay reactions, including up to 30 minutes transfer time from the QIAasympphony AS to the Rotor-Gene Q instrument.

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

- **Note:** Do not use an Elution Microtubes CL rack that has already been used on a different QIAasympphony SP instrument. Do not enter a rack ID manually.

Things to do before starting

- Before each use, all assay reagents in the *artus* GBS QS-RGQ Kit need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents.
- Prepare all required mixtures. If needed, prepare mixtures containing RNA (CARRIER) and internal controls just before starting. For more information, see “Preparation of carrier RNA (CARRIER) and internal control (GBS Internal Control)”, page 12.
- Before starting an integrated run, make sure that all instruments are clean and that the replaceable parts have been loaded (e.g., tip guards) as described in the maintenance instructions in the *QIAasympphony SP/AS User Manual — General Description*, *QIAasympphony SP/AS User Manual — Operating the QIAasympphony SP*, *QIAasympphony SP/AS User Manual — Operating the QIAasympphony AS*, and *QIAasympphony Management Console User Manual* supplied. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.
- Ensure that QIAasympphony process profile “Default Profile 1” is active. The selected profile is shown at the bottom, right corner of the touchscreen. The profile may be changed in the “Configuration” menu of the “Tools” tab by a user logged in as “Supervisor”.

Procedure

Bacterial purification on the QIAasympphony SP

1. Close all drawers and the hoods of the QIAasympphony SP/AS module.
2. Switch on the instrument, and wait until the “Sample Preparation” screen appears and the initialization procedure has finished.
The power switch is located at the bottom, left corner of the QIAasympphony SP.
3. Log in to the instrument.
4. Prepare the “Waste” drawer of the QIAasympphony SP.
 - Open the “Waste” drawer.
 - Empty and install liquid waste bottle. Make sure to remove the lid before placing the liquid waste bottle into the drawer.
 - Insert tip chute.

Note: Different tip chutes must be used for benchtop and QIAasympphony Cabinet SP/AS operation.

- Insert tip park station.
- Insert empty unit boxes (see Table 4 and Figure 1). Make sure that there is at least one empty unit box in slot 4 (closest to you).
- Install empty tip disposal bag (below waste drawer for benchtop operation or in the waste bin for QIAasympphony Cabinet SP/AS operation).
- Close the “Waste” drawer and perform an inventory scan.

Table 4. Required plasticware for 1–3 sample batches

	One batch, 24 samples	Two batches, 48 samples	Three batches, 72 samples
Empty unit boxes	2	3	4

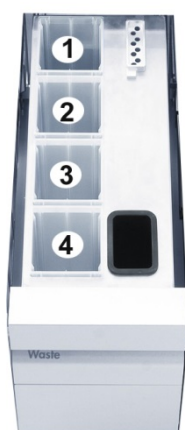


Figure 1. Position of unit boxes (1–4).

5. Load the “Eluate” drawer.

- Place the adapter (Elution Microtubes Rack QS) onto the transfer frame.
- Open the “Eluate” drawer.
- Place the assembly of adapter and transfer frame onto slot 1 of the “Eluate” drawer.
- Select “Elution Slot 1” on the touchscreen.
- Remove the bottom from the Elution Microtubes CL rack by twisting the rack until the bottom comes out.
- Scan the bar code on the Elution Microtubes CL rack using the handheld bar code scanner.

- Insert the rack in the adapter on “Elution Slot 1”.
- Remove the lid of the Elution Microtubes CL rack.
- Close the “Eluate” drawer.
- Press “OK”.
- Wait until the scan has finished.

6. Load the “Reagents and Consumables” drawer (Figure 2).

- Open the “Reagents and Consumables” drawer.
- Take the reagent cartridge (RC) and before using for the first time, check that Buffers QSL2 and QSB1 in the cartridge do not contain a precipitate. If Buffers QSL2 and QSB1 contain a precipitate, follow the instructions on page 15.

Note: Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.

- Place the reagent cartridge (RC) in the gray reagent cartridge holder.
- Ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before use. Place the trough containing the magnetic particles back in to the reagent cartridge (RC).
- Before loading the reagent cartridge (RC), remove the cover from the trough containing the magnetic particles.
- Open the enzyme tubes. Place the lids of the enzyme tubes onto the cap holders on the gray reagent cartridge holder.

Note: If enzyme tubes contain air bubbles, aspirate bubbles from the surface.

- Mount the enzyme rack (ER) on the reagent cartridge (RC).
- Mount the piercing lid (PL) onto the reagent cartridge (RC) and gently click into place.
- Place prepared reagent cartridge(s) (RC) onto position RC 1 and/or RC 2. One new reagent cartridge (RC) is sufficient for up to 96 samples.
- Press the “R+C” button on the touchscreen.
- Press the “Bottle ID” button.
- Press the text field and scan the bar code of the Buffer ATL (ATL) bottle using the handheld bar code scanner.

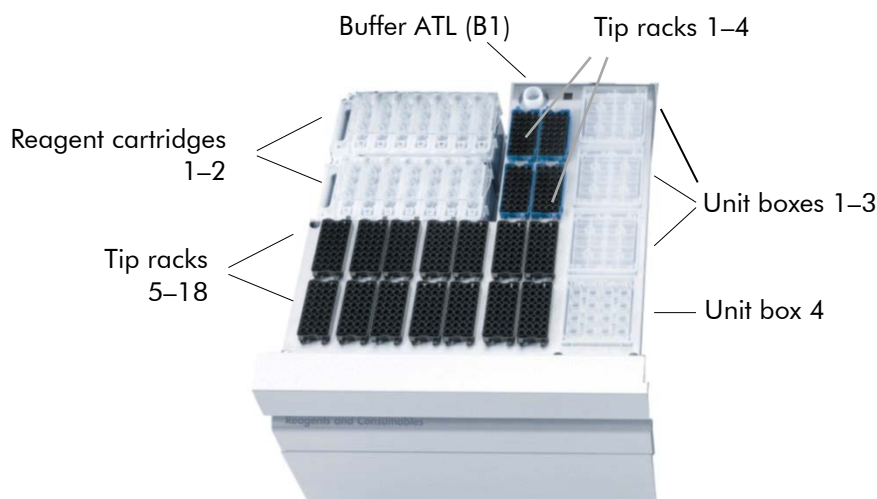


Figure 2. Position of the reagents and consumables on the QIAAsymphony SP.

- Open the bottle of Buffer ATL (ATL) and check that it does not contain a precipitate. If Buffer ATL (ATL) contains a precipitate, follow the instructions on page 15.
- Place the bottle of Buffer ATL (ATL) into position B1, which is next to the reagent cartridge slot 1 (RC 1).

Note: Try to avoid vigorous shaking of the buffer bottle otherwise foam may be generated. This can lead to liquid-level detection problems.

- Load sufficient racks of disposable 200 μ l filter-tips in tip rack holder positions 1–4 (See Table 5, page 20).
- Load sufficient racks of 1500 μ l disposable filter-tips in tip rack holder positions 5–18 (See Table 5, page 20).
- Make sure to click all racks into place.

Note: We recommend loading more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

- Remove lid of unit boxes for sample prep cartridges and load sufficient sample prep cartridges in unit box holder positions 1–3 (See Table 5, page 20).
- Remove lid of unit box for 8-Rod Covers and load unit box with sufficient 8-Rod Covers in unit box holder position 4 (See Table 5, page 20).

Note: Plastic consumables may shift during transit or storage. Check that all plastics are aligned properly inside the unit box before loading on the QIAAsymphony SP.

- Press “OK” in the consumables screen.
- Close the “Reagents and Consumables” drawer and perform an inventory scan.

Table 5. Required plasticware for 1–3 sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*
Disposable filter-tips, 200 µl ^{†‡}	34 (1 rack)	60 (2 racks)	86 (3 racks)
Disposable filter-tips, 1500 µl ^{†‡}	123 (4 racks)	205 (7 racks)	295 (10 racks)
Sample prep cartridges	18	36	54
8-Rod Covers	3	6	9

* Performing more than one inventory scan requires additional disposable filter-tips.

† There are 32 filter-tips/tip rack, 28 sample prep cartridges/unit box, and twelve 8-Rod Covers/unit box.

‡ Number of required filter-tips includes filter-tips for one inventory scan per reagent cartridge.

7. Load the “Sample” drawer (tube carrier) with the positive and negative controls.

- Place the tube with the GBS Positive Control (supplied with the *artus* GBS QS-RGQ Kit) in position 1 of the first sample carrier (use Tube Insert 3B for 2 ml micro tubes).
- Place the tube with the GBS Negative Control (supplied with the *artus* GBS QS-RGQ Kit) in position 2 of the first sample carrier (use Tube Insert 3B for 2 ml micro tubes).

Note: Make sure to load the positive and negative controls in the correct position. Rotor-Gene AssayManager will not import the result file if the positive and negative controls are placed in any other position. Do not load controls into additional carriers for the same AS batch.

Note: The position of samples and controls on the assay rack can be displayed before the start of the run. After creation of the AS batch (step 11, page 22), press the “Assays” drawer button on the touch screen and select the respective “Assay” slot. The sample type of each position will be displayed (“Type”), if the toggle button “Sample” is pressed.

8. Load the "Sample" drawer (tube carrier) with the samples.

- Load prepared samples (see page 10) in 2 ml micro tubes in the sample tube carrier already containing the controls (use Tube Insert 3B for 2 ml micro tubes).
- If required, prepare further sample tube carriers in the same way. Do not add further controls to sample tube carriers to be combined in the same AS batch (see step 11).

Note: If samples contain bar codes, orient samples in the tube carrier so that the bar codes are completely visible.

- Check that sample and control tubes are correctly loaded and clicked into place.
- Insert all sample carriers in "Sample" drawer slots 1–4. The LED light turns orange if loaded correctly.

Note: First load the sample tube carrier containing the controls and samples into slot 1. Do not load more than 72 samples and controls in one run.

9. Using the "Integrated run" setup on the QIAasympyphony touchscreen, enter the required information for each batch of samples to be processed.

- Press the "Integrated Run" tab on the touchscreen.
- Press "Define run".
- Select "SP Batch 1" (or appropriate batch number of sample carrier with "Full Process Controls", if performing continuous loading).
- Press "Edit samples".
- Make sure that the correct labware is assigned to the samples. If necessary, correct the labware assignment.
- Press "ID/Type".
- Select the first position and press "Sample ID".
- Press the text field and enter GBS Positive Control, then press "OK".
- Select the first position and press "EC+".
- Select the second position and press "Sample ID".
- Press the text field and enter GBS Negative Control, then press "OK".
- Select the second position and press "EC-".
- If necessary, resolve any bar code errors for sample and insert IDs.
- Press "OK".

Note: Do not assign the Sample Type “EC+” or “EC–” to tubes other than the positive and negative control supplied with the *artus* GBS QS-RGQ Kit. Rotor-Gene AssayManager will reject runs with incorrect control patterns. If you are additionally processing previously characterized samples along with the test samples, make sure to assign the “sample type” “sample” to these samples.

10. Define the assay(s) to run.

- Press the corresponding “SP Batch” button.
- Press “Define assays”.
- Select the samples to be processed with the assay.
- Select the assay “artus_GBS_broth200_V1” under the category “artus QS-RGQ”.
- Press “OK”.
- Repeat step 10 for all batches and samples to be processed.

11. Define the QIAasympyphony AS batch.

- Select all batches that should be processed in one integrated QIAasympyphony RGQ run.
- Press “Create AS batch”.

Note: All QIAasympyphony SP batches assigned to the same QIAasympyphony AS batch (integrated QIAasympyphony RGQ run) will be processed in the same assay setup procedure.

- Press “OK” to queue the run.

12. Load the “Sample” drawer with the internal control mixture.

- Place the previously prepared tube(s) of internal control mixture (see page 12) into the sample carrier (use Tube Insert 3B for 2 ml micro tubes).
- Insert sample carrier in slot A of the “Sample” drawer.

Note: For certain liquid levels in unlabeled 14 ml tubes (see “Consumables and reagents for the QIAasympyphony SP”), scan errors can occur due to the clear liquid and tube. To avoid this, attach a blank label to the tube or mark the tube area facing the barcode scanner with a permanent marker.

13. Define the internal control positions.

- Press the “Define ICs” button.
- Select the positions of the internal control mixture.
- Select the corresponding internal control “Complex200_V6_DSP_artus_GBS” from the folder “Required”.

- Make sure that the correct labware is assigned. If not, correct labware assignment by pressing “IC Tubes”.
- Press “OK”.
- Check the “R+C” tab to ensure all required reagent and consumables have been loaded.

14. Start the run.

- To start the run press the “Run” button.
- Read and confirm the message that appears.
- We recommend waiting beside the instrument until it has performed liquid level detection of the internal control tubes (QIA Symphony SP carrier status changes to “running”).

Note: Do not pause or stop the run during processing (unless an emergency occurs), as this will lead to the respective samples and assay reactions being flagged as “unclear”. Rotor-Gene AssayManager will invalidate “unclear” assay reactions.

Note: It is possible to continuously load samples and add them to this run (until reagents are loaded) or to a new QIA Symphony RGQ run.

Loading the QIA Symphony AS drawers for assay setup

15. Install an empty tip disposal bag and tip chutes.

- Install an empty tip disposal bag for benchtop operation or in the waste bin for QIA Symphony SP/AS Cabinet operation.
- Open the “Eluate and Reagents” drawer and the “Assays” drawer of QIA Symphony AS.
- Open the hood and insert the tip chute inside the instrument.

Note: Different tip chutes must be used for benchtop and QIA Symphony Cabinet SP/AS operation.

- Close hood, and read and confirm message.

16. Load “Assays” drawer with assay rack.

- Press slot 5 “Assay” (yellow).
- Fill the required number of strip tubes (4 tubes = 1 segment) in a precooled Rotor-Gene Strip Tubes 72 QS cooling adapter as indicated on the touchscreen.

Note: Load complete strip tubes. Do not break strip tubes.

- Load adapter with strip tubes on slot 5 of the “Assays” drawer.

- Press “Rack ID” on the touchscreen, enter a user-defined rack ID, and press “OK”.

Note: It is also possible to use the automatic ID function.

- Press “Load”.

17. Load “Assays” and “Eluate and Reagents” drawer with filter-tips.

- Load at least the number of filter-tips provided in the “Assay Setup | Loading Information” screen.

Note: Start loading tip racks from the positions at the back (near the cooling adapters). In rare cases, the pipetting head may not be able to reach some positions toward the hood and this may cause the instrument to automatically pause. We recommend loading more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

18. Load “Eluate and Reagents” drawer with reagents.

- Before each use, all assay reagents need to be thawed completely, mixed, and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents (see procedure described in “Important points before starting”, page 15).
- Press slot 3 “Reagent” (yellow) on the touchscreen.
- Prepare a precooled reagent holder as requested on the touchscreen.
- Select the tube positions on the touchscreen, load an empty tube for the master mix, and fill at least the required volume of the correct reagents in the required tubes in the corresponding positions as indicated on the touchscreen.

Note: The GBS assay is not designed to process fewer than 24 reactions per run. If the number of reactions in the run is less than 24, a full tube of GBS Master A and GBS Master B must be placed on QIA Symphony AS even if QIA Symphony AS shows a specific loading volume for the run that is less than the volumes of GBS Master A and GBS Master B in the tubes supplied with the kit.

Note: It may be necessary to combine the same reagent types (GBS Master A or GBS Master B) into one tube if required volume exceeds filling volume of the corresponding reagents. One tube each of GBS Master A and GBS Master B is sufficient for 24 QIA Symphony SP eluates (including controls).

Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the GBS Master A and GBS Master B into the respective tubes.

Note: Alternatively, select “List View” on the touchscreen and prepare the reagent adapter accordingly. A “Loading Information File” can also be downloaded via the QMC or USB port (and printed) after the QIAasympphony AS batch is defined and queued.

- Press the “Scan Kit Barcode” button on the touchscreen and press the light-blue kit bar code line.
- Press the text field and scan the kit bar code on the upper side of the *artus* GBS QS-RGQ Kit using the handheld bar code scanner.

Note: If the kit bar code is not scanned at this step, Rotor-Gene AssayManager will reject the QIAasympphony AS result file during import.

- Load the prepared reagent adapter onto slot 3 of the “Eluate and Reagents” drawer.
- Press the “Load” button.
- Close both drawers.
- Press “Scan” to enter the scan dialog.
- Press “Scan” to perform an inventory scan of all QIAasympphony AS components.

Note: We recommend waiting by the instrument until the scan is completed.

- Assay setup will start automatically when sample preparation on the QIAasympphony SP has finished.

19. Check the time for the end of the QIAasympphony AS batch to remove assay rack.

- After the QIAasympphony AS scan has finished, the calculated integrated run time is shown on the “Integrated Run Overview” screen. The maximum time permitted from the end of the QIAasympphony AS run until the start of the Rotor-Gene Q instrument run is 30 minutes. Make sure to transfer the assay rack to the Rotor-Gene Q instrument within 30 minutes of the assay run finishing.

Removal of assay rack and transfer of result file

20. Remove the QIAasympphony AS batch and the assay rack.

- Open the “Assays” and the “Eluate and Reagents” drawers.
- Remove the adapter with the strip tubes and close the tubes with the appropriate caps.
- Press slot 5 “Assay”.
- Press the “Remove” button.

- Remove the reagent adapter and discard the reagents according to your local safety regulations.
- Press slot 3 "Reagent".
- Press the "Remove" button.
- Close the "Assays" and the "Eluate and Reagents" drawers.
- Press "Scan" to enter the scan dialog.
- Press "Scan" to perform an inventory scan for adapters on the left and right (typically preselected).
- Press the "Integrated Batch" button (green) to remove the integrated run.
- Read and confirm the message.
- The final QIASymphony AS result file is created and can be transferred to either a USB stick or to a defined folder (\log\Results\AS) via the QMC.

21. Transfer the result file to a defined folder. To transfer the result file using the USB stick, follow step 21a. To transfer the result file using the QMC, follow step 21b.

21a. Transfer result file using the USB stick.

- Insert the USB stick.
- Select "Tools".
- Select "File Transfer".
- Select "Result Files" in the "Save to USB Stick" column.
- Press the "Transfer" button.
- Read and confirm the message.
- After successful transfer, press "OK" and remove the USB stick.
- Proceed to "Protocol: PCR on the Rotor-Gene Q instrument", page 28.

21b. Transfer result file using the QMC.

- Log in to the correct QIASymphony SP/AS.
- Select the transfer file icon.
- Choose file format "Result File AS".
- Select result file with the correct time stamp and batch ID from the list of "Remote Site" files (right column).
- Transfer result file to the "Local Site" (the file is saved under the path defined in "Tools", "Options", "File Transfer", under \log\Results\AS).

Note: If multiple batches on the QIAasymphony AS are configured in an integrated run, check the tip disposal bag for remaining capacity, and reload the QIAasymphony AS drawers, starting at step 14.

■ Proceed to “Protocol: PCR on the Rotor-Gene Q instrument”, page 28.

Note: We recommend marking the strip tube caps to ensure correct positioning and to use a cooled transport frame to avoid contamination.

Protocol: PCR on the Rotor-Gene Q instrument

Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- Rotor-Gene AssayManager enables automated interpretation of the PCR results.
- The *artus* GBS QS-RGQ Kit must be run on the Rotor-Gene Q instrument using automated interpretation of results with Rotor-Gene AssayManager. The cycling parameters are locked for the run.
- Download the Application Package from “Protocol Files” on the “Resources” tab of the *artus* GBS QS-RGQ Kit web catalog page (www.qiagen.com/p/artus-GBS-QS-RGQ-Kit-CE).
- After installing the plug-in and importing the assay profile (see “Things to do before starting”, below), Rotor-Gene AssayManager can use the information given in the QIA Symphony AS result file to set up a run for real-time PCR amplification and subsequent automated interpretation of results.
- For system-wide process safety it is necessary to activate the following settings for the closed mode: “Material number required”, “Valid expiry date required”, and “Lot number required”. (Under “Configuration”, “Settings”, “Global Settings”, “Work List”. User role “Administrator” is required to access “Configuration”).

Things to do before starting

- For automated interpretation of results using the *artus* GBS QS-RGQ Kit with Rotor-Gene AssayManager, the latest *artus* Basic plug-in must be installed to your Rotor-Gene AssayManager. Start the installation process by double-clicking on the **ArtusBasic.Installation.msi**, and follow the installation instructions. For a detailed description refer to “Installing Plug-ins” (see the *Rotor-Gene AssayManager Core Application User Manual* supplied).
- To use the *artus* GBS QS-RGQ Kit for LIM broth samples, the file **AP_artus_GBS_broth200_QS_V1_0_x.iap** must be imported to Rotor-Gene AssayManager. To import the assay profile into Rotor-Gene AssayManager, navigate to the “Configuration” environment and change to the “Assay Profile” tab. Click on “Import” and select the **AP_artus_GBS_broth200_QS_V1_0_x.iap** file in the open file dialog. Click “Open”, and the assay profile is loaded and added to the list of available assay profiles.

Note: The same version of an assay profile cannot be imported twice.

Procedure

1. Prepare the rotor and start the run on the Rotor-Gene Q instrument.

- Place a 72-Well Rotor on the Rotor Holder.
- Fill the rotor with strip tubes. Make sure to start at position 1 and to fill the strip tubes in the correct orientation.
- Use empty capped strip tubes to fill all unused positions.
- Attach the locking ring.
- Load the Rotor-Gene Q instrument with the rotor and locking ring.
- If using a USB stick for data transfer direct from the QIASymphony SP/AS, unzip the result file from the QIASymphony AS. The result files are stored under \log\Results\AS.

Note: On most computers, files can be unzipped by right-clicking the file and then clicking "Extract" in the menu that opens. Files must be unzipped in order to be imported into Rotor-Gene AssayManager.

- Start Rotor-Gene AssayManager.
- Log in to the closed mode.
- Select the "Setup" environment, if not already preselected.
- Import the QIASymphony AS result file at the bottom of the screen. Select the source "QIASymphony" as "Import type".
- In the "Select file" dialog, open the corresponding QIASymphony AS result file and click "Open".
- Read and confirm the message.
- After successful import, select the corresponding work list from the work list manager list and click the "Apply" button.
- Enter an experiment name.
- Select the cycler to be used in the "Cycler selection" dialog.
- Check correct attachment of locking ring and confirm on the screen that the locking ring is attached.
- Close the Rotor-Gene Q instrument lid.
- Click the "Start run" button.

Note: If using multiple cycler runs, change to the corresponding cycler environment to see the progress of the run.

- When the run is finished, click "Finish run...".

- For users logged in with the Operator role: Click “Release”.
- For users logged in with the Approver role: Click “Release and go to approval”.

2. Release and report results.

- If you have used “Release” before, select the “Approval” environment.
- Press “Apply filter” (or choose own filter options beforehand).
- Select experiment.
- Click “Start approval”.
- Approve the results of each test sample. Use the “Accepted” button for test samples whose results analyzed by Rotor-Gene AssayManager you agree with. Use the “Rejected” button if the test sample result evaluated by Rotor-Gene AssayManager is not acceptable for any reason.

Note: A result automatically set to “Invalid” by Rotor-Gene AssayManager cannot be converted to a valid result anymore, even if the result is rejected.

- Optional: Enter comments.
- Click “Release /report data...”.
- Choose a report profile and click “OK”. The report will be generated and stored automatically.

Note: The user needs approval rights to release an assay.

- Unload the Rotor-Gene Q instrument and discard the strip tubes according to your local safety regulations.

3. Perform maintenance.

- When all QIAasymphony AS batches of the integrated QIAasymphony SP/AS run have finished, perform the regular maintenance as described in the *QIAasymphony SP/AS User Manual — General Description*.

Note: This can be performed at any time before the start of the next integrated run, according to local regulations or priorities.

- Perform daily, weekly, and annual preventive maintenance as described in the *QIAasymphony SP/AS User Manual — General Description*.

Interpretation of Results

This section describes interpretation of results on the Rotor-Gene Q instrument. Review also the sample status information from the QIAasymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

The *artus* GBS QS-RGQ Kit Assay Profile contains rules for analyzing sample, positive/negative control, and run results automatically.

Every sample and control displays an independent result for each target: GBS, and Internal Control. Each result is reported as “signal detected”, “no signal”, or “INVALID”.

Positive/negative control results:

- All targets for the positive control (“Positive Control”) and negative control (“Negative Control”) must be valid in order to confirm that the assay status is successful and the test results may be reported. If any target of the positive control or negative control is invalid, results for every sample in the run will display “INVALID”. The entire assay run must be retested.
- The positive control must report a “Signal detected” result for GBS and Internal Control.
- The negative control must report a “Signal detected” result for Internal Control and “No Signal” for the specified targets.

Sample results:

- See Table 6 for a summary of results interpretation.
- A sample is considered positive for GBS if the target is detected.
- The Internal Control signal must be detected in samples where no GBS signal is detected. If the Internal Control signal is not detected or is “INVALID” in samples where no GBS signal is detected, all targets for the sample will be displayed as “INVALID”. The sample must be retested.
- The Internal Control target may be reported as “No signal” or “INVALID” in samples where GBS signal is detected. In these cases all targets for the sample will be reported. No retesting is necessary
- **Note:** It is expected that in some positive GBS samples the Internal Control PCR may be inhibited due to competition from amplifying GBS, which will cause a “No signal” or “INVALID” result for Internal Control.
- In some samples, a result for GBS may be reported as “INVALID”. In these cases, it is recommended to retest the sample.
- If the GBS target is reported as “INVALID” and the Flag says CT_ABOVE_ACCEPTED_RANGE, this sample does not need to be retested and is considered “No signal” if the Internal Control is valid.

Table 6. Summary of results interpretation

GBS	Target result		Sample status	GBS detected in sample
	Internal Control			
Signal detected	Signal detected/ No signal/ INVALID		Valid	Yes
No signal	Signal detected		Valid	No
No signal	No signal/INVALID		Invalid	Error, retest sample
INVALID*	Signal detected/ No signal/ INVALID		Valid or Invalid	Error, retest sample

*If a target is reported as invalid and the Flag says CT_ABOVE_ACCEPTED_RANGE, this sample does not need to be retested and is considered “no signal”, if the Internal Control is valid.

This automated analysis may provide the following corresponding flags.

Flag	Behavior	Description
ASSAY_INVALID	Invalid	Assay invalid because at least one external control is invalid.
CT_ABOVE_ACCEPTED_RANGE	Invalid	The detected C_T value is higher than the defined cutoff C_T (see above).
CT_BELOW_ACCEPTED_RANGE	Invalid	The detected C_T value is lower than the defined cutoff C_T .
CURVE_SHAPE_ANOMALY	Invalid	The raw data amplification curve shows a shape that is deviating from the established behavior for this assay. There is a high likelihood for wrong results or result misinterpretation.
FLAT_BUMP	Invalid	The amplification curve shows a shape like a flat bump, deviating from the established behavior for this assay. There is a high likelihood for wrong results or result misinterpretation (wrong C_T value determination).

Flag	Behavior	Description
IC_INVALID	Invalid	The internal control is invalid. Target and internal control share the same tube.
IC_NO_SIGNAL	Invalid	No internal control signal detected. Target and internal control share the same tube.
MULTI_THRESHOLD_CROSSING	Invalid	The amplification curve crosses the threshold more than once. An unambiguous C_T cannot be determined.
NO_CT_DETECTED	Invalid	No C_T is detected for this target.
NORM_FACTOR_ALTERATION	Warning	<p>Curve not normalized properly due to low signal.</p> <p>Note: If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.</p>
OTHER_TARGET_INVALID	Invalid	Another target for the same sample is invalid.
SATURATION	Invalid	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.
SATURATION_IN_PLATEAU	Warning	<p>The raw data fluorescence is saturating in the plateau phase of the amplification curve.</p> <p>Note: If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.</p>

Flag	Behavior	Description
SPIKE	Variable	A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the C_T is determined.
SPIKE_CLOSE_TO_CT	Invalid	A spike is detected in the amplification curve close to the C_T .
STEEP_BASELINE	Invalid	A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.
STRONG_BASELINE_DIP	Invalid	A strong drop in the baseline for raw data fluorescence is detected in the amplification curve.
STRONG_NOISE	Invalid	Strong noise is detected outside the growth (exponential) phase of the amplification curve.
STRONG_NOISE_IN_GROWTH_PHASE	Invalid	Strong noise is detected in the growth (exponential) phase of the amplification curve.
UNCERTAIN	Invalid	Results from the automatic data scan (AUDAS) are conflicting with results from the core analysis. An unambiguous automatic assessment of data validity is not possible.

Flag	Behavior	Description
UPSTREAM	Variable	<p>Sample status was set to invalid or unclear by an upstream process (e.g., QIA Symphony Assay Setup).</p> <p>Note: For “unclear” flags from upstream processes, the behavior of Rotor-Gene AssayManager is defined in the “Configuration” environment.</p> <p>For “invalid” flags from upstream processes Rotor-Gene AssayManager always invalidates such samples.</p>
WAVY_BASE_FLUORESCENCE	Invalid	<p>A wavy baseline for the raw data fluorescence is detected in the amplification curve.</p>

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Comments and suggestions

General handling

Error message displayed in the touchscreen

If an error message is displayed during an integrated run, refer to the user manuals supplied with your instruments.

Precipitate in reagent trough of opened cartridge of the QIASymphony DSP Virus/Pathogen Kit

a) Buffer evaporation

Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification.

b) Storage of reagent cartridge (RC)

Storage of reagent cartridge (RC) at less than 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* at 37°C for 30 minutes with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) in a water bath* at 37°C for 30 minutes with occasional shaking.

Low yield of nucleic acids

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Comments and suggestions

a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
b) Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.
c) Carrier RNA (CARRIER) not added	Reconstitute carrier RNA (CARRIER) in Buffer AVE (AVE) and mix with appropriate volume of Buffer AVE (AVE) as described in "Preparation of carrier RNA (CARRIER) and internal control (GBS Internal Control)", page 12. Repeat the purification procedure with new samples.
d) Degraded nucleic acids	Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples.
e) Incomplete sample lysis	Before use, check that Buffers QSL2 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.*
f) Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAasympyphony purification procedure. To remove insoluble material for bacterial applications, centrifuge the sample at 3000 x g for 1 minute, and transfer the supernatant to a new sample tube.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Comments and suggestions

QIAAsymphony AS detects insufficient Master

Insufficient Master transferred to tube

Combine the contents of an appropriate number of GBS Master A tubes into one tube before use. Combine the contents of an appropriate number of GBS Master B tubes into one tube before use. Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the Master in the tube.

For viscous reagents, we recommend aspirating an extra volume of 5% when using manual pipets (e.g., adjust the pipet to 840 μ l for an 800 μ l volume).

Alternatively, after slowly dispensing the liquid and performing a blowout at the target tube's wall, remove the tip from the liquid, release the pipet plunger, and wait for an additional 10 seconds. Residual liquid will flow down the tip and can be blown out by pressing the pipet plunger a second time. The use of PCR grade filter-tips labeled as "low retention" can improve the recovery of liquid.

No signal with positive control (GBS Positive Control) for target GBS

a) Incorrect configuration of the PCR

Make sure that assay setup was performed correctly and that the correct Assay Parameter Set was used. Repeat the PCR, if necessary. See "Assay Control Sets and Assay Parameter Sets", page 14.

b) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 9)

Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

c) The *artus* GBS QS-RGQ Kit has expired

Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Comments and suggestions

Weak or no signal of the internal control of a negative sample subjected to purification using the QIA Symphony DSP Virus/Pathogen Kit for target “Internal Control” and simultaneous absence of a signal for target GBS

- | | |
|--|--|
| a) The PCR conditions do not comply with the protocol | Check the PCR conditions (see above) and repeats the PCR with corrected settings, if necessary. |
| b) The PCR was inhibited | Make sure that you use the validated isolation method (see “Protocol: DNA isolation and assay setup on the QIA Symphony SP/AS”, page 15) and closely follow the instructions. |
| c) DNA was lost during extraction | <p>An absent signal of the internal control can indicate the loss of DNA during the extraction. Make sure that you use the validated isolation method (see “Protocol: DNA isolation and assay setup on the QIA Symphony SP/AS”, page 15) and closely follow the instructions.</p> <p>See also “Low yield of nucleic acids”, above.</p> |
| d) The storage conditions for one or more kit components did not comply with the instructions given in “Reagent Storage and Handling” (page 9) | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |
| e) The <i>artus</i> GBS QS-RGQ Kit has expired | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |

Signals with the negative controls for target GBS of the analytical PCR

- | | |
|---|--|
| a) Contamination occurred during preparation of the PCR | <p>Repeat the PCR with new reagents in replicates.</p> <p>If possible, close the PCR tubes directly after addition of the sample to be tested.</p> <p>Make sure that work space and instruments are decontaminated at regular intervals.</p> |
|---|--|

	Comments and suggestions
b) Contamination occurred during extraction	<p>Repeat the extraction and PCR of the sample to be tested using new reagents.</p> <p>Make sure that work space and instruments are decontaminated at regular intervals.</p>

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* GBS QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

All reagents may exclusively be used in in vitro diagnostics.

The *artus* GBS QS-RGQ Kit is to be used by laboratory professionals trained in the use of the QIAAsymphony SP/AS, Rotor-Gene Q instruments, and Rotor-Gene AssayManager.

The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only. Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

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 expired components.

Although rare, mutations within the highly conserved regions of the bacterial genome covered by the kit's primers and/or probe may result in failure to detect the presence of the bacteria in these cases. Validity and performance of the assay design are evaluated at regular intervals.

Performance Characteristics

See www.qiagen.com/p/artus-GBS-QS-RGQ-Kit-CE for performance characteristics of the *artus* GBS QS-RGQ Kit.

References

1. Fluegge, K. et al. (2006) Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. *Pediatrics*, **117**, e1139.
2. Centers for Disease Control and Prevention (USA). GBS Prevention in Newborns. <http://www.cdc.gov/groupbstrep/about/prevention.html>
3. Young, B.C., Dodge, L.E., Gupta, M., Rhee, J.S. and Hacker, M.R. (2011) Evaluation of a rapid, real-time intrapartum group B streptococcus assay. *Am. J. Obstet. Gynecol.* **205**, 372.

Symbols

The following symbols may appear on the packaging and labeling:



<N>

Contains reagents sufficient for <N> reactions



Use by



In vitro diagnostic medical device



Catalog number



Lot number



Material number



Components



Contains



Number



Global Trade Item Number

Rn

R is for revision of the Handbook and n is the revision number



Temperature limitation



Manufacturer



Consult instructions for use



Caution



Master A



Master B



Internal control



Positive control



Negative control

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
<i>artus</i> GBS QS-RGQ Kit (72)	For 72 reactions: 2 Masters, Positive Control, Internal control, Negative Control	4572366
QIAasymphony DSP Virus/Pathogen Kit		
QIAasymphony DSP Virus/Pathogen Mini Kit	For 192 preps (200 µl each): includes 2 reagent cartridges and enzyme racks and accessories	937036
QIAasymphony SP/AS Instruments		
QIAasymphony SP	QIAasymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAasymphony AS	QIAasymphony assay setup module: includes 1-year warranty on parts and labor	9001301
Rotor-Gene Q		
Rotor-Gene Q MDx 5plex HRM	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene AssayManager — for routine testing with Rotor-Gene Q and QIAasymphony RGQ instruments		
Rotor-Gene AssayManager	Software for routine testing in combination with the Rotor-Gene Q and QIAasymphony RGQ instruments; single license software for installation on one computer	9022737
Rotor-Gene AssayManager (10)	Software for routine testing in combination with the Rotor-Gene Q and QIAasymphony RGQ instruments; multi-license software for installation on up to 10 computers	9022739

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