

QIAAsymphony® RGQ Application Sheet

QIAAsymphony RGQ application artus® BK Virus QS-RGQ Kit (sample type: urine, 800 µl)



Check availability of new electronic labeling revisions at www.qiagen.com/products/artusbkvirusrgqcrkit.aspx before test execution. The current revision status is indicated by the issue date (format: month/year).

General information

| | |
|---|--|
| Kit | artus BK Virus QS-RGQ Kit, Version 1, REF 4514363 |
| Validated sample material | Urine |
| Front-end purification | QIAAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055) |
| Sample volume (including excess volume) | 1000 µl |
| Assay Parameter Set | artus_BKV_urine800_V4 |
| Default Assay Control Set | Complex800_V6_DSP_artus_BKV |
| Elution volume | 60 µl |
| Required software version | Version 4.0 or higher |
| Master mix volume | 10 µl |
| Template volume | 15 µl |
| Number of reactions | 6–24* |
| Runtime on AS module | For 6 reactions: approximately 8 minutes For 72 reactions: approximately 35 minutes |

* For assay setup for BK virus, up to 216 (9 x 24) assays can be set up in one run on the QIAAsymphony AS.

May 2012



Sample & Assay Technologies

Materials required but not provided

| | | |
|---|---|--|
| Purification kit | ■ | QIASymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055) |
| Adapters for the QIASymphony SP | ■ | Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730) |
| | ■ | Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083) |
| Consumables for the QIASymphony SP | ■ | Sample Prep Cartridges, 8-well (cat. no. 997002) |
| | ■ | 8-Rod Covers (cat. no. 997004) |
| | ■ | Filter-Tips, 1500 μ l (cat. no. 997024) |
| | ■ | Filter-Tips, 200 μ l (cat. no. 990332) |
| | ■ | Elution Microtubes CL (cat. no. 19588) |
| | ■ | Tip disposal bags (cat. no. 9013395) |
| | ■ | Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls |
| Adapters and reagent holders for the QIASymphony AS | ■ | Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090) |
| | ■ | Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089) |
| | ■ | 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)* or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005) |
| | ■ | Tube, conical, 5 ml, Qsym AS (cat. no. 997104)* or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001) |
| | ■ | Reagent Bottles, 30 ml, Qsym AS (cat. no. 997108) |
| | ■ | Elution Microtubes CL (cat. no. 19588) |
| | ■ | Filter-Tips, 1500 μ l (cat. no. 997024) |
| | ■ | Filter-Tips, 200 μ l (cat. no. 990332) |
| | ■ | Filter-Tips, 50 μ l (cat. no. 997120) |
| | ■ | Tip disposal bags (cat. no. 9013395) |
| For sample preparation (urine) | ■ | Buffer ATL, GPR (cat. no. 939016) |

* Please inquire for availability.

Specimen handling and storage

| | |
|--------------------|--|
| Sample collection | Human urine |
| Sample transport | Shatterproof transport Shipment within 6 hours Mail shipment according to legal instructions for the transport of pathogen material* |
| Sample preparation | Prevent formation of foam in or on the samples. Samples should be equilibrated to room temperature (15–25°C) before starting the run. |

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

Procedure

Preparation of carrier RNA and addition of the internal control to the samples

Using the QIA Symphony DSP Virus/Pathogen Midi Kit in combination with the *artus* BK Virus QS-RGQ Kit requires introduction of the internal control (BK Virus RG IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Internal controls must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 μ l.

The table represents the addition of internal control to the isolation 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.

| Component | Volume (μ l) (Sarstedt® tubes)* | Volume (μ l) (BD™ tubes)† |
|--|--------------------------------------|--------------------------------|
| Stock carrier RNA (CARRIER) | 3 | 3 |
| Internal control‡ | 9 | 9 |
| Buffer AVE | 108 | 108 |
| Final volume per sample (excluding dead volume) | 120 | 120 |
| Total volume for n samples | (n x 120) + 360§ | (n x 120) + 600¶ |

* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051).

‡ The calculation of the amount of internal control is based on the initial elution volumes (90 μ l). Additional void volume depends on the type of sample tube used.

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 μ l) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples. These volumes are specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

¶ Internal control mixture corresponding to 5 additional samples (i.e., 600 μ l) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples. These volumes are specific for Tubes 14 ml, 17 x 100 mm polystyrene round-bottom, Becton Dickinson, cat. no. 352051).

QIAsymphony SP setup

“Waste” drawer

| | |
|-----------------------------------|---------------------------------------|
| Unit box holder 1–4 | Empty unit boxes |
| Waste bag holder | Waste bag |
| Liquid waste bottle holder | Empty and install liquid waste bottle |

“Eluate” drawer

| | |
|------------------------|--|
| Elution rack | Use slot 1, cooling position |
| Elution volume* | Preselected elution volume: 60 μ l Initial elution volume: 90 μ l |

* The elution volume is preselected for the protocol. This is the minimum accessible volume of eluate in the final elution tube. The initial volume of elution solution is required to ensure that the actual volume of eluate is the same as the preselected volume.

“Reagents and Consumables” drawer

| | |
|---------------------------------------|--|
| Position A1 and/or A2 | Load 1 reagent cartridge (RC) for up to 48 samples or 2 new reagent cartridges (RC) for up to 96 samples |
| Position B1 | Buffer ATL (ATL) |
| Tip rack holder positions 1–17 | Load sufficient racks of disposable filter-tips, 200 μ l and 1500 μ l (see Required plasticware for 1–4 sample batches”, page 6) |
| Unit box holder position 1–4 | Load unit boxes containing sample prep cartridges and 8-Rod Covers (see Required plasticware for 1–4 sample batches”, page 6) |

“Sample” drawer

| | |
|--|--|
| Sample type | Urine |
| Sample volume (including excess volume) | 1000 μ l |
| Sample tubes | Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694) |
| Insert | Tube Insert 3B (cat. no. 9242083) |

Required plasticware for 1–4 sample batches

| | One batch, 24 samples* | Two batches, 48 samples* | Three batches, 72 samples* | Four batches, 96 samples* |
|--|-----------------------------------|-------------------------------------|---|--------------------------------------|
| Disposable filter-tips, 200 μl^{†‡} | 34 | 60 | 86 | 112 |
| Disposable filter-tips, 1500 μl^{†‡} | 123 | 205 | 295 | 385 |
| Sample prep cartridges[§] | 18 | 36 | 54 | 72 |
| 8-Rod Covers[¶] | 3 | 6 | 9 | 12 |

* Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

† There are 32 filter-tips/tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

QIASymphony AS setup

Consumables

During the setup, the appropriate positions for each consumable on the QIASymphony AS module are indicated on the touchscreen of the instrument.

| Consumables | Name on touchscreen | For use with adapter/reagent holder |
|---------------------------------------|------------------------------|--|
| Strip Tubes and Caps, 0.1 ml (250) | QIA#981103 *StripTubes 0.1 | RG Strip Tubes 72 QS |
| Tubes, conical, 2 ml, Qsym AS (500)*† | QIA#997102 *T2.0 ScrewSkirt‡ | Reagent holder 1 QS Reagent holder 2 QS |
| Tube, conical, 5 ml, Qsym AS (500)*† | QIA#997104 *T5.0 ScrewSkirt‡ | Reagent holder 1 QS Reagent holder 2 QS |
| Reagent Bottles, 30ml, Qsym AS (50)* | QIA#997108 *Bottle 30ml‡ | Reagent holder 2 QS |
| Elution Microtubes CL (24 x 96) | QIA#19588 * EMTR | Elution Microtube Rack QS |

* For master mix components, system-prepared master mix, assay standards, and assay controls.

† Alternatively, the Sarstedt tubes described in “Materials required but not provided”, page 2, can be used.

‡ The suffix “(m)” in the touchscreen indicates that liquid level calculations for the respective tube have been optimized for reagents forming a concave meniscus.

Adapters and reagent holders

| Rack/reagent holder | Name | Number required [§] |
|---------------------|---------------------------|------------------------------|
| Sample rack | Elution Microtube Rack QS | 1 |
| Reagent holders | Reagent holder 1 QS | 1 |
| Assay racks | RG Strip Tubes 72 QS | 1 |

[§] Calculated for an assay run with 72 reactions.

Filter-tips

Load tip racks starting with tip slots 1, 2, and 3 in the “Eluate and Reagents” drawer, and then load tip racks into tip slots 7, 8, and 9 in the “Assays” drawer.

| Consumable | Name on touchscreen | Minimum number for 24 reactions | Minimum number for 72 reactions |
|-------------------------------------|----------------------------|--|--|
| Filter-Tips, 1500 μ l (1024) | 1500 μ l | 3 | 4 |
| Filter-Tips, 200 μ l (1024) | 200 μ l | 5 | 5 |
| Filter-Tips, 50 μ l (1024) | 50 μ l | 25 | 73 |
| Tip Disposal Bags | – | 1 | 1 |

RT-PCR on the Rotor-Gene Q

Please refer to the software-specific protocol sheet "Settings to run *artus* QS-RGQ Kits" at www.qiagen.com/products/artusbkvirusrgpckit.aspx.

Specific settings for the *artus* BK Virus QS-RGQ Kit

Rotor-Gene AssayManager automatically sets the parameters for the *artus* BK Virus QS-RGQ Kit.

With Rotor-Gene software 2.1, the specific settings are shown below.

| | |
|--|---|
| Reaction Volume (μL) | 50 |
| Hold | Hold Temperature: 95 deg Hold Time: 10 mins |
| Cycling | 45 times 95 deg for 15 secs 65 deg for 30 secs 72 deg for 20 secs Make sure to activate the touchdown function for 10 cycles in the annealing step. |
| Auto-Gain Optimisation Setup | 65 degrees (Samples: Green; IC: Orange) |

Interpretation of results

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

Rotor-Gene AssayManager automatically interprets the PCR results and gives a conclusion and quantitative result.

The following sections describe interpretation of results using Rotor-Gene software 2.1 or higher.

Signal detection and conclusions — urine 800 µl

| Signal in channel Cycling Green | Signal in channel Cycling Orange | Quantitative result (copies/ml) | Interpretation |
|---------------------------------|----------------------------------|-------------------------------------|--|
| Yes | Yes | <78.5 | Valid result: BK virus DNA detected, <100 copies/ml Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured. |
| Yes | Yes | ≥ 78.5 and <100 | Valid result: BK virus DNA detected, <100 copies/ml Quantitation not possible since the quantitative result is below the linear range of the assay. |
| Yes | Yes | ≥ 100 and $\leq 1 \times 10^9$ | Valid result: BK virus DNA detected at the calculated concentration Quantitative result is within the linear range of the assay. |
| Yes | Yes | $> 1 \times 10^9$ | Valid result: BK virus DNA detected, $> 1 \times 10^9$ copies/ml Quantitation not possible since the quantitative result is above the linear range of the assay.* |
| No | Yes | – | Valid result: No BK virus DNA is detectable.† |
| No | No | – | Invalid result: No result can be concluded.‡ |

* If quantitation is desired, dilute the sample with BK virus-free urine and reprocess. Multiply the quantitative result from the reprocessed sample by the dilution factor.

† If the C_T value for the internal control of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run ($C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$), then the sample should be treated as invalid. No result can be concluded.

‡ Information regarding error sources and their solution can be found in “Troubleshooting guide” of the *artus BK Virus QS-RGQ Kit Handbook*.

Threshold setup for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* QS-RGQ Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. The threshold can be set at a preliminary value of 0.04 for the analysis of the first PCR run, but this value should be fine-tuned in a comparative analysis of the next runs of the workflow. The threshold should be set manually just above the background signal of the negative controls and negative samples. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. The threshold value will usually be in the range of 0.03–0.05 and should be rounded to no more than three decimal places.

Quantitation

The quantitation standards (BK Virus RG QS 1–4) in the *artus* BK Virus QS-RGQ Kit are treated as previously purified samples and the same volume is used (15 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the “Edit Samples” dialog box on the Rotor-Gene Q instrument as standards with the specified concentrations (see the instrument user manual).

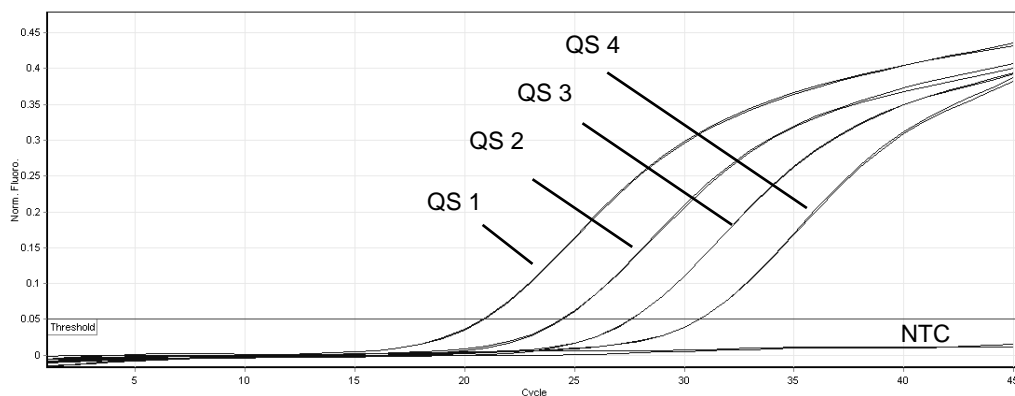
Note: The quantitation standards are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material.

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/}\mu\text{l)} \times \text{Initial Elution Volume (90 }\mu\text{l)}^*}{\text{Sample Volume (ml)}}$$

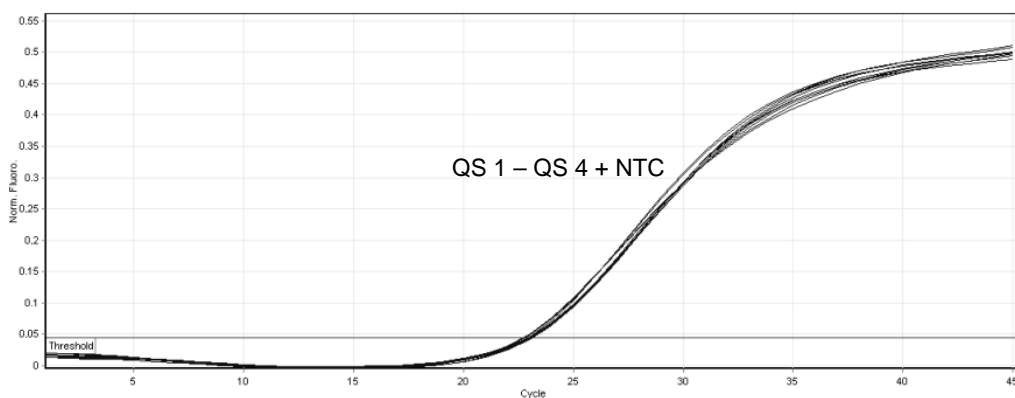
As a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

* The calculation is based on the initial elution volumes (90 µl).

Examples of positive and negative PCR reactions



Detection of the quantitation standards (BK Virus RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).



Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (BK Virus RG QS 1–4). NTC: No template control (negative control).

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