

March 2012

***mericon*[®] Automated Pathogen Detection Workflow Handbook**

For the isolation and detection of bacterial
DNA from enrichment cultures using the
QIASymphony[®] *mericon* Bacteria Kit,
mericon Pathogen Detection Kits, and the
QIASymphony SP/AS and Rotor-Gene[®] Q
Instruments



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents	4
DNA extraction	4
Real-Time PCR	4
Storage	5
Intended Use	5
Safety Information	6
Quality Control	6
Introduction	7
Principle and procedure	7
The QIASymphony RGQ pathogen detection workflow	8
Storing nucleic acids	10
Equipment and Reagents to Be Supplied by User	14
Important Notes	16
General precautions	16
Getting started on the QIASymphony SP instrument	16
Protocol: Isolation of Bacterial DNA on the QIASymphony SP	20
Protocol: Assay Setup on the QIASymphony AS	25
Protocol: PCR and Data Analysis on the Rotor-Gene Q or the Applied Biosystems 7500 Real-Time PCR cycler	33
Analyzing the Results	35
Troubleshooting Guide	37
References	41
Ordering Information	42

Kit Contents

DNA extraction

QIASymphony <i>mericon</i> Bacteria Kit	
Catalog no.	931156
Number of reactions	360
Reagent Cartridge*	2
Piercing Lid	2
TopElute Fluid (60 ml)	1
Reuse Seal Set†	2
Quick-Start Protocol	1

* Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Each Reuse Seal Set contains 8 Reuse Seal Strips.

Real-Time PCR

<i>mericon</i> PCR Detection Assays		(24)	(96)
Catalog no.		Varies	Varies
Number of reactions		24	96
Yellow	<i>mericon</i> Assay*	2 x 12 reactions	1 x 96 reactions
Red	Positive Control DNA	20 reactions	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	1.5 ml
	RNase-Free Water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 µl
	50x ROX Dye Solution	45 µl	210 µl
	Quick-Start Protocol or Handbook	1	1

* Contains target-specific primers and probes, as well as the internal control (IC)

† Contains HotStarTaq® *Plus* DNA Polymerase, dedicated multiplex real-time PCR buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP).

Storage

The QIA Symphony *mericon* Bacteria Kit should be stored at room temperature (15–25°C). The magnetic particles in the reagent cartridges remain active when stored at this temperature. Do not store the reagent cartridges at temperatures below 15°C.

When stored properly, the kit is stable until the expiration date stated on the kit box.

Partially used reagent cartridges can be stored for a maximum of 1 month, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, reseal the trough containing the magnetic particles and the reagent cartridge with the provided Reuse Seal Strips immediately after the end of the protocol run to avoid evaporation.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 48 hours (including run times) at ambient temperature.

Avoid exposure of the reagent cartridges to UV light (e.g., that used for decontamination), as exposure may cause accelerated aging of the reagent cartridges and buffers.

The *mericon* PCR Detection Assays are shipped on dry ice. The Multiplex PCR Master Mix should be stored immediately at –20°C upon receipt. All remaining kit components that are not reconstituted should be stored at 2–8°C and protected from light. Stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope. Reconstituted reagents of *mericon* Pathogen Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Intended Use

The QIA Symphony *mericon* Bacteria Kit is intended for molecular biology applications in food, animal feed, and pharmaceutical product testing. The product is not intended for the diagnosis, prevention, or treatment of a disease.

The *mericon* Pathogen Detection Kits are intended for molecular biology applications in food, animal feed, and pharmaceutical product testing. The products are not intended for the diagnosis, prevention, or treatment of a disease.

Safety Information

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



CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

For safety information regarding the instruments, see the relevant user manual.

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIASymphony *mericon* Bacteria Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

In the global food market, with its increasing demand for food safety research and monitoring, there is a need for streamlined testing solutions that are sensitive, accurate, and easy to use with a variety of starting materials.

The *mericon* food testing portfolio is a complete system of automated and manual sample preparation and assay kits that meet these demands. The technology is based on detection by real-time polymerase chain reaction (PCR). *mericon* sample preparation kits isolate pure food or pathogen DNA, and *mericon* PCR assays enable rapid and reliable pathogen detection, genetically modified organism identification, and ingredient authentication in food, animal feed, and pharmaceutical products.

Principle and procedure

The pathogen detection workflow described in this handbook covers all of the stages of the automated pathogen detection process using the QIASymphony RGQ instrument, including sample preparation, assay setup, and real-time PCR analysis.

Sample preparation

The QIASymphony *mericon* Bacteria Kit was designed for the automated isolation and detection of bacterial DNA from food enrichment cultures. Other suitable starting materials include enrichment cultures of environmental samples or bacteria concentrated by filtration. Proven magnetic-particle technology allows the extraction of DNA from Gram-negative and Gram-positive bacteria.

The extraction of bacterial DNA starts with the loading of unprocessed enrichment culture samples into the sample preparation module of the QIASymphony RGQ (QIASymphony SP module). The DNA extraction protocol is based on a short thermal lysis procedure. This is followed by a highly efficient and streamlined process of binding, washing, and elution, using silica-based magnetic particles in combination with optimized buffers. The extracted DNA is suitable for direct use in PCR.

PCR assay setup

Following DNA extraction in the QIASymphony SP module, the extracted DNA is automatically transferred to the assay setup module of the QIASymphony RGQ (QIASymphony AS module). In the QIASymphony AS module, real-time PCR based on the highly accurate *mericon* Pathogen Detection Assays is set up for a variety of different PCR output formats. Either a single-assay run with only one *mericon* PCR assay or a multi-assay run with a combination of *mericon* PCR assays can be set up for each PCR format.

Real-time PCR detection

Each *mericon* assay is an optimized mixture of PCR primer sets for a pathogen-specific target sequence and an internal control (IC), plus probes labeled with two distinct fluorescent dyes. The PCR primer set for each assay is highly specific and targets a unique and conserved DNA region of the tested pathogen genome that is verified bioinformatically and experimentally. All *mericon* real-time PCR assays use the same PCR cycling protocol, allowing automated setup and measurement of a combination of different assays in the same experiment. Although optimized for use on the QIAGEN Rotor-Gene Q real-time PCR instrument, all *mericon* assays are compatible with any real-time block cycler.

The QIASymphony RGQ pathogen detection workflow

The QIASymphony RGQ workflow begins with the isolation of nucleic acids from bacterial enrichment culture samples on the QIASymphony SP instrument (Figure 1). The DNA extraction technology of the QIASymphony SP instrument combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 2). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples, and comprises 4 steps: lysis, binding, wash, and elution.

The eluates containing the nucleic acids isolated from the sample preparation procedure are automatically transferred to the QIASymphony AS module (Figure 1), which then performs the assay setup. The assays are then transferred to the Rotor-Gene Q for PCR and data analysis (Figure 3).

A workflow overview is given on pages 11–13. For more information, refer to the detailed protocols in this handbook (pages 20, 25, and 33).

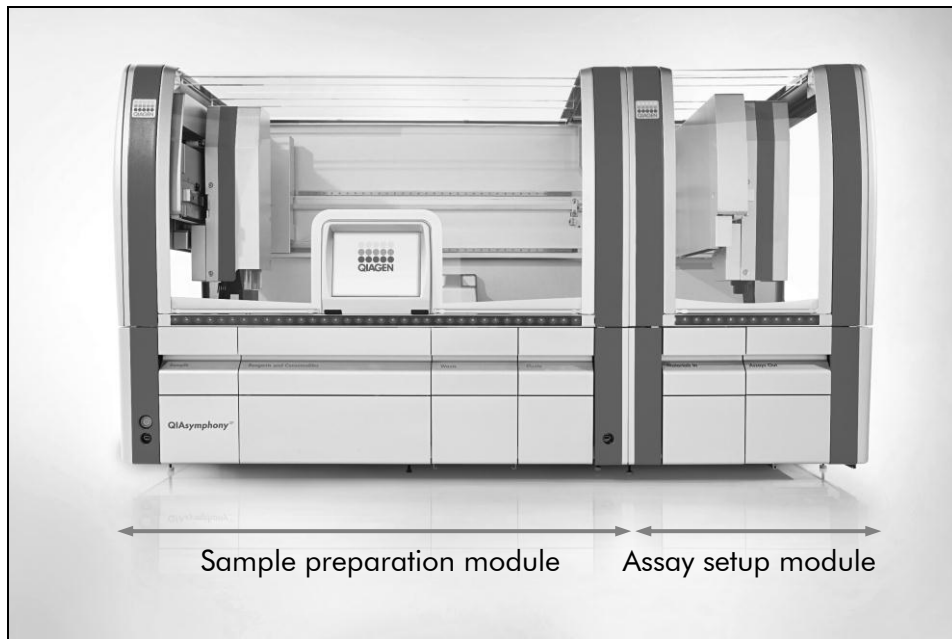


Figure 1. The QIASymphony SP/AS instruments. Samples, reagents, and consumables are loaded in the relevant drawers. Sample preparation and assay setup are then fully automated, including inter-module transfer.

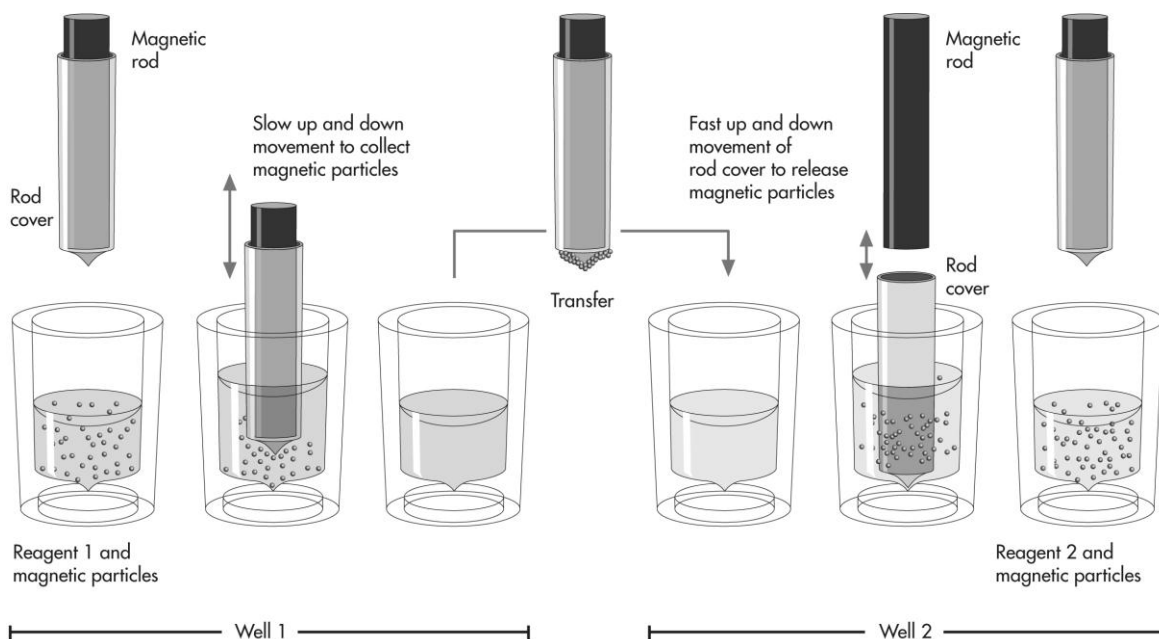


Figure 2. Schematic of the QIASymphony SP principle. The QIASymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIASymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously.



Figure 3. Transferring the samples to the Rotor-Gene Q. After sample preparation and assay setup, the reactions are loaded in the 72 Well or 72 Disc Rotor of the Rotor-Gene Q for real-time PCR analysis.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing isolated nucleic acids at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –20°C.

QIASymphony RGQ workflow overview

QIASymphony SP

Define a run/batch using the sample preparation user interface



Load the drawers with the appropriate samples, reagents, and consumables



Place the elution rack in the “Eluate” drawer

Integrated operation

Place the elution rack with adapter, including the transfer frame, on slot 1.

Independent operation

Place the elution rack with adapter, but without the transfer frame, on any elution slot. Note that only slot 1 is cooled.



Start the protocol



When sample processing is complete, either perform direct transfer of the elution rack to the QIASymphony AS via the transfer module (integrated operation) or manually remove the elution rack from the “Eluate” drawer (independent operation)

Integrated operation

Press “Transfer” to transfer the elution rack from slot 1 of the QIASymphony SP to slot 2 of the QIASymphony AS.

Independent operation

First remove the elution rack from the slot on the touchscreen, then open the “Eluate” drawer and manually remove the elution rack.



Download the QIASymphony SP result file



QIASymphony AS

Switch to the assay setup user interface on the touchscreen



Start the assay definition process

Integrated operation

After automatic transfer of the elution rack, all of the stored rack information will automatically be applied.

Independent operation

After manual transfer of the elution rack, press "New" and either select the rack file from the respective run on the QIASymphony SP or type in the required rack information manually.



Define an assay run

The screens that appear on the touchscreen will guide you step-by-step through the assay definition process.



Load the "Eluate and Reagents" and "Assays" drawer with the appropriate samples, reagents, and consumables

For detailed loading information, refer to the "Loading Information" screen. Alternatively, press "Queue".



Start the assay run



Remove the assays by pressing "Remove" and then manually removing the assay rack from the "Assays" drawer



Transfer the assay rack to the Rotor-Gene Q



Download the QIASymphony AS result file and the cycler file



Transfer the cycler file to the Rotor-Gene Q



Rotor-Gene Q

Select or create the *mericon*-specific temperature profile



Click "Start Run"



After the run is finished, analyze the results



Maintenance

Perform the appropriate maintenance procedures for the QIASymphony SP/AS and the Rotor-Gene Q according to the instrument user manuals

See Section 8 of the *QIASymphony SP/AS User Manual*, "General Description", and Section 9 of the *Rotor-Gene Q User Manual* for more details about the required daily, regular, and weekly maintenance procedures.

Equipment and Reagents to Be Supplied by User

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

For all protocols

- Pipets (adjustable) and sterile pipet tips with filters
- Vortex mixer*
- Microcentrifuge*

For sample preparation

- QIASymphony SP instrument (cat. no. 9001297)*
- QIASymphony *mericon* Bacteria Kit (cat. no. 931156)

Accessories and adapters for the QIASymphony SP

- Reagent Cartridge Holder (2) (cat. no. 997008)
- 1–4 sets of Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)
- Cooling adapter for EMT racks (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)

Consumables for the QIASymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Micro tubes 2 ml, PP, without lid (Sarstedt, cat. no. 72.608, www.sarstedt.com)
- Filter-Tips, 1500 μ l (cat. no. 997024)
- Elution Microtubes CL with cap strips (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)

For assay setup

- QIASymphony AS instrument (cat. no. 9001301)*
- *mericon* PCR Pathogen Detection Assays (e.g., *mericon* Salmonella spp Kit, cat. nos. 290013 and 290015)

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

Accessories and adapters for the QIA Symphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)

For use with the Rotor-Gene Q 72 Rotor-Disc®

- Rotor-Disc® Loading Block adapter (Adapter 2 x Rotor-Disc, Qsym, cat. no. 9242204)
- Rotor-Disc 72 Loading Block (cat. no. 9018910)

For use with the Rotor-Gene Q 72 Rotor-Strips

- RG Strip Tubes 72 adapter (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

For use with the Applied Biosystems 7500 96-well plate

- PCR Plate 96 QS adapter (cat. no. 9020732)

Consumables for the QIA Symphony AS

- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Micro tubes 2 ml, PP, without lid (Sarstedt cat. no. 72.608, www.sarstedt.com)
- Tip disposal bags (cat. no. 9013395)

For use with the Rotor-Gene Q 72 Rotor-Disc

- Rotor-Disc 72 (cat. no. 981303 (240) / 981301(24))
- Rotor-Disc Heat Sealing Film (cat. no. 981604 (600) / 981601 (60))

For use with Rotor-Gene Q 72 Rotor-Strips

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)

For use with the Applied Biosystems 7500 96-well plate

- For compatible tube and plate formats, visit www.qiagen.com/QIAymphony/Resources.

For PCR

- Rotor-Gene Q instrument*
- Rotor-Gene Q software version 2.02, or higher

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

Important Notes

General precautions

The user should always pay attention to the following:

- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- For the assay setup 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.
- Make sure that the required adapters are precooled to 2–8°C.

Getting started on the QIASymphony SP instrument

The QIASymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified DNA from the “Eluate” drawer after processing. Refer to the QIASymphony SP User Manual for operating instructions.

To begin, close all the drawers and hoods.

Switch on the QIASymphony SP/AS instrument, and wait until the “Sample Preparation” screen appears and the initialization procedure has finished.

Log into the instrument (drawers will unlock).

Loading reagent cartridges into the “Reagents and Consumables” drawer

Reagents for the purification of nucleic acids are contained in an innovative reagent cartridge (Figure 4). Each trough of the reagent cartridge contains a particular reagent, such as the magnetic particles, binding buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse, which avoids waste of leftover reagents at the end of the purification procedure.

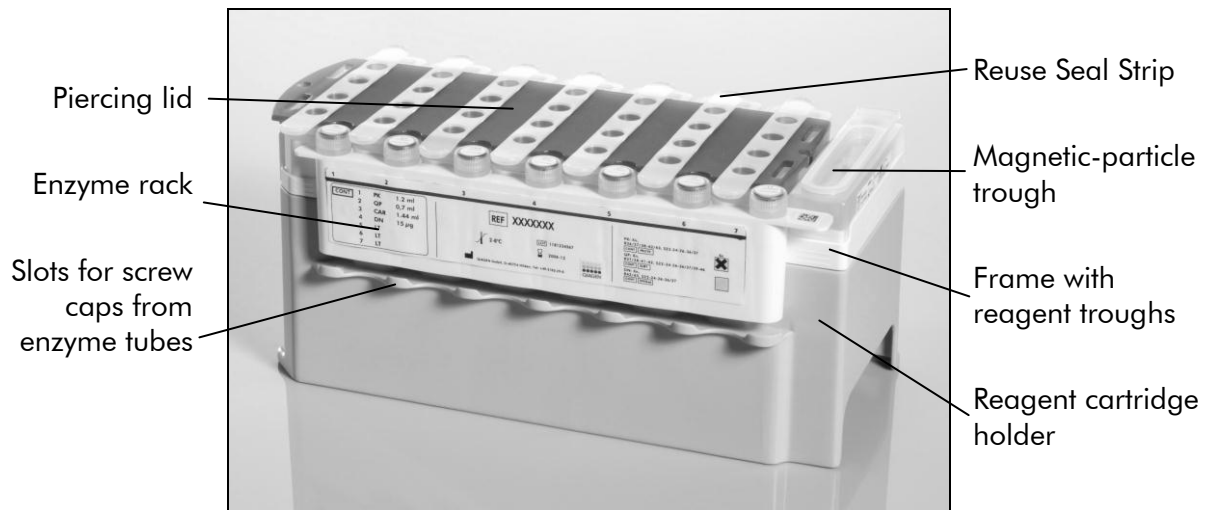


Figure 4. QIASymphony reagent cartridge. The reagent cartridge contains all of the reagents required for the purification procedure.

Note: The QIASymphony reagent cartridge shown is the standard version. The reagent cartridge for the QIASymphony *mericon* Bacteria Kit does not have an enzyme rack.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 minutes, and replace it in the reagent cartridge frame before the first use. Place the reagent cartridge into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (Figure 5).

Important: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.



Figure 5. Easy worktable setup with reagent cartridges.

After the magnetic-particle trough cover has been removed, the reagent cartridge can be loaded into the “Reagents and Consumables” drawer.

Partially used reagent cartridges can be stored until needed again (see “Storage” on page 5).

Loading plasticware into the “Reagents and Consumables” drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (1500 μ l tips provided in black racks) are loaded into the “Reagents and Consumables” drawer.

Important: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the “Reagents and Consumables” drawer.

Important: Tips have filters to help prevent cross-contamination.

Important: Do not refill tip racks or unit boxes for sample prep cartridges or 8-Rod Covers manually before starting another protocol run. The QIA Symphony SP can use partially used tip racks and unit boxes.

TopElute Fluid

The extraction procedure requires TopElute Fluid. An opened 60 ml bottle containing TopElute Fluid is placed on the worktable to the right of the reagent cartridges.

Loading the “Waste” drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the “Waste” drawer. Make sure that the “Waste” drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Important: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the “Waste” drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the “Waste” drawer.

Important: The system does not check for the presence of a tip disposal bag. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manuals provided with your instrument. Empty the tip bag after a maximum of 96 samples have been processed to avoid a tip jam.

A waste container collects liquid waste generated during the purification procedure. The “Waste” drawer can only be closed if the waste container is in place. Dispose of the liquid waste according to your local safety and environment regulations. Do not autoclave the filled waste bottle.

Loading the “Eluate” drawer

Load the required elution rack into the “Eluate” drawer. Preferably use the cooled “Elution slot 1” with the corresponding cooling adapter. As long term storage of eluates in the “Eluate” drawer may lead to evaporation of eluates, we strongly recommend using the cooling position. For integrated operation, place the elution rack with adapter, including the transfer frame, on slot 1.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch or batches have been loaded into the corresponding drawers (Tables 3–5, pages 22–23).

Protocol: Isolation of Bacterial DNA on the QIASymphony SP

The QIASymphony *mericon* Bacteria Kit (cat. no. 931156) is designed for bacterial DNA isolation from food enrichment cultures using the QIASymphony SP instrument. General information about the protocol is shown in Table 1.

Table 1. General protocol information

Kit	QIASymphony <i>mericon</i> Bacteria Kit (360)
Sample material	Food enrichment cultures
Sample volume (including excess volume)	500 μ l
Category	<i>mericon</i>
Default Assay Control Set	<i>mericon_Bacteria_400_V1</i>
Default Elution volume	200 μ l
Protocol run time	53 min
Required software version	Version 3.5

Important points before starting

- Familiarize yourself with the operation of the QIASymphony SP. Refer to the user manuals supplied with the instrument for operating instructions.
- Before beginning the procedure, read “Important Notes”, page 16.
- Before using a reagent cartridge for the first time, check that Buffer QSB1 does not contain a precipitate. If necessary, remove the trough containing Buffer QSB1 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate. Replace the trough in the correct position. If the reagent cartridge has already been pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.
- Avoid vigorous shaking of the reagent cartridge or foam may be generated, which can lead to liquid-level detection problems.
- All components of the QIASymphony *mericon* Bacteria Kit have been shown to be stable in the QIASymphony SP/AS instrument for up to 48 hours.

Things to do before starting

- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge, remove the cover from the trough containing the magnetic particles. Make sure that the piercing lid is placed on the reagent cartridge. If using a partially used reagent cartridge, make sure that the Reuse Seal Strips have been removed.
- Place an opened 60 ml bottle containing TopElute Fluid on the worktable to the right of the reagent cartridges.
- If samples are bar coded, position samples in the tube carrier so that the bar codes face the bar code reader in the “Sample” drawer at the left side of the QIASymphony SP.

Procedure

1. **Close all of the drawers and hoods of the QIASymphony SP/AS instrument.**
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 QIASymphony SP.
3. **Log into the instrument.**
4. **Ensure the “Waste” drawer is prepared properly (Table 2), and perform an inventory scan of the “Waste” drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**

Table 2. Preparing the “Waste” drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Apply waste bag
Liquid waste bottle holder	Install empty liquid waste bottle

5. **Load the required elution rack into the “Eluate” drawer.**

Use the cooled “Elution slot 1” with the corresponding cooling adapter (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730). For integrated operation, place the elution rack with adapter, including the transfer frame on slot 1.

6. Load the required reagent cartridge(s) and consumables into the “Reagents and Consumables” drawer (see Table 3 and Table 4).

Table 3. Preparing the “Reagents and Consumables” drawer

Reagent Cartridge Position 1 and 2	Load 1 reagent cartridge for up to 180 samples or 2 reagent cartridges for up to 360 samples
Tip rack holder position 1–18	Load sufficient racks of disposable filter-tips, 1500 μ l (see Table 4)
Unit box holder position 1–3	Load 3 unit boxes containing sample prep cartridges
Unit box holder position 4	Load 1 unit box containing 8-Rod Covers
TopElute Bottle	Load 1 bottle of TopElute Fluid on the worktable to the right of the reagent cartridges.

Table 4. Required plasticware for 1–4 sample batches

	1 batch, 24 samples*	2 batches, 48 samples*	3 batches, 72 samples*	4 batches, 96 samples*
Disposable filter-tips, 1500 μl^{†‡}	101 (4 racks)	193 (7 racks)	285 (9 racks)	377 (12 racks)
Sample prep cartridges[§]	15	30	45	60
8-Rod Covers[¶]	3	6	9	12

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

† There are 32 filter-tips per tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge and TopElute bottle (9 tips).

§ There are 28 sample prep cartridges per unit box.

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

7. Press the “R+C” button in the touchscreen to open the screen that shows the consumables status (“Consumables/8-RodCovers/Tubes/Filter-Tips/Reagent Cartridges”). Press the “Scan Bottle” button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Press the “OK” button.

Ensure that the TopElute bottle is scanned, opened, and placed onto the worktable before starting the inventory scan. Otherwise, the inventory scan must be repeated after scanning, opening, and placing the TopElute bottle onto the worktable.

8. Perform an inventory scan of the “Reagents and Consumables” drawer.
9. Place the samples into the appropriate tube carrier, and load them into the “Sample” drawer (see Table 5).

Table 5. Loading samples into the “Sample” drawer

Sample volume (including excess volume)	500 μ l
Sample tubes	Micro tubes 2 ml, PP, without lid (Sarstedt cat. no. 72.608, www.sarstedt.com)
Insert	Tube Insert 3B (cat. no. 9242083)

10. Using the touchscreen, enter the required information for each batch of samples to be processed.

Enter the following information:

- Sample information
- Protocol to be run (choose “mericon_Bacteria_400_V1”)
- Elution volume 200 μ l and output position “slot 1”

After information about the batch has been entered, the status changes from “LOADED” to “QUEUED”. As soon as one batch is queued the “Run” button appears.

11. Press the “Run” button to start the purification procedure.

All processing steps are fully automated. The protocol process is indicated by a progress bar and the batch run time. At the end of the protocol run, the status of the batch changes from “RUNNING” to “COMPLETED”.

- 12. When sample processing is complete, either perform direct transfer of the elution rack to the QIASymphony AS via the transfer module (integrated operation) or manually remove/transfer the elution rack from the “Eluate” drawer (independent operation).**

For integrated operation, press “Transfer” to transfer the elution rack from slot 1 of the QIASymphony SP to slot 2 of the QIASymphony AS.

For independent operation, remove the elution rack from the slot on the touchscreen, then open the “Eluate” drawer and manually transfer/remove the elution rack.

For short-term storage of up to 24 h, we recommend storing the isolated nucleic acids at 2–8°C. For long-term storage of over 24 h, we recommend storage of isolated nucleic acids at –20°C.

Result files are generated for each elution rack and can be downloaded via the QIASymphony Quality Management Console (QMC).

- 13. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.**

Important: For more information about storage of partially used reagent cartridges, see “Storage” on page 5.

- 14. Discard used sample tubes, plates, and waste according to your local safety regulations.**

See page 6 for safety information.

- 15. Close the instrument drawers, and proceed with assay setup on the QIASymphony AS (page 25).**

- 16. Clean the QIASymphony SP during the assay setup on the QIASymphony AS or later.**

For integrated operation, clean all instruments at the end of the completed workflow.

Follow the maintenance instructions in the *QIASymphony SP/AS User Manual — General Description*. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.

Protocol: Assay Setup on the QIASymphony AS

The QIASymphony AS carries out setup of the *mericon* PCR Detection Assays for the Rotor-Gene Q Rotor-Disc 72, Rotor-Gene Q strip tubes, and 96-well plates for the Applied Biosystems® 7500 cycler. For each of these PCR formats, either a single-assay run with only one *mericon* PCR assay or a multi-assay run with a combination of *mericon* PCR assays can be set up.

Important: While the Rotor-Gene Q does not require ROX as a passive reference dye, for the Applied Biosystems 7500 cycler, the use of a ROX passive reference dye during the PCR is necessary. For cycler-dependent preparation of the *mericon* assays, please refer to the corresponding preparation instructions in “Things to do before starting” on page 26.

Samples processed on the QIASymphony SP can be either transferred automatically (integrated operation) or manually (independent operation) to the QIASymphony AS. For extra flexibility, the QIASymphony SP and AS can be operated independently.*

Details of the assay setup are shown in Table 6.

Table 6. Assay parameters for the assay setup on the QIASymphony AS

Cycler PCR format	Rotor-Gene Q 72-Disc or 72-Well	Applied Biosystems 7500 Standard 96-well plate
ROX required	No	Yes
Master mix volume	10 μ l	10.4 μ l
Template volume	10 μ l	9.6 μ l
Runtime	Approx. 72 minutes	Approx. 72 minutes
Required software version	Version 3.5	Version 3.5

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 16.
- Familiarize yourself with the operation of the QIASymphony AS module. Refer to the user manuals supplied with your instrument for operating instructions.

* The QIASymphony SP and AS remain physically connected during independent operation.

Things to do before starting

- If using the Rotor-Gene Q cycler, prepare the *mericon* Assay(s) without ROX:

24 sample kit: Add 130 μ l Multiplex PCR Master Mix (tube with blue lid) to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly. Transfer each or both of the reconstituted *mericon* Assays to a labeled, fresh 2 ml microtube.

96 sample kit: Add 1040 μ l Multiplex PCR Master Mix (tube with blue lid) to the vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

Note: If the reconstituted *mericon* Assay will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- If using the Applied Biosystems 7500 cycler, prepare the *mericon* Assay(s) with ROX:

24 sample kit: Add 130 μ l Multiplex PCR Master Mix (tube with blue lid) and 5.2 μ l ROX dye to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly. Transfer each or both of the reconstituted *mericon* Assay to a labeled, fresh 2 ml microtube.

96 sample kit: Add 1040 μ l Multiplex PCR Master Mix (tube with blue lid) and 41.6 μ l ROX dye to the vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

Note: If the reconstituted *mericon* Assay will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Dissolve the dried Positive Control DNA (tube with red lid). For both kit sizes and all cyclers add 200 μ l of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing and centrifuge briefly. Transfer the reconstituted Positive Control to a labeled, fresh 2 ml microtube.

Note: If the dissolved positive control will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

Before each use, all reagents must be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

1. Insert the tip chute into its position on the right hand side in the front part of the QIASymphony AS module.
2. Install an empty tip disposal bag in the bag holder under the "Assays" drawer.
3. Switch from the sample preparation user interface to the assay setup user interface by pressing the "Switch" button in the status bar.
4. Start the assay definition process.
5. For integrated operation (elution rack is automatically transferred from the QIASymphony SP into the AS module) the "Sample Rack(s)" screen will appear directly.
6. For independent operation manually place the elution rack into slot 2 of the QIASymphony AS module and press "New" to access the "Sample Rack(s)" screen.

The screens on the touchscreen guide the user through the assay definition process in a step-by-step fashion. All necessary information is added for each step once the "Next" button is activated.

7. For integrated operation, all stored sample information (sample status, sample ID, sample volume, and rack ID) are transferred to the QIASymphony AS module together with the elution rack and will automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface.

For independent operation, select the rack file of the corresponding QIASymphony SP run. This will load all stored sample information (sample status, sample ID, sample volume, and rack ID) and automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface.

8. If the assay setup is independent from a former QIASymphony SP run, select the rack type of your elution rack for the highlighted "Sample" position (slot 2) and then either manually type in the "Rack ID" of your elution rack or choose "Automatic ID" for a new ID.

The "Reagents" position (slot 1) can optionally be assigned to an additional sample rack ("S") or an additional reagent rack ("R"). When applied, the software allows the separate implementation of the additional samples/reagents into the overall PCR setup.

9. In the “Sample Rack Layout” screen of the assay setup user interface the elution rack in slot 2 is pictured.

For integrated operation, or for independent operation in combination with a loaded rack file, sample IDs and sample volumes are automatically assigned to the corresponding positions.

For independent operation without a rack file, select the positions to be processed from the elution rack. Define the highlighted positions as “Sample” then reselect the defined samples and assign sample volumes.

If an additional sample rack was implemented, switch to “Slot 1” and assign corresponding samples and sample volumes.

10. In the “Assay Selection” screen select the Assay Parameter Set(s) to use in the run (see Table 7, page 29).

Select the preferred PCR format from the corresponding categories. The featured PCR formats include the Rotor-Gene Q Rotor-Disc 72, the Rotor-Gene Q strip tubes, and a 96-well plate for the Applied Biosystems 7500 cycler. Each category features Assay Parameter Sets for the *mericon* Pathogen Detection Assays, such as the *mericon* Salmonella spp Assay.

Assign the assay(s) to the samples either for “Slot 1”, “Slot 2”, or “Slot1/2”. For each PCR format, a single-assay run with one *mericon* PCR assay or a multi-assay run with a combination of *mericon* PCR assays can be set up. When two assay sets are selected, the option “Assign each assay individually” appears. If “Yes” is selected, the separate assays can be assigned to specific samples in the next screen. If “No” is selected, all samples will be processed with both assays.

Table 7. Example PCR format and Assay Parameter Set information for the *mericon* Salmonella spp. Assay

PCR format	Protocol information	Details
All formats	<i>mericon</i> PCR Assays	e.g., <i>mericon</i> Salmonella spp assay
	Category 1	<i>mericon</i> 96 well
ABI 96-well plate	Assay Parameter Set 1	e.g., <i>mericon_Salmonella_spp_QIAGEN-PCR-96_V1</i>
	Category 2	<i>mericon</i> QS RGQ
RGQ 72 Rotor-Strips	Assay Parameter Set 2	e.g., <i>mericon_Salmonella_spp_QS_RGQ_RS_V1</i>
	Category 3	<i>mericon</i> QS RGQ Rotor Disc
RGQ 72 Rotor-Disc	Assay Parameter Set 3	e.g., <i>mericon_Salmonella_spp_QS_RGQ_RD72_V1</i>

11. In the “Assay Assignment” screen assign the Assay Parameter Sets to samples.

If a single-assay run was chosen with only one *mericon* PCR assay, select the samples to process from the depicted elution rack and press “Assign”. If a multi-assay run with both *mericon* PCR assays was chosen, a separate sheet for each assay will be displayed in which samples from the depicted elution rack can be separately assigned to either one or the other *mericon* PCR assay. If an additional sample rack next to the elution rack was previously defined during the Assays Definition Process for slot 1, assign assays accordingly.

12. In the “Assay Rack(s)” screen define the assay rack ID. Either type in the assay rack ID manually or choose “Automatic ID” for a new ID.

13. The cooling of samples and reagents will start automatically. Check the temperature of the cooling positions.

When the target cooling temperatures are reached, the slots will appear green.

14. The “Loading Information” screen displays the working table of the QIASymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.

15. Place the reconstituted *mericon* Assay(s), the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters (see Table 8).

Important: Make sure that the reconstituted *mericon* assay(s) of the 24 sample kits as well as the Positive Control(s) have been transferred to 2 ml micro tubes before they are loaded into the AS reagent adapter. If the reconstituted 12-reaction assays of the 24 sample kit have been transferred to separate 2 ml tubes, make sure to only load one assay tube.

Important: Before each use, all reagents need to be thawed completely, mixed by pipetting up and down 5 times or vortexing and centrifuge briefly. Avoid bubbles or foaming, which could cause detection errors.

Important: For the automated assay setup the QIASymphony AS pipetting dead volume has to be considered. To ensure reliable and accurate pipetting a dead volume of 6 reactions occurs per kit (24 and 96 sample kits)

Table 8. Positions of the PCR reagents in the reagent adapter

Reagent positions in cooling adapter	
A1	A2
<i>mericon</i> Assay 1	<i>mericon</i> Assay 2
B1	B2
Positive Control 1	Positive Control 2
C1	C2
Negative Control 1	Negative Control 2

16. Open the “Eluate and Reagents” and “Assays” drawers.

17. Load the prepared reagent adapter into slot 3 of the “Eluate and Reagents” drawer according to the illustration in the “Loading Information” screen. Place the PCR assay consumable (disc, wells, or plate) in the appropriate adapter and load the adapter into the designated slot of the “Assays” drawer (see Table 9).

If an additional sample rack next to the elution rack was previously defined during the Assays Definition Process place the samples in the appropriate cooling adapter and into slot 1 of the “Eluate and Reagents” drawer.

Table 9. Adapters and reagent holders

PCR format	Rack/reagent holder	Slot
RG Q 72 disc	Rotor-Disc Loading Block adapter	1–3
	Rotor-Disc 72 Loading Block	Front
RG Q 72 well	RG Strip Tubes 72 adapter	2
ABI 7500 96-well plate	PCR Plate 96 QS adapter	2

18. If not already done after the purification procedure (see step 12 of the purification protocol, page 24) transfer the elution rack together with the adapter to the QIASymphony AS.
19. For integrated operation, press “Transfer” to transfer the elution rack from slot 1 of the QIASymphony SP to slot 2 of the “Eluate and Reagents” drawer of the QIASymphony AS.
20. For independent operation, remove the elution rack from the slot in the sample preparation user interface on the touchscreen, then open the “Eluate” drawer of the QIASymphony SP and manually transfer elution rack and adapter to slot 2 of the “Eluate and Reagents” drawer of the QIASymphony AS.
21. Load disposable filter-tips into the “Eluate and Reagents” and “Assays” drawers, according to the required number of each tip type.
22. Close the “Eluate and Reagents” and “Assays” drawers.
23. Upon closing each drawer, press “Yes” to start the inventory scan for each drawer.

The inventory scan checks the slots, adapters, filter-tips, and the tip chute. If required, correct any errors.

24. Press “Queue”. Monitoring of the cooling starts.
25. Press “Run” to start the run.

The protocol process is indicated by a progress bar and the protocol run time.

- 26. After the run is finished, press “Remove” in the assay setup “Overview” screen. Open the “Assays” drawer and unload the PCR assay adapter.**
- 27. Download the result and cycler files via the QIASymphony Quality Management Console (QMC).**
- 28. Proceed to “PCR and Data Analysis on the Rotor-Gene Q or the Applied Biosystems 7500”, page 33.**
- 29. Perform the regular maintenance/cleaning of the QIASymphony AS during the PCR run on the Rotor-Gene Q or later.**

For integrated operation, clean all instruments at the end of the completed workflow.

Follow the maintenance instructions in the *QIASymphony SP/AS User Manual — General Description*. Be sure to carry out maintenance regularly to minimize the risk of cross-contamination.

Protocol: PCR and Data Analysis on the Rotor-Gene Q or the Applied Biosystems 7500 Real-Time PCR cyclers

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 16.
- Familiarize yourself with the operation of the Rotor-Gene Q or the Applied Biosystems 7500 Real-Time PCR cycler. Refer to the user manual supplied with the instrument for operating instructions.
- Make sure that at least one positive and one negative control are included per PCR run.

Procedure

1. **Seal or close the PCR tubes or strips and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.**

If using the Rotor-Gene Q, make sure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.

2. **Transfer the cycler file from the QIASymphony AS to the Rotor-Gene Q or the Applied Biosystems 7500.**

All specifications refer to the Rotor-Gene Q software version 2.02. Please find further information about programming Rotor-Gene Q instruments in the instrument user manual.

3. **Program the thermal cycler. If using the Rotor-Gene Q, use the cycling protocol in Table 10 (page 34). For the Applied Biosystems 7500, use the cycling protocol in Table 11 (page 34).**
4. **For the Rotor-Gene Q make sure that “Perform Optimisation Before 1st Acquisition” in the Gain Optimisation menu is activated.**
5. **Start the PCR run.**
6. **Proceed to “Analyzing the Results” on page 35.**

Table 10. Cycling protocol for Rotor-Gene Q

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq Plus DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	
Annealing	15 s	60°C	Data collection at 60°C
Extension	10 s	72°C	
Number of cycles	40		
Detection	Reporter	Excitation/emission	Channel
Target	FAM™	495/520 nm	Green
Internal control	MAX™	524/557 nm	Yellow

Table 11. Cycling protocol for the Applied Biosystems 7500

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq Plus DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	
Annealing	32 s	60°C	Data collection at 60°C
Extension	10 s	72°C	
Number of cycles	40		
Detection	Reporter	Excitation/emission	Channel
Target	FAM	495/520 nm	Green (FAM)
Internal control	MAX	524/557 nm	Yellow (VIC)

Analyzing the Results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. Figures 6–8 are examples of possible outcomes, which are summarized in Table 12 (page 36).

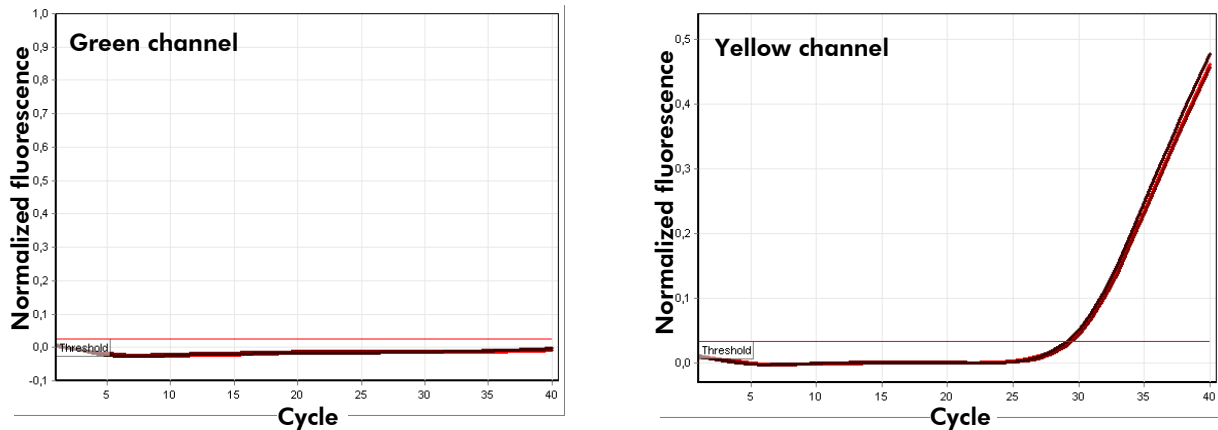


Figure 6. The sample is negative for tested pathogen. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

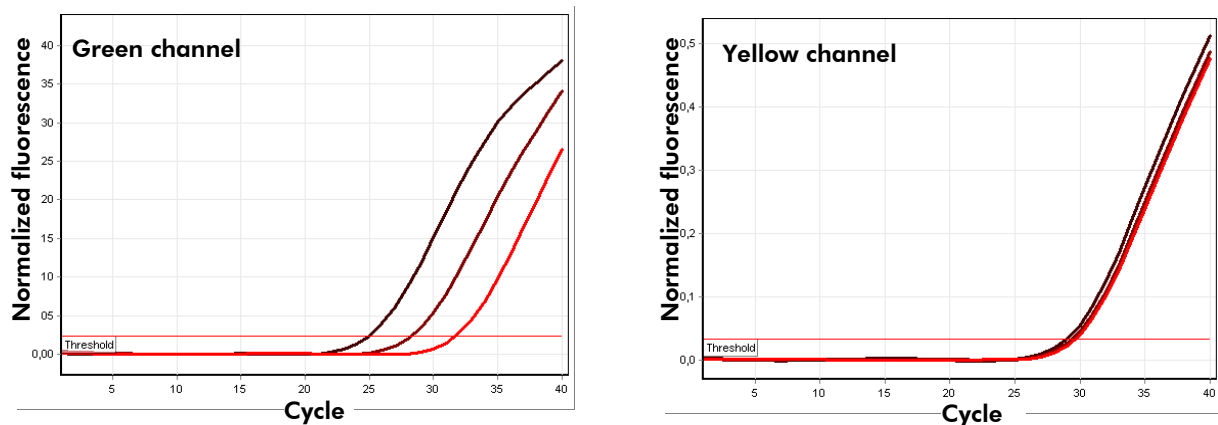


Figure 7. The sample is positive for tested pathogen. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

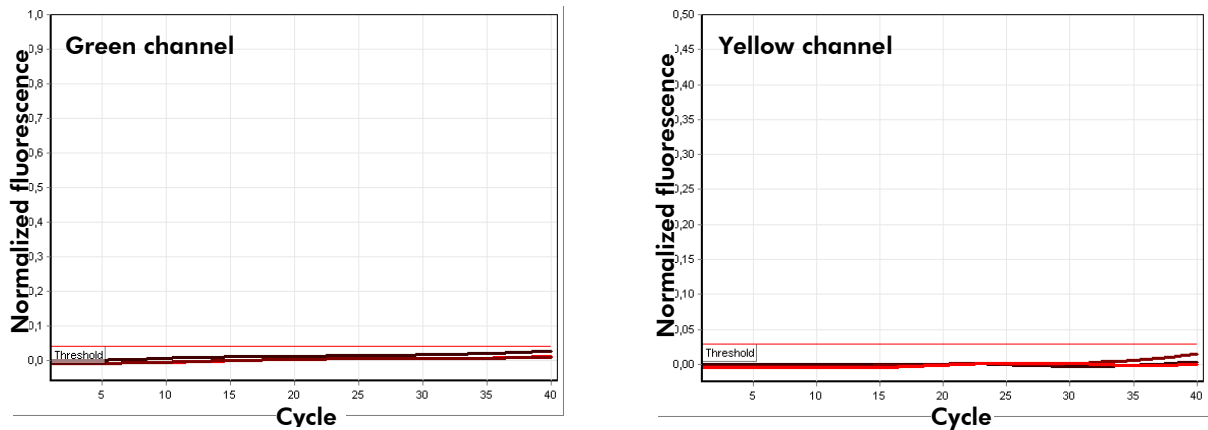


Figure 8. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 12. Summary of possible outcomes

Amplification of internal control	Amplification of sample	Result
+	+	Sample is positive
+	–	Sample is negative
–	–	PCR failed

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the internal control to higher quantification cycle (C_T) values. As a guideline, the uninhibited internal control should give a quantification cycle value ranging between 28 and 32. A quantification cycle value above 33 indicates inhibition.

In the event of PCR inhibition, dilute the extracted samples 1:10 with RNase-free water and repeat the test.

If DNA template concentration in the PCR reaction is very high, a shift of the Internal Control to lower cycle values might occur, which does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

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 of the QIASymphony SP and AS module

Error message displayed on the touchscreen	If an error message is displayed during a protocol run, refer to the user manuals supplied with your instruments.
--------------------------------------------	-------------------------------------------------------------------------------------------------------------------

Precipitate in the reagent trough of an opened cartridge of the QIASymphony mericon Bacteria Kit

- | | |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Buffer evaporation | Excessive evaporation may lead to an increased salt concentration or decreased alcohol concentration in the buffers. All of the components of the reagent cartridge have been shown to be stable for at least 48 hours when open and unsealed. Make sure that the buffer troughs of partially used reagent cartridges are sealed with Reuse Seal Strips when not being used for purification. |
| b) Storage of reagent cartridge | Storing the reagent cartridge at temperatures below 15°C may lead to the formation of precipitates. If necessary, remove the troughs containing Buffer QSB1 from the reagent cartridge and incubate in a water bath* at 37°C for 30 min with occasional shaking to dissolve any precipitate. If the reagent cartridge has already been pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge in a water bath* at 37°C for 30 min with occasional shaking. |

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

Comments and suggestions

Low yield of nucleic acids

- a) Magnetic particles were not completely resuspended Before starting the procedure, ensure that the magnetic particles have been fully resuspended. Vortex for at least 3 minutes before use.
- b) Frozen samples were not mixed properly after thawing If the samples were frozen, thaw them with mild agitation to ensure thorough mixing.
- c) Clogging of pipet tip by insoluble material Insoluble material, such as food debris from the enrichment culture, may have been co-transferred with the sample to the sample tube prior to starting the QIA Symphony DNA extraction procedure. To avoid transfer of insoluble material, use filter bags to prepare the enrichment culture. A sample that is suitable for the automated DNA extraction can be drawn from the separate filter compartment of the filter bag.

Unprocessed sample

- Not enough sample in the tube The QIA Symphony SP instrument performs a liquid level detection before each sample transfer. If the sample volume is below 399 μl (the protocol processes 400 μl for each DNA extraction) the sample will not be transferred or processed. The corresponding well of the elution plate will be left empty by the instrument. Specifications for the sample transfer vary with the applied labware. When larger tubes are used than the specified 2 ml micro tubes, an increased dead volume is needed, and thus it is necessary to apply a greater overfill (>500 μl).

Comments and suggestions

Sample spilling in the “Sample” unit of the QIA Symphony SP

Too much sample in the tube	The QIA Symphony SP instrument performs a liquid level detection before each sample transfer. For this, the pipet tips enter the sample tube for 2 mm. If you have the specified 2 ml micro tubes filled with the maximal 2 ml volume, spillage is unlikely. However, depending on the degree of tube overfill (>2 ml), even a small sample displacement can lead to spillage of the highly concentrated and infectious sample material
-----------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

No signal with the positive control in the green fluorescence channel

- | | |
|----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) The selected fluorescence channel for PCR data analysis does not comply with the protocol | For data analysis, select the green fluorescence channel for the target and the yellow fluorescence channel for the internal control. |
| b) Incorrect programming of the temperature profile of the real-time PCR cycler | Compare the temperature profile with the corresponding cycling protocol. See Tables 10 and 11, page 34. Repeat the PCR with corrected settings, if necessary. |
| c) 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 “Protocol: Assay Setup on the QIA Symphony AS”, page 25. |
| d) The storage conditions for one or more kit components did not comply with the instructions given in “Storage” (page 5). | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |

Weak or no signal in the internal amplification control

- | | |
|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Incorrect programming of the temperature profile of the real-time PCR cycler | Compare the temperature profile with the corresponding cycling protocol. See Tables 10 and 11, page 34. Repeat the PCR with corrected settings, if necessary. |
|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|

Comments and suggestions

- b) The PCR was inhibited Inhibition might occur in the case of carry-over of strong sample-derived PCR inhibitors from the DNA extraction procedure. In this case prepare 10-fold dilutions of inhibited sample eluates and repeat the PCR.
- c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 5) Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
- d) The *mericon* PCR Assay 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 in the green fluorescence channel

- a) Contamination occurred during preparation of the PCR Repeat the PCR with new reagents in replicates.
If possible, seal the PCR tubes directly after the assay setup has finished.
Make sure that the work space and instruments are decontaminated at regular intervals.
- b) Contamination occurred during extraction Repeat the extraction and PCR of the sample using new reagents.
Make sure that the work space and instruments are decontaminated at regular intervals.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
QIASymphony <i>mericon</i> Bacteria Kit	Reagent Cartridge, Piercing Lid, TopElute Fluid, Reuse Seal Set	931156
<i>mericon</i> Pathogen Detection Assays		
<i>mericon</i> Campylobacter spp Kit (24)*	For 24 reactions: PCR Assay Campylobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290033
<i>mericon</i> Campylobacter triple Kit (24)*	For 24 reactions: PCR Assay Campylobacter triple, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290043
<i>mericon</i> Cronobacter spp Kit (24)*	For 24 reactions: PCR Assay Cronobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290063
<i>mericon</i> Legionella spp Kit (24)*	For 24 reactions: PCR Assay Legionella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290083
<i>mericon</i> L. pneumophila Kit (24)*	For 24 reactions: PCR Assay L. pneumophila, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290093
<i>mericon</i> Listeria spp Kit (24)*	For 24 reactions: PCR Assay Listeria spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290123

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> L. monocytogenes Kit (24)*	For 24 reactions: PCR Assay L. monocytogenes, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290023
<i>mericon</i> Salmonella spp Kit (24)*	For 24 reactions: PCR Assay Salmonella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290013
<i>mericon</i> Shigella spp Kit (24)*	For 24 reactions: PCR Assay Shigella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290103
<i>mericon</i> S. aureus Kit (24)*	For 24 reactions: PCR Assay S. aureus, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290073
<i>mericon</i> VTEC stx1/2 Kit (24)*	For 24 reactions: PCR Assay VTEC stx1/2, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290053
<i>mericon</i> Y. enterocolitica Kit (24)*	For 24 reactions: PCR Assay Y. enterocolitica, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290113
QIASymphony RGQ system		
QIASymphony RGQ, System	QIASymphony SP, QIASymphony AS, Rotor-Gene Q 5plex HRM, required accessories and consumables, installation, and training	9001850

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
QIASymphony SP/AS instruments and accessories		
QIASymphony SP	QIASymphony sample prep module, 1-year warranty on parts and labor	9001297
QIASymphony AS	QIASymphony assay setup module, 1-year warranty on parts and labor	9001301
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIASymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIASymphony SP	997004
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks; for use with the QIASymphony SP/AS instruments (software version 3.1)	9020730
Reuse Seal Set (20)	Reuse seal sets for sealing partly used QIASymphony reagent cartridges	997006
Insert, 2.0ml v2, samplecarr. (24), Qsym	Secondary tube adapter (for 2 ml screw-cap tubes, tube insert 3b) for use with the QIASymphony SP tube carrier	9242083
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Cooling Adapter, Reagent Holder 1, Qsym	Adapter for holding 18 x 2 ml conical tubes, and 6 x 5 ml conical tubes; for use with the QIASymphony SP/AS instruments (software version 3.1)	9018090
Cooling Adapter, Reagent Holder 2, Qsym	Adapter for holding 18 x 2 ml conical tubes, 2 x 5 ml conical tubes, and 2 x Reagent Bottles, 30 ml; for use with the QIASymphony SP/AS instruments (software version 3.1)	9018089
Cooling Adapter, RG Strip Tubes 72, Qsym	Adapter for holding 18 strips of 4 tubes; for use with the QIASymphony SP/AS instruments (software version 3.1)	9018092

Product	Contents	Cat. no.
Tubes, conical, 2 ml, Qsym AS (500)	Conical tubes (2 ml) for holding reagent; for use with the QIASymphony AS	997102*
Tube, conical, 5 ml, Qsym AS (500)	Conical tubes (2 ml) for holding reagent; for use with the QIASymphony AS	997104*
Reagent Bottles, 30 ml, QSym AS (50)	Reagent bottles (30 ml) with lids; for use with the QIASymphony AS	997108
Filter-Tips, 1500 μ l (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIASymphony SP	997024
Filter-Tips, 200 μ l (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube [®] and the QIASymphony SP	990332
Filter-Tips, 50 μ l (1024)	Disposable Filter-Tips, racked (8 x 128). For use with the QIASymphony AS	997120
Tip Disposal Bags (15)	Tip disposal bags for use with the QIASymphony SP/AS instruments	9013395
Rotor-Gene Q and accessories		
Rotor-Gene Q 5plex HRM System	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor	9001650
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor; includes installation and training	9001580

* Please inquire for availability.

Product	Contents	Cat. no.
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: QIAGEN®, QIAcube®, QIAAsymphony®, HotStarTaq®, *mericon*®, QuantiTect®, Rotor-Disc®, Rotor-Gene® (QIAGEN Group); Bagmixer® (Interscience); MAX™ (Integrated DNA Technologies, Inc.); Applied Biosystems®, FAM™ (Applied Biosystems Corporation or its subsidiaries); Stomacher® (Seward Ltd).

Limited License Agreement for QIAAsymphony *mericon* Bacteria Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

© 2012 QIAGEN, all rights reserved.

www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

