January 2016

mericon™ DNA Bacteria Handbook

mericon DNA Bacteria Kit

mericon DNA Bacteria Plus Kit

For extraction of DNA from Gram-negative and Gram-positive bacteria

QIAGEN®

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

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Kit Contents

	mericon DNA Bacteria Kit (100)	*mericon* DNA Bacteria Plus Kit (50)
Catalog no.	**69525**	**69534**
Number of preps	**100**	**50**
Fast Lysis Buffer	1 x 25 ml	2 x 25 ml
Pathogen Lysis Tubes L	–	5 x 10
Handbook	1	1

Storage

Fast Lysis Buffer should be stored dry at room temperature (15–25°C).

Pathogen Lysis Tubes L (*mericon* DNA Bacteria Plus Kit only) should be stored dry at room temperature (15–25°C).

When stored dry at room temperature (15–25°C), the *mericon* DNA Bacteria Kits are stable for up to 1 year without any reduction in performance.

Product Use Limitations

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

Quality Control

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the *mericon* DNA Bacteria Kit, the *mericon* DNA Bacteria Plus Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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

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.

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

Introduction

In a globalized food market with increasing demand for food 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 sample preparation and assay kits that meet the demands listed above. Based on detection by real-time polymerase chain reaction (PCR), *mericon* sample preparation kits and PCR Assays enable fast and reliable detection of a broad range of pathogens, genetically modified organisms, allergens, and plant and animal matter in food, animal feed, or pharmaceutical products.

Principle and procedure

To provide a streamlined and straightforward method for microbial sample preparation from pathogen enrichment cultures, QIAGEN has designed protocols based on mechanical lysis (using beads) and thermal lysis (by boiling) that deliver optimal disruption force for different bacteria types.

The thermal lysis procedure of the protocol "Purification of DNA from Food-borne Pathogens Using the *mericon* DNA Bacteria Kit" is optimized for the majority of easily lysed Gram-negative bacteria (e.g., *Salmonella* spp., *Camplylobacter* spp., and *Cronobacter* spp.).

The advanced features of the mechanical sample preparation of the protocol "Purification of DNA from Food-borne Pathogens Using the *mericon* DNA Bacteria Plus Kit" create the lysis conditions needed for difficult Gram-positive bacteria, such as *Listeria* spp.

Developed to be combined with *mericon* PCR Assays, both sample preparation systems deliver a DNA suspension derived from pathogen enrichment cultures, for optimal real-time PCR analysis. Carefully formulated chemistry included in the *mericon* DNA Bacteria Kit and the *mericon* DNA Bacteria Plus Kit maximizes lysis efficiency, stabilizes the extracted DNA, and removes inhibitors that may interfere with downstream applications.

DNA extracted using these sample preparation kits (even from low-count samples) is suitable for sensitive, real-time PCR.

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 use with the *mericon* DNA Bacteria Kit

- Lab pedal blender (e.g., Stomacher® 400 Circulator, available from Seward or BagMixer® 400, available from Interscience)
- Homogenizing bags (e.g., Stomacher Bags, available from Seward or BagMixer Bags, available from Interscience)
- Vortex
- Microcentrifuge tubes with screw caps (2 ml)
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes
- Thermomixer or heating block suitable for 1.5 or 2 ml tubes and capable of attaining a temperature of 100°C. Alternatively, a water bath may be used
- Pipets and pipet tips

For use with the *mericon* DNA Bacteria Plus Kit

- Lab pedal blender (e.g., Stomacher 400 Circulator, available from Seward or BagMixer 400, available from Interscience)
- Homogenizing bags (e.g., Stomacher Bags, available from Seward or BagMixer Bags, available from Interscience)
- Vortexer
- A vortex adapter plate for horizontal or vertical fixation of 2 ml screw-cap tubes (e.g., Shaking Plate [P509.1] with vortex adapter [P510.1] available from Carl Roth) may be used
- Microcentrifuge tubes with screw caps (2 ml)
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes
- Pipets and pipet tips

Protocol: Purification of DNA from Food-borne Pathogens Using the *mericon* DNA Bacteria Kit

This protocol is designed for the extraction of total genomic DNA from Gram-negative bacteria from pathogen enrichment cultures.

Important notes before starting

- If working with infectious pathogens (e.g., salmonella), the protocols and subsequent sample analysis (e.g., PCR) must be carried out in a suitable laboratory area, designated for working with infectious bacteria. Samples should be processed in accordance with official national or local guidelines.
- Samples may still contain infectious material, even after sample lysis. Samples should therefore be treated as infectious throughout the procedure. To test for the presence of viable bacteria in the DNA solution, see step 9 of the protocol.

Things to do before starting

- Prewarm a Thermomixer or heating block to 100°C for use in step 5.

Procedure

1. **Place 25 g of a food sample into a homogenizing bag and add 225 ml enrichment culture medium.**

   Note: If bacterial food samples are being prepared for food control reasons, refer to the local official guidelines for specified enrichment media for different bacteria.

2. **Homogenize the food sample using a lab pedal blender at 230 rpm for 1.5 min. Incubate the homogenate according to the recommended temperature and time recommended for the bacteria.**

   Note: If bacterial food samples are being prepared for food control reasons, refer to the local official guidelines for incubation temperatures and times for different bacteria.

3. **After the recommended incubation, pipet 1 ml enrichment culture into a 2 ml microcentrifuge screw-cap tube (not supplied) and centrifuge at 13,000 x *g* for 5 min.**
4. **Discard the supernatant using a pipet, taking care to not disrupt the pellet.**

5. **Add 200 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.**

   Note: Residues of some enrichment media may strongly color the bacterial pellet. If small amounts of colored media are carried over into the DNA solution, fluorescence detection during PCR reaction will be affected. In this case, wash the bacterial pellet at least twice by repeated centrifugation at 13,000 x *g* for 5 min and resuspension in 500 µl Fast Lysis Buffer. Washing should be repeated until the bacterial suspension is colorless.

6. **Place the microcentrifuge tube into a heating block or thermal shaker (800 rpm) set to 100°C. Heat the sample for 10 min.**
7. **Remove the sample and allow it to cool to room temperature (15–25°C) for 2 min.**
8. **Centrifuge the tube at 13,000 x *g* for 5 min.**
9. **Transfer 100 µl of the supernatant to a fresh 1.5 ml microcentrifuge tube. Use an aliquot of the collected supernatant directly in a PCR reaction. Discard the remaining supernatant, unless testing for the presence of viable bacteria.**

   Note: Supernatants can be stored for 1 week at 2–8°C or for 3 weeks at –20°C.

   Note: To test for the presence of viable bacteria in the DNA solution, plate out 20 µl of the DNA solution from the remaining supernatant onto an agar plate suitable for the respective pathogen (use time and temperature settings as for the enrichment culture). Bacterial growth on the agar will not be inhibited by the 'low' inhibitor concentration that may be present in this solution.

Protocol: Purification of DNA from Food-borne Pathogens Using the *mericon* DNA Bacteria Plus Kit

This protocol is designed for the extraction of total genomic DNA from Gram-positive bacteria from pathogen enrichment cultures, or other pathogens that are difficult to lyse.

Important notes before starting

- If working with infectious pathogens (e.g., salmonella), the protocols and subsequent sample analysis (e.g., PCR) must be carried out in a suitable laboratory area, designated for working with infectious bacteria. Samples should be processed in accordance with official national or local guidelines.
- Samples may still contain infectious material, even after sample lysis. Samples should therefore be treated as infectious throughout the procedure. To test for the presence of viable bacteria in the DNA solution, see step 8 of the protocol.

Procedure

1. **Place 25 g of a food sample into a homogenizing bag and add 225 ml enrichment culture medium.**

   Note: If bacterial food samples are being prepared for food control reasons, refer to the local official guidelines for specified enrichment media for different bacteria.

2. **Homogenize the food sample using a lab pedal blender at 230 rpm for 1.5 min. Incubate the homogenate according to the recommended temperature and time recommended for the bacteria.**

   Note: If bacterial food samples are being prepared for food control reasons, refer to the local official guidelines for incubation temperatures and times for different bacteria.

3. **After the recommended incubation, pipet 1 ml enrichment culture into a 2 ml microcentrifuge tube (not supplied) and centrifuge at 13,000 x *g* for 5 min.**
4. **Discard the supernatant using a pipet, taking care to not disrupt the pellet.**

5. **Add 400 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.**

   Note: Residues of some enrichment media may strongly color the bacterial pellet. If small amounts of colored media are carried over into the DNA solution, fluorescence detection during PCR reaction will be affected. In this case, wash the bacterial pellet at least twice by repeated centrifugation at 13,000 x *g* for 5 min and resuspension in 500 µl Fast Lysis Buffer. Washing should be repeated until the bacterial suspension is colorless.

6. **Transfer the entire mixture to a Pathogen Lysis Tube (supplied). Tightly cap the tube, secure it vertically or horizontally to a vortex adapter, and vortex at maximum speed for 10 min.**
7. **Centrifuge the tube at 13,000 x *g* for 5 min.**
8. **Transfer 100 µl of the supernatant to a fresh 1.5 ml microcentrifuge tube. Use an aliquot of the collected supernatant directly in a PCR reaction. Discard the remaining supernatant, unless testing for the presence of viable bacteria.**

   Note: Supernatants can be stored for 1 week at 2–8°C or for 3 weeks at –20°C.

   Note: To test for the presence of viable bacteria in the DNA solution, plate out 20 µl of the DNA solution from the remaining supernatant onto an agar plate suitable for the respective pathogen (use time and temperature settings as for the enrichment culture). Bacterial growth on the agar will not be inhibited by the 'low' inhibitor concentration that may be present in this solution.

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
Occurrence of a thin top layer on supernatant after pelleting bacteria	
Food components (e.g., fats) have been carried over from enrichment culture	Make sure that the top layer does not settle on the bacteria pellet when removing the supernatant. Remove the top layer before supernatant is discarded.
Occurrence of a colored bacterial suspension after addition of Fast Lysis Buffer in step 3 of the *mericon* DNA Bacteria or *mericon* DNA Bacteria Plus protocol	
a) Inclusion of food debris inside the bacteria pellet	Wash the bacterial pellet at least twice, or until the pellet is colorless, by repeated centrifugation at 13,000 x *g* for 5 min and resuspension of the colored pellet in 500 µl Fast Lysis Buffer. Discard supernatant and apply the washed pellet in step 3 of the *mericon* DNA Bacteria or *mericon* DNA Bacteria Plus protocol.
b) Inclusion of enrichment medium inside the bacteria pellet	Wash the bacterial pellet at least twice, or until the pellet is colorless, by repeated centrifugation at 13,000 x *g* for 5 min and resuspension of the colored pellet in 500 µl Fast Lysis Buffer. Discard supernatant and apply the washed pellet in step 3 of the *mericon* DNA Bacteria or *mericon* DNA Bacteria Plus protocol.
Low DNA yield	
Insufficient lysis or disruption	Carry out a second thermal (*mericon* DNA Bacteria protocol, step 4) or mechanical (*mericon* DNA Bacteria Plus protocol, step 4) lysis cycle for a further 5 min, to ensure complete lysis of bacteria.

	Comments and suggestions
DNA does not perform well in downstream applications	
a) Food-derived inhibitor carryover from sample preparation	Dilute the sample at least 1:10 before PCR analysis.
b) Sample too old or incorrect storage of DNA	We recommend using the DNA in downstream applications directly after preparation. If necessary, store the DNA solution for a maximum of 1 week at 2–8°C or 3 weeks at –20°C. After this time interval, DNA can be degraded.

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.
***mericon* Sample Preparation Kits**		
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
DNeasy *mericon* Food Kit (50)	50 QIAquick Spin Columns, Proteinase K, buffers	69514
***mericon* Assay Kits**		
mericon Salmonella spp Kit (24)*	For 24 reactions: *mericon* Salmonella Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect® Nucleic Acid Dilution Buffer, RNase-free water	290013
mericon L. monocytogenes Kit (24)*	For 24 reactions: *mericon* L. monocytogenes Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290023
mericon Campylobacter spp Kit (24)*	For 24 reactions: *mericon* Campylobacter spp. Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290033
mericon Campylobacter triple Kit (24)*	For 24 reactions: *mericon* Campylobacter triple Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290043
mericon VTEC stx1/2 Kit (24)*	For 24 reactions: *mericon* VTEC stx1/2 Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290053

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon C. sakazakii Kit (24)*	For 24 reactions: *mericon* C. sakazakii Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290063
mericon S. aureus Kit (24)*	For 24 reactions: *mericon* S. aureus Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290073
mericon Legionella spp Kit (24)*	For 24 reactions: *mericon* Legionella spp. Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290083
mericon L. pneumophila Kit (24)*	For 24 reactions: *mericon* L. pneumophila Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290093
mericon Shigella spp Kit (24)*	For 24 reactions: *mericon* Shigella spp Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290103
mericon Y. enterocolitica Kit (24)*	For 24 reactions: *mericon* Y. enterocolitica Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290113

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Related products		
***mericon* GMO Detection Assays**		
mericon Screen 35S Kit (24)*	For 24 reactions: *mericon* Screen 35S Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291013
mericon Screen Nos Kit (24)*	For 24 reactions: *mericon* Screen Nos Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291043
mericon RR Soy (24)*	For 24 reactions: *mericon* RR Soy Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291113
***mericon* Animal and Plant Identification Assays**		
mericon Pig Kit (24)*	For 24 reactions: *mericon* Pig Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	292013
mericon Soy Kit (24)*	For 24 reactions: *mericon* Pig Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	293013

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* Larger kit sizes available; please inquire.

Trademarks: QIAGEN®, *mericon*™, QuantiTect® (QIAGEN Group); BagMixer® (Interscience) Stomacher® (Seward Ltd.).

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Use of this product signifies the agreement of any purchaser or user of the *mericon* DNA Bacteria Kit and the *mericon* DNA Bacteria Plus Kit to the following terms:

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Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

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