June 2017

# QIAamp® BiOstic® Bacteremia DNA Kit Handbook

For the isolation of bacterial DNA from cultured blood, fecal and cervical swabs

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAamp BiOstic Bacteremia DNA Kit** | **(50)** |
|---|---|
| **Catalog no.** | **12240-50** |
| **Number of preps** | **50** |
| PowerBead Tubes, Garnet 100 | 50 |
| MB Spin Columns | 50 |
| Solution MBL | 25 ml |
| Solution IRS | 15 ml |
| Solution BB | 2 x 28 ml |
| Solution CB | 2 x 30 ml |
| Solution EB | 9 ml |
| Collection Tubes (2 ml) | 5 x 50 |
| Quick Start Protocol | 1 |

# Storage

The QIAamp BiOstic Bacteremia DNA Kit reagents and components can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

# Intended Use

All QIAamp products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

# Safety Information

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

**Warning:** Solution CB contains alcohol and is flammable.

| | |
|---|---|
| CAUTION  | **DO NOT add bleach or acidic solutions to directly to the sample preparation waste** |

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

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp BiOstic Bacteremia DNA Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

The QIAamp BiOstic Bacteremia DNA Kit is optimized for the extraction of total DNA (host and microbial) from cultured blood tubes. The resulting DNA can be used for the detection of bacterial infection in blood. The kit includes Inhibitor Removal Technology® (IRT), which removes antibiotic neutralizers, such as charcoal, from blood cultures. This reduces the chances of false negatives caused by PCR inhibitors during the analysis of the DNA by PCR, real-time PCR and Rep PCR.

## Principle and procedure

Blood samples are inoculated into the BioMerieux BacT/Alert® SA Culture Bottle for growth of potential pathogens. These blood culture tubes neutralize antibiotics in the patient sample, allowing bacteria to grow, if present. The culture bottle indicates positive growth when the bacteria are in the range of $10^5$–$10^6$ bacteria/ml of culture. When extracting DNA from these samples with the QIAamp BiOstic Bacteremia DNA Kit, all inhibitors from the medium are removed using our Inhibitor Removal Technology. The purity of the extracted DNA allows for its use in sensitive detection assays, including Agilent BioAnalyzer assays for multiplex PCR products and real-time PCR.

Silica spin-column technology provides a robust and fast way to purify nucleic acids without the use of organic solvents or cesium chloride gradients.

Using the QIAamp BiOstic Bacteremia DNA Kit, cells from a 1.8 ml sample of cultured blood are pelleted for extraction. Lysis conditions combine mechanical lysis using 2 ml PowerBead Tubes and heat to lyse microbes and release DNA. The inhibitor removal solution removes heme as well as components of the culture medium that inhibit PCR. Charcoal is used to neutralize antibiotics in blood culture tubes. The IRT in this kit removes charcoal, and the lysate is purified using a silica spin column using chaotropic buffers and ethanol wash buffers. The final DNA is concentrated and ready to use in genotyping or real-time PCR.

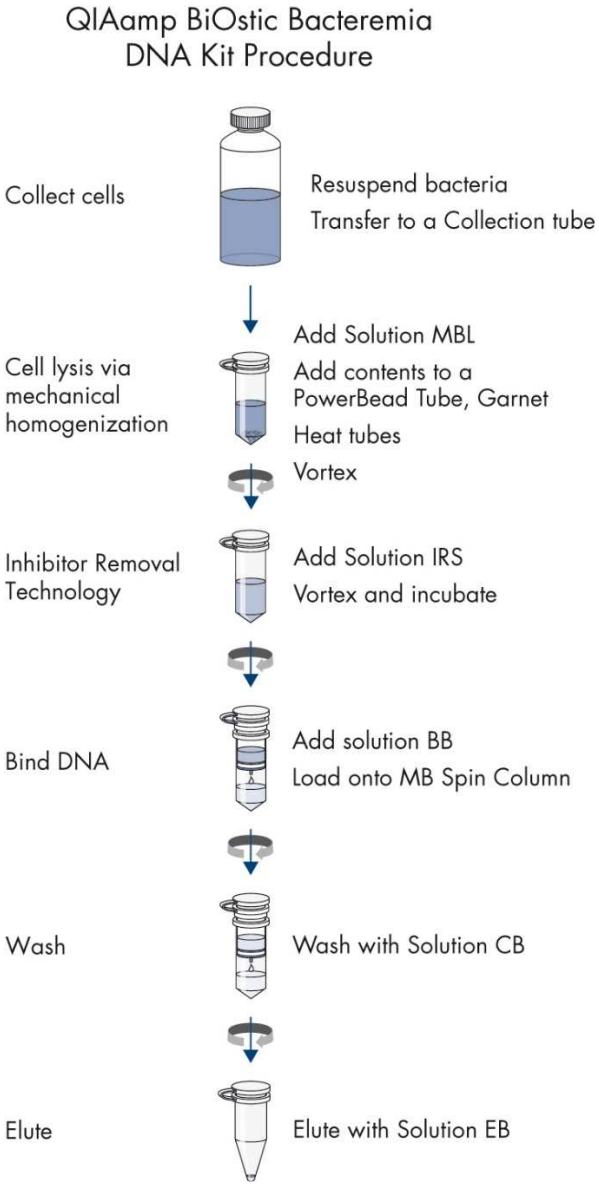


**Figure 1. QIAamp BiOstic Bacteremia DNA Kit procedure.**

# 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 safety data sheets (SDSs) available from the product supplier.

- Microcentrifuge (10,000 x *g*)
- Pipettor (50–200 µl; 100–1000 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)

# Important Notes

- The Centers for Disease Control and Prevention, the Food and Drug Administration and the American Hospital Association recommend **universal precautions** when working with blood and body fluids. To prevent contact with potentially infectious pathogens, we recommend that workers protect themselves from contact with these fluids by using suitable and effective barrier protection, which includes gloves.
- You will need five 2 ml Collection Tubes and one MB Spin Column per sample preparation. To make processing more efficient, prepare 2 ml Collection Tubes and MB Spin Columns in advance by labeling the tops and aligning them in a row in a microcentrifuge tube rack.
- If BacT/Alert SA Culture Bottles have been used to store cultured blood, use a needle and syringe to remove the liquids and aliquot into 2 ml tubes. Make sure to swirl the culture before removing samples to obtain a homogeneous mixture of bacteria. Centrifuge at 13,000 x *g* for 2 minutes to pellet bacteria and cells. Use a pipet to remove the supernatant, and store samples at –20°C until ready to extract DNA. Residual blood or culture medium (up to 100 µl) will not negatively impact the prep.

# Protocol: Experienced User

## Important points before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use.

## Procedure

1. Swirl the cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or pipet and dispense into a 2 ml Collection Tube (provided).
2. Centrifuge at 13,000 x *g* for 2 min to pellet the bacteria and pipet to remove the supernatant and dispose in biohazard waste.
3. Add 450 µl of Solution MBL to the pellet and resuspend by pipetting. Transfer the lysate into a 2 ml PowerBead Tube and close. Vortex for 10 s to mix and place in a 70°C heat block or water bath for 15 min.
4. Secure the PowerBead Tube horizontally using the Vortex Adapter tube holder for the vortex (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
5. Centrifuge the PowerBead Tube to pellet debris at 10,000 x *g* for 1 min. Transfer the supernatant to a new 2 ml Collection Tube (provided).
6. Add 100 µl of Solution IRS and vortex to mix. Incubate for 5 min at room temperature. **Note:** Longer incubation in Solution IRS does not affect DNA yield or purity (sample may be incubated up to 10 min in Solution IRS).
7. Centrifuge at 10,000 x *g* for 1 min to pellet debris. Transfer the supernatant to a new 2 ml Collection Tube (provided).
8. Add 1 ml of Solution BB. Pipette or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.
9. Load 600 µl of lysate onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube. Repeat this step until all the lysate has been loaded onto the MB Spin Column.

10. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided) and wash by adding 500 µl of Solution CB. Centrifuge 10,000 x *g* for 1 min. Discard the flow-through and put the MB Spin Column back into the 2 ml Collection Tube.
11. Wash with another 500 µl of Solution CB and spin at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube.
12. Centrifuge at 13,000 x *g* for 2 min to dry the MB Spin Column membrane.
13. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided).
14. Elute by adding 50 µl of Solution EB directly in the center of the membrane. Allow the MB Spin Column to sit at room temperature for up to 5 min to maximize the elution.
    **Note:** Do not heat the elution buffer.
15. Centrifuge at 10,000 x *g* for 1 min.
16. Discard the MB Spin Column and cap the 2 ml Collection Tube containing the genomic DNA. The DNA is now ready for downstream applications.
    **Note:** We recommend storing DNA frozen (–20° to –80°C) as Solution EB does not contain EDTA.

# Protocol: Detailed

## Important points before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use.

## Procedure

1. Swirl the cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or pipet and dispense into a 2 ml Collection Tube (provided).
2. Centrifuge at 13,000 x *g* for 2 min to pellet the bacteria and pipet to remove the supernatant and dispose in biohazard waste.
   **Note:** Bacteria and red and white blood cells are collected in the pellet.
3. Add 450 µl of Solution MBL to the pellet and resuspend by pipetting. Transfer the lysate into a 2 ml PowerBead and close. Vortex for 10 s to mix and place in a 70°C heat block or water bath for 15 min.
   **Note:** The lysis and inhibitor removal steps are enhanced in the presence of Solution MBL. The heating step enables efficient mechanical lysis.
4. Secure the PowerBead Tube horizontally using the Vortex Adapter tube holder for the vortex (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   **Note:** This mechanical homogenization step completes the lysis of the bacteria in the sample.
5. Centrifuge the PowerBead Tube to pellet debris at 10,000 x *g* for 1 min. Transfer the supernatant to a new 2 ml Collection Tube (provided).
   **Note:** Debris and PowerBeads are removed from the supernatant, which contains the DNA.
6. Add 100 µl of Solution IRS and vortex to mix. Incubate for 5 min at room temperature.
   **Note:** Longer incubation in Solution IRS does not affect DNA yield or purity (sample may be incubated up to 10 min in Solution IRS).
7. Centrifuge at 10,000 x *g* for 1 min to pellet debris. Transfer the supernatant to a new 2 ml Collection Tube (provided).

**Note:** Solution IRS removes all inhibitors from the sample.

8. Add 1 ml of Solution BB. Pipet or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.
   **Note:** The addition of Solution BB allows genomic DNA to bind to the MB Spin Column filter membrane.
9. Load 600 µl of lysate onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube. Repeat this step until all the lysate has been loaded onto the MB Spin Column.
   **Note:** The DNA is bound to the MB Spin Column silica filter membrane. Due to the large volume of lysate, it takes multiple loads to bind all the DNA.
10. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided) and wash by adding 500 µl of Solution CB. Centrifuge 10,000 x *g* for 1 min. Discard the flow-through and put the MB Spin Column back into the 2 ml Collection Tube.
11. Wash with another 500 µl of Solution CB and spin at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube.
12. Centrifuge at 13,000 x *g* for 2 min to dry the MB Spin Column membrane.
    **Note:** Solution CB washes impurities and salt off the MB Spin Column filter membrane. The dry spin ensures that all of the ethanol is removed, so that DNA can be released from the membrane in subsequent steps.
13. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided).
14. Elute by adding 50 µl of Solution EB directly in the center of the membrane. Allow the MB Spin Column to sit at room temperature for up to 5 min to maximize the elution.
    **Note:** Do not heat the elution buffer.
15. Centrifuge at 10,000 x *g* for 1 min.
    **Note:** The DNA is eluted in 10 mM Tris (pH 8.0) buffer.
16. Discard the MB Spin Column and cap the 2 ml Collection Tube containing the genomic DNA. The DNA is now ready for downstream applications.
    **Note:** We recommend storing DNA frozen (–20° to –80°C) as Solution EB does not contain EDTA.

# Protocol: Fast

## Important points before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use.
- You will need five 2 ml Collection Tubes and one MB Spin Column per sample for this procedure. To make processing more efficient, prepare the 2 ml Collection Tubes and the MB Spin Column in advance by labeling the tops and aligning them in a row in a microcentrifuge tube rack.
- Dispense 100 µl of Solution IRS in the second of the pre-labeled 2 ml Collection Tubes in a rack and 1 ml of Solution BB in the third labeled 2 ml Collection Tube in the rack.

## Procedure

1. Swirl the cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or pipet and dispense into a 2 ml Collection Tube (provided).
2. Centrifuge at 13,000 x *g* for 2 min to pellet the bacteria and pipet to remove the supernatant and dispose in biohazard waste.
3. Add 450 µl of Solution MBL to the pellet and resuspend by pipetting. Transfer the lysate into a 2 ml PowerBead Tube and close. Vortex for 10 s to mix and place in a 70°C heat block or water bath for 15 min.
4. Secure the PowerBead Tube horizontally using the Vortex Adapter tube holder for the vortex (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
5. Centrifuge the PowerBead Tube to pellet debris at 10,000 x *g* for 1 min. Transfer the supernatant to the 2 ml Collection Tube containing 100 µl of Solution IRS.
6. Vortex to mix. Incubate for 5 min at room temperature.
   **Note:** Longer incubation in Solution IRS does not affect DNA yield or purity (sample may be incubated up to 10 min in Solution IRS).
7. Centrifuge at 10,000 x *g* for 1 min to pellet debris. Transfer the supernatant to the 2 ml Collection Tube containing 1 ml of Solution BB.

8. Pipet or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.
9. Load 600 µl of lysate onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube. Repeat this step until all the lysate has been loaded onto the MB Spin Column.
10. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided) and wash by adding 500 µl of Solution CB. Centrifuge 10,000 x *g* for 1 min. Discard the flow-through and put the MB Spin Column back into the 2 ml Collection Tube.
11. Wash with another 500 µl of Solution CB and spin at 10,000 x *g* for 1 min. Discard the flow-through and place the MB Spin Column back into the 2 ml Collection Tube.
12. Centrifuge at 13,000 x *g* for 2 min to dry the MB Spin Column membrane.
13. Transfer the MB Spin Column to a new 2 ml Collection Tube (provided).
14. Elute by adding 50 µl of Solution EB directly in the center of the membrane. Allow the MB Spin Column to sit at room temperature for up to 5 min to maximize the elution.
    **Note:** Do not heat the elution buffer.
15. Centrifuge at 10,000 x *g* for 1 min.
16. Discard the MB Spin Column and cap the 2 ml Collection Tube containing the genomic DNA. The DNA is now ready for downstream applications.
    **Note:** We recommend storing DNA frozen (–20° to –80°C) as Solution EB does not contain EDTA.

# 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/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

| | | Comments and suggestions |
|---|---|---|
| **DNA** | | |
| a) | DNA does not amplify: PCR for the organism of interest fails | The sample will contain human genomic DNA from white blood cells. To check for PCR inhibition, test the sample with primers for a human gene.<br>To ensure that your primers for the organism of interest are correct, include a PCR reaction that is expected to work. You can use the QIAamp BiOstic Bacteremia DNA Kit to prepare a batch of control DNA from a pellet of bacterial cells from an overnight culture. |
| a) | DNA does not amplify: Positive-control samples are amplified but PCR for the organism of interest fails | Make sure the culture tube was incubated long enough to indicate positive growth of the bacteria. Culture time may vary based on the type of organism, the level of infection and the amount of antibiotics in the sample.<br>Use primers to amplify across a wide range of bacterial species (for example, 16s rRNA primers) to ensure detection of microbial DNA before performing a species-specific assay. It is possible for more than one microorganism to be present in the sample. |
| b) | DNA floats out of a well when loading a gel | This usually occurs because residual ethanol remains in the final sample. Avoid transferring any Solution CB to the elution step. To ensure complete drying of the membrane after addition of Solution CB, centrifuge the MB Spin Column (in a 2 ml Collection Tube) for an additional minute.<br>Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual ethanol. |
| c) | Concentrating eluted DNA | The final volume of eluted DNA will be 50 µl. The DNA may be concentrated by adding 5 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution EB). |

| | | Comments and suggestions |
|---|---|---|
| d) | Storing DNA | DNA is eluted in Solution EB (10 mM Tris) and must be stored at –20°C to –80°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. |
| e) | DNA has low $A_{260/280}$ ratio | The ratio for pure DNA should be 1.7–1.9. Any $A_{260/280}$ reading below 1.6 may indicate significant protein contamination.<br>A low $A_{260/280}$ ratio may also occur when the sample is measured by UV spectrophotometry in water. The low pH of water can influence the 280 reading and cause reduced sensitivity to protein contamination. Remeasure the 260/280 by diluting the DNA in 10 mM Tris pH 7.5.<br>If using a Nanodrop, blank the instrument with Solution EB. |

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAamp BiOstic Bacteremia DNA Kit (50) | For 50 preps: Isolation of bacterial DNA from cultured blood, fecal and cervical swabs | 12240-50 |
| **Related Products** | | |
| DNeasy® UltraClean® Microbial Kit (50) | For 50 preps: Isolation of high-quality DNA from microbial cultures | 12224-50 |
| DNeasy UltraClean Microbial Kit (250) | For 250 preps: Isolation of high-quality DNA from microbial cultures | 12224-250 |
| DNeasy UltraClean 96 Microbial Kit (384) | For 384 preps: High-throughput isolation of DNA from microbial cultures | 10196-4 |
| MagAttract® Microbial DNA Kit (384) | For 384 preps: Automated isolation of DNA from microbial and food cultures using automated processing and liquid handling systems | 27200-4 |
| DNeasy PowerFood® Microbial Kit (100) | For 100 preps: Isolation of inhibitor-free DNA from a variety of cultured foods | 21000-100 |
| DNeasy PowerLyzer® Microbial Kit (50) | For 50 preps: Isolation of high-quality DNA from microbial cultures using a bead-based homogenizer | 12255-50 |
| Vortex Adapter | For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex | 13000-V1-24 |

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.

**Limited License Agreement for QIAamp BiOstic Bacteremia DNA Kit**

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

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