BiOstic® Bacteremia DNA Isolation Kit

(For use with cultured blood for the enrichment of bacteria)

Catalog No.	Quantity
12240-50	50 preps

Instruction Manual

Please recycle

Version: 01152013

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Introduction

The Bi*O*stic® Bacteremia DNA Isolation 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 the blood culture. 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.

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 Bi*O*stic® Bacteremia DNA Isolation Kit, all inhibitors from the medium are removed using MO BIO's patented Inhibitor Removal Technology®. The purity of DNA allows for use in sensitive detection assays including Agilent BioAnalyzer assays for multiplex PCR products and real-time PCR.

Protocol Overview

Bi*O*stic® silica spin column products utilize the novel MO BIO Laboratories flat bottom spin column design, which provides improved sample processing and yields. The bucket configuration allows for enhanced sample flow and membrane drying after wash steps since the entire membrane is accessible to air flow. Silica technology provides a robust and fast way to purify nucleic acids without the use of organic solvents or cesium chloride gradients.

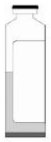
Using the Bi*O*stic® Bacteremia DNA Isolation Kit, cells from a 1.8 ml sample of cultured blood are pelleted for extraction. Lysis conditions combine mechanical lysis using 2 ml MicroBead Tubes and heat to break microbes and release DNA. MO BIO's inhibitor removal solution removes heme as well as components of the culture medium that inhibit PCR. Charcoal is used to neutralize antibiotics in blood culture tubes. The Inhibitor Removal Technology® in this kit removes this charcoal, and the lysate is purified in 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 detection assays.

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
Bi*O*stic® Blood Total RNA Isolation Kit	12230-50	50 preps
Vortex-Genie® 2 Vortex	13111-V	1 unit
Vortex Adapter for 1.5 – 2.0 ml tubes	13000-V1 13000-V1-24	holds 12 tubes holds 24 tubes

BiOstic® Bacteremia DNA Isolation Kit

Collect Cells



- Resuspend bacteria
- Transfer to Microcentrifuge Tube

Centrifuge

Cell Lysis via Mechanical Homogenization

- Add Solution CB1
- Add contents of a MicroBead Tube
- Heat tubes
- Vortex

Centrifuge

Inhibitor Removal Technology®

- Add Solution CB2
- Vortex and Incubate

Centrifuge

Bind DNA

- Add Solution CB3
- Load into Spin Filter

Centrifuge

Wash

- Wash with Solution CB4

Elute

- Elute with Solution CB5

Equipment Required

Microcentrifuge (≥13,000 x *g*)
Pipettors
Waterbath or heat block set at 70° C
Vortex
Vortex adapter for 2.0 ml tubes (MO BIO Catalog# 13000-V1 or 13000-V1-24):
This is not required but will allow for thorough and reproducible lysis of samples. If you do not have a vortex adapter, secure your bead tubes to your vortex horizontally with adhesive tape.
2.0 ml Collection Tubes (cat#1200-250-T):
2.0 ml Collection Tubes are provided in this kit for your prep but for storing additional samples for later extraction, you will need a microcentrifuge tube capable of holding 2.0 ml of liquid.

Reagents Required but not Included:

BacT/Alert® SA Culture Bottles

Kit Contents

	Kit Catalog# 12240-50	
Component	**Catalog#**	**Amount**
Solution CB1	12240-50-1	25 ml
Solution CB2	12240-50-2	6 ml
Solution CB3	12240-50-3	2 x 28 ml
Solution CB4	12240-50-4	2 x 30 ml
Solution CB5	12240-50-5	6 ml
2 ml MicroBead Tubes	12240-50-BT	50
Spin Filters	12240-50-SF	50
2 ml Collection Tubes	12240-50-T	250

Kit Storage

Store all reagents and kit components at room temperature (15-30°C).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solutions CB3 and CB4 contain ethanol. They are flammable.

Important Notes Before Starting

This protocol describes how to extract genomic DNA (host and microbial) from cultured blood for the enrichment of bacterial DNA.

- The Center for Disease Control, 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, it is recommended that workers protect themselves from contact with these fluids by using suitable barrier protection which includes gloves. Effective barrier protection should be used during this procedure.

- A precipitate will form in Solution CB1. Warm the reagent at 55^{o}C for 5-10 minutes and swirl to dissolve all the components back into solution. Solution CB1 can be used warm.

- This protocol uses five 2 ml Collection Tubes and one Spin Filter per sample preparation. To make processing more efficient prepare 2 ml Collection Tubes and Spin Filters in advance by labeling the tops and align them in a row in a microcentrifuge tube rack. This will make sample transfer faster and easier.

- For storage of entire volume of cultured blood (40-50 ml), if using BacT/Alert® SA Culture Bottles, use a needle and syringe to remove the liquids and aliquot into 2 ml tubes. Make sure to swirl the culture before removing sample to obtain a homogeneous mixture of bacteria. Centrifuge at 13,000 x *g* for 2 minutes to pellet bacteria and cells. Use pipetting to remove the supernatant and store samples at -20°C until ready to extract DNA. Residual blood or culture medium on the pellet (up to 100 µl) does not negatively impact the prep.

Experienced User Protocol – Extraction of bacterial DNA from cultured blood

Please wear gloves at all times

Note: A precipitate will form in Solution CB1. Pre-warm the Solution at 55°C for 5-10 minutes.

1. Swirl the cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or pipette and dispense into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 2 minutes to pellet the bacteria and pipette to remove the supernatant and dispose in biohazard waste.
2. Add **450 µl of Solution CB1** to the pellet and resuspend with pipetting. Transfer the lysate into the 2 ml MicroBead Tube and close. Vortex for 10 seconds to mix and place in a 70° C heat block or water bath for 15 minutes.
3. Secure the 2 ml MicroBead Tube horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tube horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
4. Centrifuge the 2 ml MicroBead Tube to pellet debris at 10,000 *x g* for 1 minute. Transfer the supernatant to a new 2 ml Collection Tube (provided).
5. Add **100 µl of Solution CB2** and vortex to mix. Incubate 5 minutes at room temperature and centrifuge at 10,000 *x g* for 1 minute to pellet debris. Transfer the supernatant to a new 2 ml Collection Tube (provided).
   Note: Longer incubation in Solution CB2 does not affect DNA yield or purity. (Sample may be incubated up to 10 minutes with Solution CB2.)
6. Add **1 ml of Solution CB3.** Pipette or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.
7. Load 600 µl of lysate onto the Spin Filter and centrifuge at 10,000 *x g* for 1 minute to bind. Discard the flow-through liquid and place the **Spin Filter** back into the 2 ml Collection Tube. Repeat this step 2 more times until all the lysate has been loaded onto the Spin Filter.
8. Transfer the Spin Filter to a new 2 ml Collection Tube (provided) and wash by adding **500 µl of Solution CB4** to the Spin Filter Column. Centrifuge 10,000 *x g* for 1 minute. Discard the flow-through and put the Spin Filter back into the 2 ml Collection Tube.
9. Wash with another **500 µl of Solution CB4** and spin at 10,000 *x g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.
10. Centrifuge at 13,000 *x g* for 2 minutes to dry the Spin Filter membrane.
11. Transfer the Spin Filter to a new 2 ml Collection Tube (provided). Elute by adding **50 µl of Solution CB5** directly in the center of the membrane. Allow the Spin Filter Column to sit at room temperature for up to 5 minutes to maximize the elution. Centrifuge 10,000 *x g* for 1 minute.
    Note: **Do not heat the elution buffer.**
12. Discard the Spin Filter and cap the 2 ml Collection Tube containing the genomic DNA. Store at -20°C or -80°C.

Thank you for choosing the Bi*O*stic® Bacteremia DNA Isolation Kit!

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

Note: Pre-warm the Solution CB1 at 55°C for 5-10 minutes.

1. Swirl the cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or pipette and dispense into a 2 ml Collection Tube (provided). Centrifuge at 13,000 *x g* for 2 minutes to pellet the bacteria and pipette to remove the supernatant and dispose in biohazard waste.

   What's happening: The bacteria will be collected in the pellet along with the white and red blood cells.

2. Add **450 µl of Solution CB1** to the pellet and resuspend with pipetting. Transfer the lysate into the 2 ml MicroBead Tube and close. Vortex for 10 seconds to mix and place in a 70° C heat block or water bath for 15 minutes.

   What's happening: The lysis steps and inhibitor removal steps are enhanced in the presence of Solution CB1. The heating step enables an efficient mechanical lysis with the MicroBeads.

3. Secure the 2 ml MicroBead Tube horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tube horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   What's happening: The mechanical homogenization step completes the lysis of the bacteria in the sample.

4. Centrifuge the 2 ml MicroBead Tube to pellet the debris at 10,000 *x g* for 1 minute. Transfer the supernatant to a new 2 ml Collection Tube (provided).

   What's happening: Debris and the MicroBeads are removed from the sample containing DNA.

5. Add **100 µl of Solution CB2** and vortex to mix. Incubate 5 minutes at room temperature and centrifuge at 10,000 *x g* for 1 minute to pellet the debris. Transfer the supernatant to a new 2 ml Collection Tube (provided).
   Note: Longer incubation in Solution CB2 does not affect DNA yield or purity. (Sample may be incubated up to 10 minutes with Solution CB2.)

   What's happening: The Solution CB2 removes all of the inhibitors from the sample.

6. Add **1 ml of Solution CB3.** Pipette or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.

   What's happening: The addition of Solution CB3 allows for binding of genomic DNA to the Spin Filter membrane.

7. Load 600 µl of lysate onto the **Spin Filter** and centrifuge at 10,000 *x g* for 1 minute to bind. Discard the flow-through liquid and place the Spin Filter back into the 2 ml Collection Tube. Repeat this step 2 more times until all the lysate has been loaded onto the Spin Filter.

   What's happening: The DNA is bound to the silica Spin Filter. Due to the large volume of lysate, it takes multiple loads to bind all the DNA present.

8. Transfer the Spin Filter to a new 2 ml Collection Tube (provided) and wash by adding **500 µl of Solution CB4** to the Spin Filter Column. Centrifuge 10,000 *x g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.

9. Wash with another **500 µl of Solution CB4** and spin at 10,000 *x g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.

10. Centrifuge at 13,000 *x g* for 2 minutes to dry the Spin Filter membrane.

    What's happening: The Solution CB4 washes the impurities and salt off the Silica Filter membrane. The dry spin ensures all the ethanol is removed so the DNA can be released.

11. Transfer the Spin Filter to a new 2 ml Collection Tube (provided). Elute by adding **50 µl of Solution CB5** directly in the center of the membrane. Allow the Spin Filter Column to sit at room temperature for up to 5 minutes to maximize the elution. Centrifuge 10,000 *x g* for 1 minute.
    Note: **Do not heat the elution buffer.**

    What's happening: The DNA is eluted in a 10 mM Tris pH 8.0 buffer and is ready to use or can be stored.

12. Discard the Spin Filter and cap the 2 ml Collection Tube containing the genomic DNA. Store at -20°C or -80°C.

Thank you for choosing the Bi*O*stic® Bacteremia DNA Isolation Kit!

Fast Protocol – Extraction of bacterial DNA from cultured blood

Important Notes Before Starting

- You will use five 2 ml Collection Tubes per sample in this procedure. To make processing more efficient, for the number of preps you will process, prepare the 2 ml Collection Tubes in advance by labeling the tops and align them in a row in a microcentrifuge tube rack. This will make sample transfers faster and easier.

- Dispense **100 µl of Solution CB2** in the second of the pre-labeled 2 ml Collection Tubes in your rack and **1 ml of Solution CB3** in the third labeled 2 ml Collection Tube in your rack.

- Take out and label the tops of the Spin Filter Columns for the number of preps required and place in a microcentrifuge tube rack.

Protocol:

Note: Pre-warm the Solution CB1 at 55°C for 5-10 minutes.

1. Swirl your cultured blood to resuspend the bacteria and remove 1.8 ml with a syringe and needle or by pipetting and dispense into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 2 minutes to pellet the bacteria. Use pipetting to remove the supernatant and dispose in biohazard waste.
2. Add **450 µl of Solution CB1** to the pellet and resuspend with pipetting. Transfer the lysate into the 2 ml MicroBead Tube and close. Vortex for 10 seconds to mix and place in a 70° C heat block or water bath for 15 minutes.
3. Secure the 2 ml MicroBead Tube horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tube horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
4. Centrifuge the homogenized sample to pellet the debris at 10,000 *x g* for 1 minute.
5. Transfer the supernatant to the 2 ml Collection Tube containing **100 µl of Solution CB2**. Vortex to mix. Incubate 5 minutes at room temperature.
   Note: Longer incubation in Solution CB2 does not affect DNA yield or purity. (Sample may incubated up to 10 minutes with Solution CB2.
6. Centrifuge at 10,000 *x g* for 1 minute to pellet the debris. Transfer the supernatant to the 2 ml Collection Tube containing **1 ml of Solution CB3** and mix by pipetting or pulse vortexing. If necessary, centrifuge briefly to collect all the liquids.
7. Load 600 µl of lysate onto the **Spin Filter** and centrifuge at 10,000 *x g* for 1 minute to bind. Discard the flow-through liquid and place the Spin Filter back into the 2 ml Collection Tube. Repeat this step 2 more times until all the lysate has been loaded onto the Spin Filter.
8. Transfer the Spin Filter to a new 2 ml Collection Tube (provided) and wash by adding **500 µl of Solution CB4** to the Spin Filter Column. Centrifuge 10,000 *x g* for 1 minute. Discard the flow-through and put the Spin Filter back into the 2 ml Collection Tube.
9. Wash with another **500 µl of Solution CB4** and spin at 10,000 *x g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.
10. Centrifuge at 13,000 *x g* for 2 minutes to dry the membrane.

11. Transfer the Spin Filter to a new labeled 2 ml Collection Tube (provided). Elute by adding **50 µl of Solution CB5** directly in the center of the membrane. Allow the Spin Filter Column to sit at room temperature for up to 5 minutes to maximize the elution. Centrifuge 10,000 *x g* for 1 minute.
    Note: **Do not heat the elution buffer.**
12. Discard the Spin Filter and cap the 2 ml Collection Tube containing the genomic DNA. Store at -20°C or -80°C.

Thank you for choosing the Bi*O*stic® Bacteremia DNA Isolation Kit!

Hints and Troubleshooting Guide

DNA Does Not Amplify

The DNA purity and yields are typically in a range that allows for successful PCR without dilution ($A_{260/280}$ 1.7-1.9 and $A_{260/230}$ >1.5).

If PCR for the organism of interest fails, test the following controls:

- Human DNA PCR- your sample will contain human genomic DNA from the white blood cells. To check for PCR inhibition, test the sample with primers for a human gene.
- Positive control DNA- to make sure your primers for the organism of interest are correct, include a reaction that is expected to work. You can prepare a batch of control DNA by using the Bi*O*stic® Bacteremia DNA Isolation Kit to extract DNA from a pellet of bacterial cells from an overnight culture.

If the positive control samples amplify and your sample prepared using the Bi*O*stic® Bacteremia DNA Isolation Kit is not detected using real-time PCR for a specific organism:

- 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.
- Use primers for amplification 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.

Concentrating the DNA

The DNA concentration will range between 20 ng/µl and 200 ng/µl depending on the density of the bacteria in the sample and how long it was cultured. If you desire more concentrated DNA, use the following protocol:

Per 50 µl of eluted DNA, add 5 µl of 3M Sodium Acetate (1/10th volume) and mix. Then add 2 volumes of 100% cold ethanol. Mix, and incubate at -70°C for 15 minutes or -20^{o}C for 2 hours to overnight. Centrifuge at 13,000 x *g* for 10-15 minutes at 4°C. Decant all liquid. Wash the DNA pellet with 100-200 µl of 70% ethanol and centrifuge for another 5 minutes at 13,000 x *g*. Decant the wash and allow to drain on a kemwipe or towel for several minutes. Dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in the desired volume of buffer (Solution CB5).

DNA Floats Out of Well When Loaded on a Gel

Residual Solution CB4 Wash Buffer may be in the final sample. To ensure complete drying of the membrane after Solution CB4, centrifuge the Spin Filter in a clean 2 ml Collection Tube for an additional minute.

- Ethanol precipitation is the best way to remove residual Solution CB4. (See “Concentrating the DNA” above.)
- If you live in a humid climate, you may experience increased difficulty with drying of the membrane in the centrifuge. Increase the centrifugation times at step 10 by another minute.

Hints and Troubleshooting Guide cont.

DNA has Low $A_{260/230}$ Ratio

The ratio for pure DNA should be 1.7-1.9. A 260/280 reading below 1.6 may have significant protein contamination.

- A low 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(1) . Re-measure the 260/280 diluting the RNA for measurement in 10 mM Tris pH 7.5.
- If using a Nanodrop, blank the instrument with Solution CB5.

Storing DNA

DNA is eluted in sterile 10 mM Tris Buffer pH 8.0 to protect the DNA from the acidic conditions of water. The DNA can be used immediately or stored at -20°C or -80°C. For long-term storage, a buffer containing 1 mM EDTA may be used to inhibit potential DNase activity.

Technical Guide

References

1. **Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.*

Contact Information

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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
BiOstic® Stabilized Blood RNA Isolation Kit	12231-50	50 preps
BiOstic® Blood Total RNA Isolation Kit	12230-50	50 preps
UltraClean® Tissue & Cells RNA Isolation Kit	15000-50 15000-250	50 preps 250 preps
UltraClean® BloodSpin® DNA Isolation Kit	12200-50 12200-250	50 preps 250 preps
UltraClean® Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps
Vortex-Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for Vortex Genie® 2	13000-V1 13000-V1-24	Holds 12 (2 ml) Tubes Holds 24 (2 ml) Tubes
Dye Dots®	15020-10 15020-20	10 plates 20 plates