



## QIAGEN Supplementary Protocol:

### Purification of bacterial DNA from primary samples using the MagAttract® DNA Mini M48 Kit

This protocol is designed for the purification of bacterial DNA from primary sample material (e.g., nasopharyngeal secretions, tracheal secretions, tissue, blood, CSF, urine, and sputum samples) using the MagAttract DNA Mini M48 Kit in combination with the BioRobot® M48 workstation.

#### Introduction

The BioRobot M48 system allows automated purification of bacterial DNA from primary samples. The magnetic-particle technology used by the BioRobot M48 system provides high-quality DNA, which is well suited for direct use in downstream applications, such as PCR amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the DNA purification procedure. The eluted bacterial DNA is ready for use in amplification reactions or storage at  $-20^{\circ}\text{C}$ .

This protocol describes first how to prepare the sample material and then follows with the procedure for setting up the BioRobot M48 and starting a run.

**IMPORTANT:** This protocol can only be used on BioRobot M48 workstations, which have been updated using the M48 Software Upgrade Tool version 2.0 (cat. no. 9016241) or received after 1 January 2004, in combination with App. Package, M48, Infectious Disease version 2.0 (cat. no. 9016145).

**IMPORTANT:** Please consult the "Safety Information" and "Important Notes" sections in the *MagAttract DNA M48 Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

#### Determining pathogen DNA yield

The yield of DNA depends on the sample type and the number of DNA-containing cells in the sample. Genomic DNA from contaminating host cells will co-purify with pathogen DNA. Presence and yield of pathogens from primary samples are typically quantified by downstream analysis such as PCR or real-time PCR.

#### Addition of internal control

Using the MagAttract M48 procedure in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal-control DNA should be added to the lysis buffer (Buffer MTL). If the internal-control DNA is purified in the absence of primary sample material, additional carrier RNA should be added. Dilute the carrier RNA to a final concentration of  $10\ \mu\text{g}/\text{ml}$  in Buffer AVL (cat. no. 19073; supplied together with carrier RNA). For optimal purification, internal-control DNA molecules should be at least 200 nucleotides long, as smaller molecules are not recovered efficiently. Refer to the manufacturer's instructions in order to

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determine the optimal concentration. Using a concentration other than that recommended may reduce amplification efficiency.

### Reagents and equipment to be supplied by the user

- MagAttract DNA Mini M48 Kit (cat. no. 953336)
- BioRobot M48 workstation and disposables (see *MagAttract DNA M48 Handbook*)
- Sample tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692).
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692) or 2 ml (Sarstedt, cat. no. 72.693)
- Optional: Phosphate-buffered saline (PBS)
- Optional: Dithiothreitol (DTT); see “Viscous or mucous samples” below
- Optional: Carrier RNA (cat. no. 19073); see “Addition of internal control” above
- Optional: Lysozyme (50 mg/ml in water) and/or lysostaphin (5 mg/ml in water); see “Blood and blood culture”, page 3

### Important notes before starting

- This protocol can only be used on BioRobot M48 workstations, which have been updated using the M48 Software Upgrade Tool version 2.0 (cat. no. 9016241) or received after 1 January 2004, in combination with App. Package, M48, Infectious Disease version 2.0 (cat. no. 9016145).
- Check that Buffer MW1 has been prepared according to the instructions given in the “Important Notes” section of the kit handbook.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.

### Starting material

This procedure is suitable for use with a wide range of biological primary material, including body fluids, secretions, tissues, and stool specimens. Depending on the starting material, preparation of the sample may be required. Please read the next section concerning preparation of sample material. The MagAttract DNA Mini M48 Kit may be used for purification of DNA from a broad range of bacterial species, including both Gram-negative and Gram-positive strains.

**Table 1. Starting and Elution Volumes for the M48 Primary Sample Procedure**

| Sample type     | Protocol   | Starting volume | Elution volume                            |
|-----------------|------------|-----------------|---|
| Primary samples | Bact_200ul | 200 $\mu$ l     | 50–200 $\mu$ l<br>100 $\mu$ l recommended |

## Preparation of sample material

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling. This section describes some recommendations for processing primary samples.

### Body fluid and secretion swabs

Submerge the swab tip in 290  $\mu$ l 1x PBS. Add 10  $\mu$ l Proteinase K. Incubate for 15 min at 56°C with continuous mixing. If mixing is not possible, vortex before and after incubation. Remove the swab and squeeze out all liquid by pressing the swab against the inside of the tube. Transfer 200  $\mu$ l of the sample to a 1.5 ml sample tube.

### Viscous or mucous samples

Low viscosity: No preparation needed.

Medium to high viscosity: Dilute the sample 1:1 with 1x PBS and add freshly prepared DTT to a final concentration of 0.15% (w/v) (optional). Incubate at 37°C until the sample can be pipetted.

### Urine

It is possible to use 200  $\mu$ l of urine directly, without any preparation. However, if a more concentrated DNA sample is required, centrifuge 1 ml urine for 5 min at 3000 rpm. Resuspend the cell pellet in 200  $\mu$ l Buffer G2 (supplied with the kit).

### Stool

Add 500  $\mu$ l 1x PBS to 50 mg of stool sample and vortex for 1 min. Incubate at room temperature (15–25°C) for 10 min. Vortex for 1 min. Centrifuge for 30 s at 3000 rpm. Transfer 200  $\mu$ l of the liquid phase to a 1.5 ml sample tube.

### Blood and blood culture

For Gram-negative bacteria, pretreatment of blood samples is not required, use 200  $\mu$ l of blood or blood culture medium directly.

For Gram-positive bacteria, add 20  $\mu$ l lysozyme (50 mg/ml) and/or 10  $\mu$ l lysostaphin (5 mg/ml), and incubate the sample at 37°C for 10 minutes.

### Soft tissue biopsies

Please follow "Protocol: Isolation of Total DNA from Soft Tissue Using the MagAttract DNA M48 Kit" in the *MagAttract DNA M48 Handbook*.

### Bacterial suspension culture or plate-grown bacterial colonies

Please read QIAGEN Supplementary Protocol "Purification of DNA from bacterial culture samples using the MagAttract DNA Mini M48 Kit", available at [www.qiagen.com/literature/protocols/MagAttractDNAM48.aspx](http://www.qiagen.com/literature/protocols/MagAttractDNAM48.aspx).

## Procedure

1. **Ensure that the 1.5 ml sample tube containing the bacterial cells is prepared as described above.**
2. **Ensure that the BioRobot M48 is switched on.**  
The power switch is on the left side of the instrument.
3. **Switch on the computer and monitor.**
4. **Launch the QIAsoft M Operating System.**
5. **Select the protocol group "Infectious Disease" from the drop-down menu.**
6. **Select the protocol subgroup "Bacteria DNA"**
7. **Select the protocol "Bact\_200µl", and click the "Select" button to choose the elution tube type. Enter the number of samples and the elution volume into the software.**

The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48. Follow the steps detailed in the protocol messages before continuing. Wear gloves when loading the required items on the worktable.

8. **Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.**
9. **Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.**

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see the appendix of the kit handbook) in order to minimize the risk of magnetic-particle carryover.

## Troubleshooting

For general troubleshooting, please consult the “Troubleshooting Guide” in the kit handbook. The troubleshooting described here is specific for the bacterial cells application.

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### Comments and suggestions

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#### Low DNA purity

Sample overload

Follow the recommendations in the “Preparation of sample material” section.

#### Clumping of beads

High sample viscosity or sample overload

Follow the recommendations in the “Preparation of sample material” section.

#### Poor amplification sensitivity

Inhibition of amplification

Add an internal control to the sample.  
Reduce the amount of sample material.  
Increase the elution volume.

Inefficient lysis

For Gram-positive bacteria, pretreat the sample with lysozyme and/or lysostaphin as described in “Blood and blood culture”, page 3. If necessary, extend the incubation time up to 2 hours.

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

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