

## QIAGEN Supplementary Protocol:

### Isolation of DNA from large volume human whole blood using the MagAttract® DNA Blood Midi M48 Kit

This protocol is designed for the isolation of total (genomic and mitochondrial) DNA from human whole blood using the MagAttract DNA Blood Midi M48 Kit in combination with the BioRobot® M48 workstation.

#### Introduction

The MagAttract DNA Blood M48 system allows fully automated purification of DNA from human whole blood and blood products. MagAttract technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material.

**IMPORTANT:** Please read the *MagAttract DNA Blood M48 Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

#### Starting material

Fresh or frozen whole blood samples treated with EDTA, ACD, or heparin can be used. Frozen samples should be thawed and equilibrated at room temperature (15–25°C), then mixed thoroughly before beginning the procedure. Yield and quality of the purified DNA depends on storage conditions of the blood. Fresh blood samples may yield better results.

The starting and elution volumes to use in this procedure are shown in Table 1, below. The sample and elution volume for the protocol can be scaled within the ranges shown to give a yield and concentration of high-quality DNA appropriate for the intended downstream application.

**Table 1. Amounts of starting material and elution volumes for the MagAttract DNA M48 large volume whole blood procedure**

Sample	Kit	Protocol	Amount of starting material	Elution volume
Whole blood	MagAttract DNA Blood Midi M48 Kit	700 µl Blood	500–700 µl	200–400 µl

## Yield of purified DNA

DNA yields depend on the number of nucleated cells in the sample, and the storage conditions of the blood. Fresher blood samples may yield better results. Table 2 shows typical yields obtained from different sample volumes and sample types. Elution in smaller volumes increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

**Table 2. DNA yields obtained from whole blood samples using the MagAttract DNA M48 system**

Sample type	Sample volume	DNA yield ( $\mu\text{g}$ )
Whole blood*	700 $\mu\text{l}$	9–20

\* Whole blood with  $3\text{--}8 \times 10^6$  white blood cells/ml; elution volume 200  $\mu\text{l}$

## Reagents and equipment to be supplied by the user

- MagAttract DNA Blood Midi M48 Kit, cat. no. 951356
- BioRobot M48 workstation and disposables (see the *MagAttract DNA Blood M48 Handbook*)
- Sample tubes, 2 ml, without lids (Sarstedt, cat. no. 72.608) or with screw caps (Sarstedt, cat. no. 72.693)
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692) or 2 ml (Sarstedt, cat. no. 72.693)

## Important points before starting

- Thaw and equilibrate up to 48 whole blood samples at room temperature (15–25°C).
- Check that Buffer MW1 has been prepared according to the instructions given in the “Important Notes” section of the *MagAttract DNA Blood M48 Handbook*.
- Before use, check that Buffer ML does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer ML into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.

## Procedure

1. **Place 700  $\mu$ l of blood into 2 ml sample tubes.**

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.**

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software start-up window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft® Windows® "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

5. **Select the protocol group "Genotyping" from the drop-down menu, by clicking on the dark green arrow, then select "gDNA".**

6. **Select the protocol "700  $\mu$ l Blood" and click the "Select" button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol; these steps include the option of entering names for your samples. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

7. **Place the sample tubes on the worktable, plus reagent containers and plasticware, according to the software.**

8. **Close the workstation door and start the purification protocol. All steps are fully automated, and a software message on the screen will indicate when the protocol 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 fluorescent capillary sequencing, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see appendix of the *MagAttract DNA Blood Mini M48 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 large volume blood application.

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### Comments and suggestions

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#### Low DNA yield

Clogging of pipet tip due to sample overload

Reduce the sample input volume.

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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