QIAGEN Supplementary Protocol:

Isolation of DNA from dried blood using the BioRobot® M48 and the MagAttract® DNA Mini M48 Kit

This protocol is designed for the isolation of total (genomic and mitochondrial) DNA from dried blood using the MagAttract DNA Mini M48 Kit in combination with the BioRobot M48 workstation.

Introduction

The BioRobot M48 system allows fully automated purification of total DNA from dried blood. The magnetic particle technology used by the BioRobot M48 system provides high-quality DNA, which is ideal for genotyping and epidemiological studies as well as forensic analyses. The isolated DNA is well suited for direct use in downstream applications, such as amplification or other enzymatic reactions.

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

Starting material

Drying blood on filter paper is an effective form of storage and samples prepared in this manner are cheaper and safer to transport. The amount of starting material and the elution volumes to use in this procedure are given in Table 1, below.

Table 1. Amount of starting material and elution volumes for the MagAttract DNA M48 dried blood procedure

Sample type	Protocol	Starting volume	Elution volume
Dried blood spots*	Dried Blood	4 discs†	50–400 µl

* See the "Sample collection" subsection of the procedure.

† A 3.25 mm (1/8 in.) diameter disc punched out from 903 Specimen Collection Paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.

Equipment and reagents required by user

- BioRobot M48 workstation, cat. no. 9000708, and disposables (see the *MagAttract DNA Mini M48 Handbook)*
- MagAttract DNA Mini M48 Kit, cat. no. 953336
- 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 2.0 ml (Sarstedt, cat. no. 72.693)

- Filter paper (e.g., 903 Specimen Collection Paper (Schleicher & Schuell, cat. no. 10538414), 903 Generic Blood Collection Card (Schleicher & Schuell, cat. no. 10538019), FTA Classic Card (Whatman, cat. no. WB120205), FTA Indicating Classic Card (Whatman, cat. no. WB120206))
- Manual paper punch, 3 mm (Schleicher & Schuell, cat. no. 10495010) or, alternatively, DBS puncher (Perkin Elmer, cat. no. 1296-071)

Important notes before starting

- Check that Buffer MW1 has been prepared according to the instructions given in the "Important Notes" section of the *MagAttract DNA Mini M48 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.

Things to do before starting

- Prepare a 70°C shaking water bath or thermomixer for use in step 5.

Procedure

Sample collection

1. **Collect 70 µl of each blood sample onto a ring marked on 903 Specimen Collection Paper. Allow the blood to air-dry.**

   Either untreated blood or blood containing anticoagulant (EDTA, ACD, or heparin) can be used.
2. **For each dried blood sample, use the manual paper punch to cut out four 3 mm diameter discs.**
3. **Transfer each set of 4 discs to a 1.5 ml sample tube.**
4. **Add 720 µl Buffer MTL to the sample tubes.**
5. **Incubate the tubes with continuous mixing (i.e. in a shaking water bath or thermomixer) at 70°C for 20 min.**

DNA isolation

6. **Ensure that the BioRobot M48 is switched on.**

   The power switch is on the left side of the instrument.
7. **Switch on the computer and monitor.**
8. **Launch the QIAsoft™ M Operating System.**

   Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup 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.

9. **Select the protocol group "Genetic Screening" from the drop-down menu by clicking on the arrow, then select "gDNA".**
10. **Select the protocol "Dried 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 dried blood protocol. Follow the steps detailed in the protocol message before continuing. Wear gloves when loading the required items on the worktable.
11. **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.**
12. **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 *MagAttract DNA Mini M48 Handbook*) in order to minimize the risk of magnetic-particle carryover.

Troubleshooting

For troubleshooting, please consult the "Troubleshooting Guide" in the *MagAttract DNA Mini M48 Handbook*.