

QIAGEN Supplementary Protocol:

Isolation of genomic DNA from paraffin-embedded sections using the MagAttract® DNA Mini M48 Kit

This protocol is designed for the isolation of total (genomic and mitochondrial) DNA from paraffin-embedded sections using the MagAttract DNA Mini M48 Kit in combination with the BioRobot® M48 workstation.

Introduction

The MagAttract M48 system allows fully automated purification of genomic DNA from paraffin sections. MagAttract technology provides high-quality genomic 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 DNA isolation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material.

This protocol describes first how to digest paraffin-embedded tissue samples and then follows with the simple procedure for setting up the BioRobot M48 and starting a run.

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

For most tissue types, a sample size of one to five 10 μm -thick paraffin sections is recommended; however, up to 10 or more very small sections such as needle biopsies may be used.

Note: The wax block should preferably be trimmed prior to sectioning in order to reduce the amount of wax in the sample material. It is recommended to trim the paraffin wax block if more than 6 sections are to be used in the DNA purification procedure. This is especially important for needle biopsy samples.

The starting and elution volumes to use in this procedure are given in Table 1 below.

Table 1. Starting and elution volumes for the MagAttract DNA M48 paraffin-embedded procedure

Sample type	Protocol	Amount of starting material	Elution volume
Paraffin-embedded tissue samples	Paraffin Sections	100 or 200 μl digestion	75–200 μl

* Depending on the preparation and age of the sample, some degree of DNA degradation prior to DNA isolation is to be expected.

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 Mini M48 Handbook*)
- Sample tubes, 1.5 ml, with screw caps (Sarstedt, cat. no. 72.692), or 2 ml, 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

- Digest up to 48 paraffin section samples (see “Proteinase K digestion of paraffin-embedded tissue sections”, below).
- 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.

Things to do before starting

- Prepare a 75°C shaking water bath or thermomixer for use in step 3.

Procedure

Proteinase K digestion of paraffin-embedded tissue sections

- 1. Transfer sections of paraffin-embedded tissue into a 1.5 or 2 ml sample tube (with screw-cap). For most tissue types, a sample size of one to five 10 µm thick paraffin sections is recommended; however, up to 10 or more very small sections such as needle biopsies may be used.**
- 2. Add 190 µl of Buffer G2 to the sample tubes.**
Ensure that the paraffin sections are fully submerged into Buffer G2.
- 3. Incubate for 5 min at 75°C with vigorous mixing (i.e., in a shaking water bath or thermomixer).**
- 4. Lower the temperature to 56°C and allow the sample to cool to 56°C.**
- 5. Add 10 µl of Proteinase K solution, and mix by tapping the tube gently.**
- 6. Incubate overnight at 56°C, with continuous vigorous mixing (i.e., in a shaking water bath or thermomixer).**

Note: To ensure complete digestion of the sample, overnight incubation is strongly recommended. Shorter incubation may result in incomplete digestion of the sample, which may lead to clogging of the tips during the automated DNA isolation procedure.

7. **Centrifuge the tube briefly to remove drops from inside the lid.**
8. **If necessary, homogenize the sample by pipetting up and down several times. Large pieces of paraffin or insoluble tissue material, which could clog pipet tips, should be removed.**

To remove insoluble material, centrifuge the sample at 300 x g for 1 min, and transfer the supernatant to a new 2 ml sample tube.

If the predigested sample is to be stored at 2–8°C or at –20°C it is recommended to heat the samples up again to 56°C with vigorous mixing prior to isolation on the BioRobot M48.

9. **Proceed to the automated DNA isolation procedure given below (step 10 onwards).**

If the digested samples were stored at 2–8°C or –20°C after step 8, remove samples from storage and heat to 56°C, with vigorous mixing, before proceeding to step 10.

DNA isolation

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

The power switch is on the left side of the instrument.

11. **Switch on the computer and monitor.**

12. **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.

13. **Select the protocol group “Pathology” from the drop-down menu by clicking on the dark green arrow, then select “gDNA”.**
14. **Select the protocol “Paraffin Sections”. Click the “Select” button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the paraffin-embedded section protocol. Follow the steps detailed in the protocol message before continuing. Wear gloves when loading the required items on the worktable.

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

16. **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.**
17. **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 or fluorescent capillary sequencing, 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 general troubleshooting, please consult the “Troubleshooting Guide” in the *MagAttract DNA Mini M48 Handbook*. The troubleshooting described here is specific for the paraffin-embedded DNA application.

Comments and suggestions

Low DNA yield

Clogging of pipet tip due to sample overload

Ensure complete sample digestion. Overnight incubation is strongly recommended.

Remove excess wax prior to DNA purification. This is particularly important when more than 6 sections are to be used.

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.

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