QIAGEN Supplementary Protocol

Purification of genomic DNA from whole blood, optimized for use in MRC-Holland MLPA® assays, using EZ1® DNA Blood Kits

This protocol is designed for isolation of genomic DNA from whole blood using the QIAGEN® EZ1 DNA Blood 200 µl Kit or the EZ1 DNA Blood 350 µl Kit in combination with EZ1 instruments and EZ1 DNA Blood Cards. This protocol has been optimized for use in MRC-Holland multiplex ligation-dependent probe amplification (MLPA) assays.

Introduction

EZ1 instruments enable fully automated purification of genomic DNA from whole blood. Magnetic-particle technology used by EZ1 instruments provides high-quality genomic DNA, which is suitable for direct use in downstream applications, such as PCR or other enzymatic reactions. EZ1 instruments perform all steps of the DNA isolation procedure, and the procedure allows purification from varying amounts of starting material.

This protocol describes first how to prepare a simple additional reagent and how to add this to the workstation in order to get optimal downstream results from assays using MLPA technology.

IMPORTANT: Please read the *EZ1 DNA Blood Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. Ensure that you are familiar with operating the EZ1 instrument. See the respective EZ1 instrument user manuals.

Equipment and reagents required

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioRobot EZ1, EZ1 Advanced (cat. no. 9001410) or EZ1 Advanced XL (cat. no. 9001492) and disposables (see the *EZ1 DNA Blood Handbook*)
- EZ1 DNA Blood 200 µl Kit (cat. no. 951034) or EZ1 DNA Blood 350 µl Kit (cat. no. 951054)
- EZ1 DNA Blood Card v1.3 (cat. no. 9015585), EZ1 Advanced DNA Blood Card (cat. no. 9018293), or the EZ1 Advanced XL DNA Blood Card (cat. no. 9018695)
- 2 ml Sarstedt microcentrifuge tubes (Sarstedt, cat. no. 72.693 or 72.694)
- 80% (v/v) ethanol* in molecular-biology grade water

* Do not use denatured alcohol because this contains other substances such as methanol or methylethylketone.

Procedure

1. **Pipet 200 µl or 350 µl whole blood (depending on protocol and kit used) into 2 ml sample tubes.**

   Thawed whole blood samples should be thoroughly resuspended prior to pipetting.

2. **Insert the appropriate EZ1 DNA Blood Card completely into the EZ1 Card slot of the EZ1 instrument.**
3. **Switch on the EZ1 instrument.**
4. **Press "START" to display the "Protocols" menu.**
5. **Press "1" or "2" to start worktable setup for the 200 µl protocol or the 350 µl protocol, respectively.**
6. **Press "1" to select an elution volume of 50 µl, "2" to select an elution volume of 100 µl, or "3" to select an elution volume of 200 µl.**
7. **Press "2" to select the ethanol wash step, optimized for MLPA assays.**
8. **Press any key to proceed through the text displayed in the LCD and start worktable setup.**

   The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.

9. **Open the instrument door.**
10. **Invert reagent cartridges 4 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.**
11. **Load the reagent cartridges into the cartridge rack.**

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

    If there are fewer than 6 reagent cartridges for the EZ1 Advanced or BioRobot EZ1, or fewer than 14 reagent cartridges for the EZ1 Advanced XL, you can load them in any order on the rack. However, when loading the other labware in steps 12–15, ensure that they also follow the same order. When using the data tracking option on the EZ1 Advanced or EZ1 Advanced XL, always start loading samples in position 1 and place the remaining samples one after the other into the next available positions on the worktable.

12. **Add 1800 µl of 80% ethanol to an empty 2 ml Sarstedt tube, and place the filled tube into the third row in the tip rack. Repeat for each reagent cartridge added to the workstation.**
13. **Load opened elution tubes into the first row of the tip rack.**
14. **Load tip holders containing filter-tips into the second row of the tip rack.**

15. **Load opened sample tubes containing 200 µl or 350 µl blood into the back (fourth) row of the tip rack.**
16. **Close the instrument door.**
17. **Press "START" to start the purification procedure.**
18. **The automated purification procedure takes 17–23 min depending on the protocol version and the EZ1 instrument.**
19. **When the protocol ends, the LCD displays "Protocol finished". Open the workstation door.**
20. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C or –20°C.**

    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 *EZ1 DNA Handbook*) in order to minimize the risk of magnetic-particle carryover.
21. **To run another protocol, press "ESC", prepare samples as described in step 1, and follow the procedure from step 5 onward. Otherwise, press "STOP" twice to return to the first screen of the LCD, close the workstation door, and switch off the EZ1 instrument.**
22. **Clean the EZ1 instrument.**

    Follow the maintenance instructions in the respective EZ1 instrument user manuals.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *EZ1 DNA Blood Handbook*.

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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