

QIAGEN Supplementary Protocol

Purification of normalized DNA from forensic samples, including solid material, using the EZ1 Advanced XL

This protocol is designed for purification of total (genomic and mitochondrial) DNA from forensic reference samples (e.g., buccal swabs, dried blood spots), using the EZ1 DNA Investigator Kit in combination with the EZ1 Advanced XL and EZ1 Advanced XL DNA Investigator Card. Solid samples (e.g., blood card punches, swabs) can also be processed using this protocol. Using the normalization protocol, DNA yields can be limited uniformly to 150–250 ng.

The EZ1 Advanced XL instrument and the EZ1 DNA Investigator Kit reproducibly automate purification of genomic DNA from samples encountered in forensic, human identity, and biosecurity applications. Purification is efficient and purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios. Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as STR analysis or other enzymatic reactions. The EZ1 Advanced XL instrument performs all steps of the sample preparation procedure, and up to 14 samples can be processed in a single run.

In the “tip dance” protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

IMPORTANT: Please read the EZ1 DNA Investigator 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 Advanced XL (see the *EZ1 Advanced XL User Manual*). 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.

Equipment and reagents

- EZ1 DNA Investigator Kit (cat. no. 952034)
- EZ1 Advanced XL (cat. no. 9001492)
- EZ1 Advanced XL DNA Investigator Card (cat. no. 9019933)

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced XL instrument), PC (can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), and monitor (cat. no. for PC and monitor 9016643)



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- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced XL instrument) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
 - Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read the "Important Notes" section of the *EZ1 DNA Investigator Handbook*.
- Reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents that contain bleach. See the *EZ1 DNA Investigator Handbook* for safety information.
- Perform all protocol steps at room temperature (15–25°C). During the setup procedure, work quickly.

Things to do before starting

- Lyse the sample according to the pretreatment protocol outlined in the *EZ1 DNA Investigator Handbook*.
- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See the "Equilibrating reagent cartridges" section of the *EZ1 DNA Investigator Handbook*.
- The lysis buffer in the reagent cartridges may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. **Insert the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL.**
2. **Switch the instrument on.**
3. **Press "START" to start the protocol setup. Follow the onscreen instructions for data tracking.**
4. **Press "5" (for Norm TD protocol).**
5. **Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE buffer. Then press "1" to elute in 40 µl, "2" to elute in 50 µl, "3" to elute in 100 µl, or "4" to elute in 200 µl.**
6. **Press any key to proceed through the onscreen text 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.
7. **Open the instrument door.**

8. Invert reagent cartridges 4 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

9. Load the reagent cartridges into the cartridge rack.

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

10. Load opened elution tubes into the first row of the tip rack.

11. Load tip holders containing filter-tips into the second row of the tip rack.

12. Load opened sample tubes containing digested samples into the back row of the tip rack.

Pretreat the samples by following the individual protocols in the EZ1 DNA Investigator Handbook.

Note: To avoid mixing up samples when using the data tracking option, ensure that sample IDs follow the same order as the samples.

13. Close the instrument door.

14. Press "START" to start the purification procedure.

The automated purification procedure takes 15–20 min.

15. When the protocol ends, the display shows "Protocol finished". Press "ENT" to generate the report file.

The EZ1 Advanced can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. Open the instrument door.

17. Retrieve the elution tubes containing the purified DNA. The DNA is ready-to-use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample preparation waste.*

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 then the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

19. To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onwards. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See the EZ1 DNA Investigator Handbook for safety information.

20. Clean the EZ1 instrument.

Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

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.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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