



QIAGEN Supplementary Protocol:

Automated preparation of up to 10 ml cell lysate on the Autopure LS®

This protocol is designed for preparation of cell lysates from 1–5 ml or 5–10 ml fresh or frozen whole blood, buffy coat, or packed cell samples using Autopure reagents on the Autopure LS.

The Autopure LS provides automated purification of archival-quality DNA from a variety of large samples. Proven Gentra® Puregene® chemistries and optimized protocols provide high yields of pure DNA ready for use in sensitive downstream applications or for DNA archiving. Purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9. Either 8 or 16 samples can be processed per run.

This protocol stops at the end of the cell lysis step, enabling overnight incubation of lysates. The overnight incubation helps to disperse cell clumps and maximize DNA yield. The protocol also provides a convenient stopping point for the purification procedure. Lysates can be prepared from many different sample types including fresh or frozen whole blood and fresh or frozen buffy coat.

IMPORTANT: Please read the *Autopure LS User Manual*, paying careful attention to the safety information, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The Autopure LS instrument is intended to be used only in combination with Autopure reagents for applications described in the *Autopure LS User Manual*.

Equipment and reagents to be supplied by user

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.

- Autopure LS, cat no. 9001340
- Autopure RBC Lysis Solution (9500 ml), cat. no. 949004
- Autopure Cell Lysis Solution (3800 ml), cat. no. 949006
- Autopure Qubes® E (192), cat. no. 949020 or Autopure Qubes D (192), cat. no. 949022
- Autopure Waste Container, cat. no. 9017686

Important point before starting

- Ensure that you are familiar with operating the Autopure LS. Refer to the *Autopure LS User Manual* for operating instructions.

Procedure

1. **Make sure that the Autopure LS is switched on. The power switch is located at the back left side of the instrument.**
2. **Log in to the instrument software. Prepare the samples and the rack, and follow the steps for starting sample processing described in the *Autopure LS User Manual*.**
3. **Select the protocol "Cell Lysate Preparation" and select the sample volume "1–5 ml" or "5–10 ml".**
Important: When running the protocol for 1–5 ml samples of lysate, the sample volume must not exceed 5 ml. When running the protocol for 5–10 ml samples of lysate, the sample volume must not exceed 10 ml.
4. **Select "Run Rack" to start the run. The Autopure LS will then perform the automated purification procedure. For more detailed information about the procedure, see "Steps performed by the Autopure LS", below.**

Steps performed by the Autopure LS

The amount of reagent used depends on the protocol being run. Reagent volumes for processing 1–5 ml of lysate are denoted by ■ and reagent volumes for processing 5–10 ml of lysate are denoted by ◆.

RBC lysis

1. **Scans and verifies the input and output cap bar codes and weighs the tubes to check that input tubes contain samples and that output tubes are empty.**
2. **Dispenses ■ 15–19 ml or ◆ 30–35 ml Autopure RBC Lysis Solution (Reagent 1) into each input tube.**
Note: The system uses Reagent 1 to balance the tubes before centrifugation. The amount dispensed into each tube varies depending on the initial sample volume. The total volume of sample and Reagent 1 is ■ 20 ml or ◆ 40 ml.
3. **Incubates the sample in Autopure RBC Lysis Solution for 6 min 30 s to lyse the red blood cells. The samples are rotated gently to mix during the incubation.**
4. **Centrifuges the samples at 3000 x g for 2 min to pellet the white blood cells.**
5. **After centrifugation, the supernatant is poured into the waste tray.**
6. **Dispenses ■ 5 ml or ◆ 10 ml Autopure Cell Lysis Solution (Reagent 2) into each input tube to lyse the white blood cells.**
7. **Places input tubes back into sample loading rack.**
8. **Display message to inform user that the protocol run has finished.**

Removal from instrument

1. **Manually remove the samples from the Autopure LS.**
2. **To facilitate cell lysis, mix vigorously by vortexing at high speed for 10 s.**
3. **Incubate samples until homogeneous. Samples must be homogeneous before continuing with DNA purification. If cell clumps are visible, incubate samples at 37°C for several hours or at room temperature (15–25°C) until samples are homogeneous.**

Note: Samples are stable for at least 2 years at room temperature in Cell Lysis Solution.

4. **Continue with the protocol “Cell Lysate” on the Autopure LS.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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