

QIAGEN Supplementary Protocol

Automated purification of DNA from 8 or 16 samples of fresh or frozen whole blood on the Autopure LS[®]

This protocol is designed for purification of DNA from 1–5 ml or 5–10 ml samples of fresh or frozen whole blood 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 and is up to 200 kb in size.

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.

Equipment and reagents

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 Precipitation Soln. (3800 ml), cat. no. 949008
- Autopure DNA Hydration Soln. (3800 ml), cat. no. 949010 or DNA Hydration Solution (500 ml), cat. no. 158916
- Autopure 100% Isopropanol (3800 ml), cat. no. 949016
- Autopure 70% Ethanol (3800 ml), cat. no. 949018
- Autopure Qubes[®] E (192), cat. no. 949020 or Autopure Qubes D (192), cat. no. 949022
- Autopure Waste Container, cat. no. 9017686
- Water bath heated to 65°C



Important point before starting

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

Things to do before starting

- Heat water bath to 65°C for use in step 6 of the procedure.

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 appropriate protocol for the sample type and size. See the table below for more information.**

Important: When running protocols for 1–5 ml whole blood, the sample volume must not exceed 5 ml. When running protocols for 5–10 ml whole blood, the sample volume must not exceed 10 ml.

Protocols for processing up to 10 ml fresh or frozen blood

Protocol name	Sample size	Sample storage
Fresh Whole Blood	1–5 ml or 5–10 ml	Samples stored for <24 h at room temperature (15–25°C) or <5 days at 4°C. Samples have not been frozen.
Frozen Whole Blood	1–5 ml or 5–10 ml	Samples frozen at –80°C directly after collection and stored for less than 2 years at –80°C. Samples have not been thawed and refrozen.

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”, page 3.**
5. **When instructed to do so by the software, remove the purified DNA from the Autopure LS.**
6. **After removing samples from the instrument, incubate at 65°C for 1–2 h.**

7. Incubate at room temperature overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Steps performed by the Autopure LS

The amount of reagent used depends on the protocol being run. Reagent volumes for processing 1–5 ml whole blood are denoted by ■ and reagent volumes for processing 5–10 ml blood 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 (6.5 min for fresh whole blood samples or 5 min for frozen whole blood samples) to lyse the red blood cells. The samples are rotated gently to mix.
4. Centrifuges the samples at 3000 x g for 2 min to pellet the white blood cells.
5. When running 16 samples, during centrifugation in step 4, the Autopure LS dispenses ■ 5 ml or ◆ 10 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C.
6. After centrifugation, the supernatant from step 4 is poured into the waste tray.

Cell lysis and protein precipitation

7. Dispenses ■ 1.67 ml or ◆ 3.34 ml Autopure Precipitation Soln. (Reagent 3) vigorously into the center of the input tubes to disperse the white blood cell pellet.
8. Dispenses ■ 5 ml or ◆ 10 ml Autopure Cell Lysis Solution (Reagent 2) into each input tube to lyse the white blood cells.
9. Mixes the samples vigorously to precipitate the proteins.
10. Centrifuges the samples at 3000 x g for 2 min. The precipitated proteins will form a tight pellet at the bottom of the input tube.
11. During the centrifugation in step 4, the instrument dispenses ■ 5 ml or ◆ 10 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C when processing 8 samples or Row C and D when processing 16 samples.
12. Pours the DNA-containing supernatant from step 4 into the output tubes that contain Autopure 100% Isopropanol.

DNA precipitation

- Rotates the output tubes gently 50 times to precipitate the DNA.
- Centrifuges the samples at 3000 x g for 2 min to pellet the DNA.
- Pours the isopropanol supernatant into the waste tray, and inverts the output tubes for 1 min to evaporate any remaining alcohol.

DNA wash

- Dispenses ■ 5 ml or ◆ 10 ml Autopure 70% Ethanol (Reagent 5) into the output tubes.
- Centrifuges the samples at 3000 x g for 1 min to pellet the DNA.
- Pours the ethanol supernatant into the waste tray, and inverts the output tubes for 1 min to evaporate any remaining alcohol.

DNA hydration

- Dispenses the volume of Autopure DNA Hydration Soln. or DNA Hydration Solution (Reagent 6) selected by the user into the output tubes to rehydrate the DNA.
- Displays message to inform user that the protocol run has finished.

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

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