Maxwell® HT 96 gDNA Blood Isolation System

Instructions for Use of Products A2670 and A2671.

Quick Protocol

Purification of Genomic DNA from Anticoagulated Whole Blood

The following protocol describes manually purifying gDNA from whole blood, which mimics the steps required for a completely automated process. If interested in evaluating in a 96-well format, please contact Promega. Contact information available at: **www.promega.com**

Perform the following steps at room temperature unless noted.

1. **Whole Blood Addition.** Add 350µl of whole blood (fresh or frozen) to a clean 1.5ml processing tube.

   Note: To ensure whole blood is homogeneous, place 10ml tube or aliquot tube on a tube rotor and allow whole blood to mix for 10 minutes before adding 350µl to a clean 1.5ml processing tube.

2. **Lysis Buffer Addition.** Add 350µl of Cell Lysis Buffer (1:1 ratio) to the 1.5ml processing tube. Gently vortex or shake for 60 seconds. Be careful when vortexing or shaking since the lysis buffer will generate bubbles.

   Note: Spin the 1.5ml processing tube using a microcentrifuge to remove liquid or bubbles from tube cap. Perform this step as necessary throughout the protocol. This step is not required for automation setup.

3. **Proteinase K Addition.** Add 35µl of Proteinase K (20mg/ml) to each 1.5ml processing tube. Mix by pipetting 5X, and then vortex or shake for 30 seconds.

4. **Lysis Incubation.** Place the 1.5ml processing tube in a dry or wet heat block and incubate for 10 minutes at 75°C. (Keep the tube cap open to allow heat transfer.) When complete, vortex or shake the 1.5ml processing tube (with tube cap closed) for 30 seconds and continue incubating (with tube cap open) for 10 minutes at 75°C. Total incubation time = 20 minutes. Remove the 1.5ml processing tube from the heat block, and allow lysate to cool for 5 minutes before moving to the next step.

5. **Binding Buffer Addition.** Add 420µl of Binding Buffer to the 1.5ml processing tube. Mix 10X by pipetting 800µl using a P1000 filtered tip. Make sure blood lysate is homogeneous with binding buffer before adding Resin (no visible liquid phases).

   Note: If RNase treatment is desired, prepare a 0.1mg/ml solution of RNase A Solution (Cat.# A7974; 4mg/ml) in Binding Buffer when preparing and dispensing reagents. If running multiple samples, add enough Binding Buffer to a separate tube, and add RNase A Solution to the Binding Buffer. Mix RNase A Solution and Binding Buffer by pipetting 10X.

6. **Resin Addition.** Add 35µl of Resin to the 1.5ml processing tube.

   Note: Make sure the Resin is completely resuspended in the bottle. Vigorously shake or vortex the stock Resin to ensure no Resin remains at the bottom of the bottle. Complete resuspension is required.

7. **DNA Binding.** Incubate the 1.5ml processing tube at room temperature for 20 minutes. After Resin is added, mix 10X by pipetting 800µl using a P1000 filtered tip, and continuously vortex or shake the 1.5ml processing tube for 10 minutes. Mix an additional 10X by pipetting 800µl using a P1000 filtered tip, and continue to vortex or shake for another 10 minutes. Total incubation time = 20 minutes. Alternatively a rotisserie mixer can be used; alternative mixers can be used if they keep the Resin in solution throughout the binding process.

   Note: If you observe Resin settling to the bottom of the 1.5ml tube, increase shaking speed until Resin remains in suspension.

8. **Cleared Lysate Removal.** Move the 1.5ml processing tube to the 1.5ml Magnetic Separation Device, and allow lysate to clear for 5 minutes to capture magnetic Resin. When the lysate is cleared, remove the cleared lysate to the waste.

   Note: It is important to remove all supernatant lysate.

9. **Wash Buffer Addition #1.** Add 50µl of 50% EtOH to the 1.5ml processing tube. Make sure to use molecular biology-grade ethanol and RNase- and DNase-free water. Pipette mix 5X and vortex or shake for 1 minute or until Resin is completely broken up. Add 400µl of Wash Buffer to the 1.5ml processing tube. Mix the samples in a series of shaking and pipetting steps:
   a. Mix the 1.5ml processing tube for 45 seconds by vortexing or shaking.
   b. Mix the 1.5ml processing tube 8X by pipetting 125µl.
   c. Mix the 1.5ml processing tube for 45 seconds by vortexing or shaking.
   d. Mix the 1.5ml processing tube 8X by pipetting 125µl.
   e. Mix the 1.5ml processing tube for 30 seconds by vortexing or shaking.
10. **Wash Buffer Removal #1.** Move the 1.5ml processing tube to the 1.5ml Magnetic Separation Device. Allow wash buffer to clear for 120 seconds. Remove the Wash Buffer to waste.
11. **Wash Buffer Addition/Removal #2.** Repeat Steps 9–10.
12. **Ethanol Wash Addition.** Add 250µl of 50% ethanol to the 1.5ml processing tube. Mix the samples in a series of shaking and pipetting steps:
   a. Mix the 1.5ml processing tube for 45 seconds by vortexing or shaking.
   b. Mix the 1.5ml processing sample tube 12X by pipetting 125µl.
   c. Mix the 1.5ml processing sample tube for 30 seconds by vortexing or shaking.
13. **Ethanol Wash Removal.** Move the 1.5ml processing tube to the 1.5ml Magnetic Separation Device. Allow the 50% EtOH wash to clear for 120 seconds. Remove the 50% EtOH wash to waste. Keep the 1.5ml processing tube on the 1.5ml Magnetic Separation Device for 5 minutes. When complete, remove any residual 50% EtOH to waste.
14. **Heat Block Drying.** Move the 1.5ml processing tube to the heat block (75–85°C) for 45 seconds with tube cap open.
15. **Tris Buffer Addition.** Elution at 75–85°C. Add 50–110µl of 25mM Tris-HCl (pH 8.0) to the 1.5ml processing tube. Mix the samples in a series of shaking and 75°C-heated incubation steps to elute gDNA from the Resin into the Tris buffer:
   a. Mix the 1.5ml processing tube for 30 seconds by vortexing or shaking.
   b. Incubate the 1.5ml processing tube for 3 minutes at 75°C.
   c. Mix the 1.5ml processing tube for 30 seconds by vortexing or shaking.
   d. Incubate the 1.5ml processing tube for 3 minutes at 75°C.
   e. Mix the 1.5ml processing tube for 30 seconds by vortexing or shaking.
16. **Elution.** Move the 1.5ml processing tube to the 1.5ml Magnetic Separation Device. Allow the elution buffer to clear for 2 minutes. When elution buffer is clear, transfer eluted gDNA to a clean 1.5ml tube.
17. **Method Ends.** The manual Maxwell® HT 96 gDNA Blood Isolation System method is now complete. Process the purified gDNA samples in the elution tube immediately or store at 4°C. For prolonged stability, add 10µl of the 10mM EDTA (pH 8.0) included with the Maxwell® HT 96 gDNA Blood Isolation System. Add the EDTA only after completion of absorbance readings (check compatibility of EDTA with downstream application first).

Additional protocol information is in Technical Manual #TM368, available online at: **www.promega.com**

