Maxwell® HT 96 gDNA Blood Isolation System

Instructions for Use of Products A2670 and A2671.

Quick Protocol

Purification of Genomic DNA from Saliva Samples in Oragene®•Discover Devices

The The following protocol describes manually purifying gDNA from Oragene® saliva samples, 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**

Note: Collection procedures recommend no smoking, gum chewing, eating, brushing teeth or mouthwash 30 minutes prior to collecting the saliva.

Perform the following steps at room temperature unless noted.

1. **Oragene Lysate Addition.** Add 700µl of Oragene lysate to a clean 1.5ml processing tube.

   Note: After the saliva is collected via the Oragene device, be sure to snap shut the top tube to allow the blue lysis buffer to mix with the raw saliva. Invert the tube 5–10X and incubate in a water bath for 1 hour at 56–65°C to complete the lysis step. If using a dry heat source, incubate for >2 hours at 56–65°C.

2. **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 Oragene saliva 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 1.5ml processing tubes, 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.

3. **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.

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

5. **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.

6. **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 complete 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.
7. **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.
8. **Wash Buffer Addition/Removal #2.** Repeat Steps 6–7.
9. **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.
10. **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.
11. **Heat Block Drying.** Move the 1.5ml processing tube to the heat block (75–85°C) for 45 seconds with tube cap open.
12. **Tris Buffer Addition.** Elution at 75–85°C. Add 50–110µl of 25mM Tris-HCl (pH 8.0) to each 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.
13. **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.
14. **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**

  • PRINTED IN USA 12/16 • PART #FB214