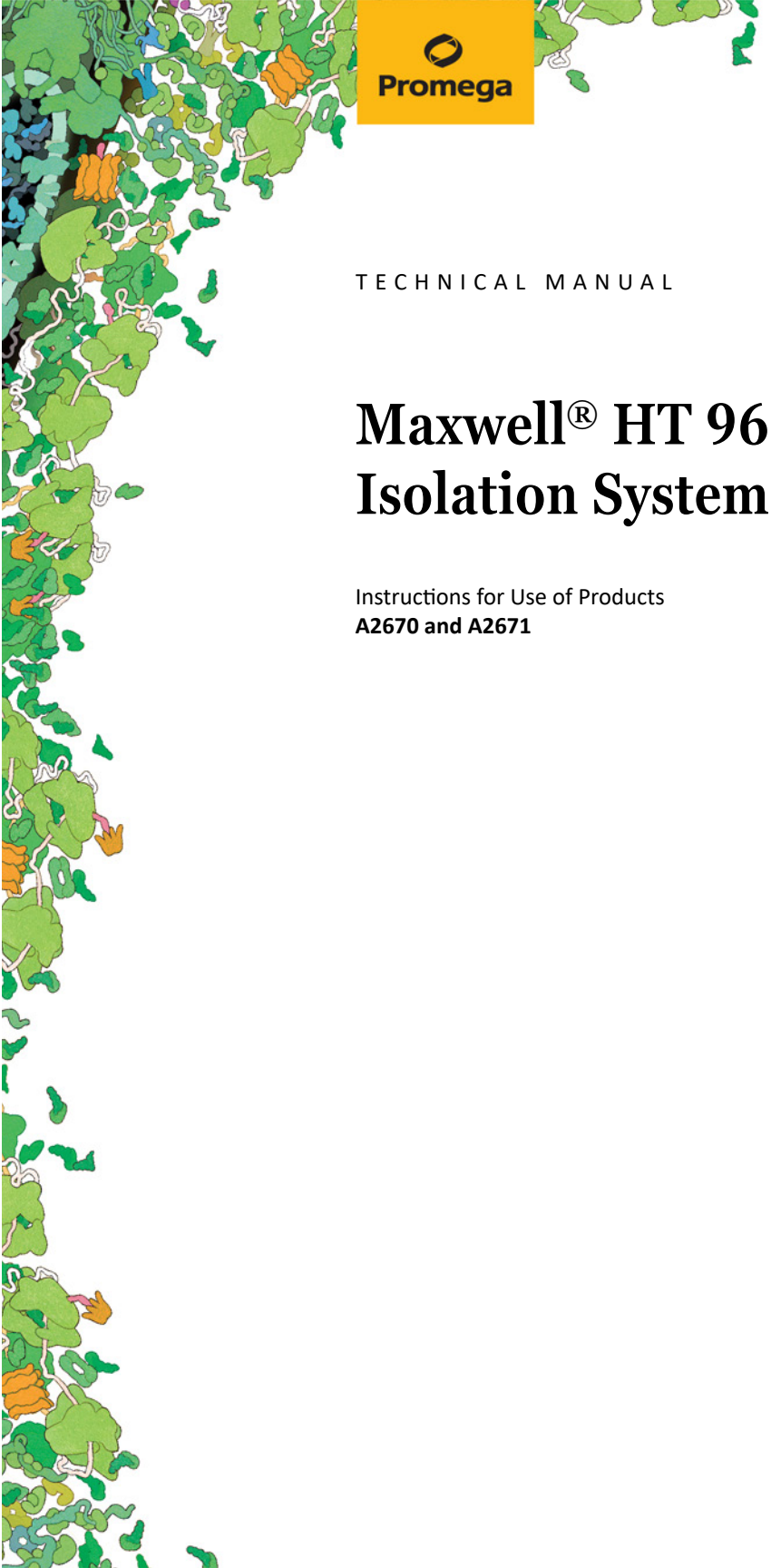

TECHNICAL MANUAL

Maxwell® HT 96 gDNA Blood Isolation System

Instructions for Use of Products
A2670 and A2671

Revised 4/21
TM368

Promega

Maxwell® HT 96 gDNA Blood Isolation System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Maxwell® HT 96 gDNA Blood Isolation System[a] provides a simple and reliable method for the rapid isolation of genomic DNA (gDNA) in a multiwell format. Because the system is robust, gDNA may be purified from anticoagulated whole blood or saliva collected in Oragene®•Discover devices. The purified gDNA can be used directly in PCR assays, microarrays, and next-generation sequencing applications. The use of paramagnetic particles for DNA capture eliminates the need for centrifugation or vacuum manifolds, making the system suitable for full automation. In addition, the system does not require phenol or chloroform, making it safe and convenient.

The Maxwell® HT 96 gDNA Blood Isolation System uses Proteinase K and a specially formulated Cell Lysis Buffer to liberate sample DNA. (**Note:** The Cell Lysis Buffer is only required for blood samples.) Released DNA is bound to the ReliaPrep™ particles in the presence of the Binding Buffer. The DNA bound to Resin is captured by a magnet, and contaminants are removed by washing with the Wash Buffer and ethanol. The DNA is then eluted from the particles with 25mM Tris-HCl. The process involves the following simple steps:

- cell lysis with Proteinase K and Lysis Buffer (for blood samples only)
- gDNA capture to paramagnetic Resin in Binding Buffer
- washes with Wash Buffer and ethanol
- elution with Tris-HCl

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® HT 96 gDNA Blood Isolation System	**1 × 96**	**A2670**

For in vitro Research Use Only. Sufficient reagents to process one 96-well plate of samples. Includes:

- 5.5ml Proteinase K (PK) Solution
- 40ml Cell Lysis Buffer (CLD)
- 5.5ml Resin
- 50ml Binding Buffer (BBA)
- 85ml Wash Buffer (WBA)
- 60ml 25mM Tris-HCl (pH 8.0)
- 10ml 10mM EDTA (pH 8.0)
- 1ml RNase A Solution, 4mg/ml

PRODUCT	SIZE	CAT.#
Maxwell® HT 96 gDNA Blood Isolation System	**4 × 96**	**A2671**

For in vitro Research Use Only. Sufficient reagents to process four 96-well plates of samples. Includes:

- 23ml Proteinase K (PK) Solution
- 160ml Cell Lysis Buffer (CLD)
- 4 × 5.5ml Resin
- 200ml Binding Buffer (BBA)
- 350ml Wash Buffer (WBA)
- 60ml 25mM Tris-HCl (pH 8.0)
- 10ml 10mM EDTA (pH 8.0)
- 5ml RNase A Solution, 4mg/ml

Storage Conditions: Store all Maxwell® HT 96 gDNA Blood Isolation System components at +15°C to +30°C.

Items Available Separately

PRODUCT	SIZE	CAT.#
Heat Block Adapter	**1 each**	**A2661**
RNase A Solution, 4mg/ml	**5ml**	**A7974**
25mM Tris-HCl (pH 8.0)	**60ml**	**A2641**
10mM EDTA (pH 8.0)	**10ml**	**A2631**
MagnaBot® FLEX 96 Magnetic Separation Device	**1 each**	**VA1290**

Note: Throughout the remainder of this document, the supplied Cell Lysis Buffer (CLD), Binding Buffer (BBA) and Wash Buffer (WBA) are referred to as Cell Lysis Buffer, Binding Buffer and Wash Buffer, respectively.

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3. System Requirements for Liquid Handling Instruments

For automated processing, this protocol can be performed on any liquid handling instrument capable of an integrated shaking and heating device. Additional requirements include movement of SLAS plates around the deck. Please contact Promega for additional deck and hardware configurations.

Materials to Be Supplied by the User for Liquid Handling Implementation

Reagents

- 47.5–50% ethanol (**Note:** Prepare by diluting 95–100% USP/ACS- or molecular biology-grade ethanol with an equal volume of molecular biology grade water. Using denatured ethanol that contains methanol or isopropanol may decrease DNA purity or yield.)

Development Supplies and Equipment Options

This protocol requires the use of a liquid handler with integrated shaking, heating and SLAS plate-moving capabilities. Please contact Promega to discuss hardware configuration options. The following hardware and supplies were used during development:

- integrated shaker (Tecan–Te Shake with 3mm orbit or QInstruments BioShake 3000 elm DWP with 2mm orbit)
- heat block (Torrey Pines EchoTherm™ RIC20)
- Deep Well Heat Transfer Block (Cat.# V6741; for use with 1.2ml Round Bottom Deep-Well Plates)
- Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
- 3/16 inch Spacer (Cat.# V8381)
- 3 × 2.2ml square deep-well, 96-well plate (Cat.# V6781)
- 1 × 1.2ml round-bottom deep-well, 96-well plate (sample processing plate; Cat.# V6771)

Automation of this chemistry with other integrated heater shaker units or independent heaters and shakers may be possible but will require performance testing during method development.

Other Supplies and Equipment Options

- integrated heater shaker (QInstruments—BioShake D30-T elm 3mm Cat.# 50047-101 or Hamilton Heater Shaker (HHS) 3mm Cat.# 199034)
- 1 × 2.2ml deep-well, 96-well plate (for sample waste; Cat.# V6781)
- 1 × 2.0ml deep-well, 96-well plate (sample processing plate; Cat.# AS9307 or AS9309 [Non-Sterile])
- Heat Block Adapter (QInstruments Cat.# 2016-1151 or Hamilton Cat.# 199028) that fits Cat.# AS9307, AS9309 or Nunc Cat.# 278743
- MagnaBot® 96 FLEX Magnetic Separation Device (Cat.# VA1290)
- elution plate (customer-specified)

4. Description of the Manual Maxwell® HT 96 gDNA Blood Isolation System Protocols

The following manual protocols describe the purification of genomic DNA from whole blood or Oragene® saliva samples. These manual steps will follow liquid handler adoption. If interested in evaluating in a 96-well format, please contact Promega. Contact information available at: **www.promega.com**. E-mail: **HTGenomics@promega.com**. Find additional High Throughput product information at:
www.promega.com/products/dna-purification-quantitation/high-throughput-dna-purification/

Materials to Be Supplied by the User for 1.5ml Manual Evaluation

- Eppendorf ThermoMixer® C or F (device used for heating and shaking samples)
- 1.5ml microtubes
- 47.5–50% ethanol
  (**Note:** Prepare by diluting 95–100% USP/ACS- or molecular biology-grade ethanol with an equal volume of molecular biology-grade water. Using denatured ethanol that contains methanol or isopropanol may decrease DNA purity or yield.)
- 1.5ml magnetic separation device (e.g., MagneSphere® Technology Magnetic Separation Stand [two-position]; Cat.# Z5332)

Notes:

1. The heat block temperature must be set to 75–85°C before starting the method and should remain at that temperature for the duration of the method. Using a heat block temperature other than 75–85°C will result in suboptimal performance of the chemistry.
2. Since different heat blocks work at different ramp temperatures, make sure the temperature is set to a point that the internal lysate is at 56–65°C. Setting the heat block to a higher temperature may be necessary for proper heat transfer and is heater unit-dependent. A water bath set to 75°C will work as well.

4.A. Manual Purification of Genomic DNA from Anticoagulated Whole Blood

The following manual protocol describes the purification of genomic DNA from whole blood for evaluation purposes. If interested in evaluating in a 96-well format, please contact Promega. Contact information available at: **www.promega.com**. E-mail: **HTGenomics@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. **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.
3. **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.

4.A. Manual Purification of Genomic DNA from Anticoagulated Whole Blood (continued)

4. **Lysis Incubation.** Place the 1.5ml processing tube in a dry or wet heat block and incubate for 10 minutes on heat block at 75–85°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 on heat block at 75–85°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 the Resin. When the lysate is cleared, remove the cleared lysate to the waste.

   Note: 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 75–85°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 on a heat block at 75–85°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 on a heat block at 75–85°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. Process the purified gDNA samples in the elution tube immediately or store at 4°C. For prolonged stability, add 1/10th eluate volume of the 10mM EDTA (pH 8.0; 5–11µl) 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).

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4.B. Manual Purification of Genomic DNA from Saliva Samples in Oragene®•Discover Devices

The following manual protocol describes the purification of genomic DNA from Oragene® saliva samples for evaluation purposes. If interested in evaluating in a 96-well format, please contact Promega. Contact information available at: **www.promega.com** . E-mail: **HTGenomics@promega.com**.

Note: Collection procedures recommend no smoking or gum chewing 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 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 the Resin. When the lysate is cleared, remove the cleared lysate to the waste.

   Note: 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 75–85°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 75–85°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 75–85°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. Process the purified gDNA samples in the elution tube immediately or store at 4°C. For prolonged stability, add 1/10th eluate volume of the 10mM EDTA (pH 8.0; 5–11µl) 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).

5. Description of the Automated Maxwell® HT 96 gDNA Blood Isolation System Protocols

The following protocols describe the automated purification of genomic DNA from whole blood or Oragene® saliva samples.

Notes:

- When adding Proteinase K, Lysis Buffer, Binding Buffer and Resin, the reagents are predispensed from the trough into a 96-well plate with the 8-channel LiHa arm. Then the MCA head transfers the reagents to the sample plate to ensure all samples are getting the same amount of time in all reagents. These steps are denoted with an asterisk (*) in the method steps below. Washes and elution reagents are dispensed directly into the sample plate.
- All tip mixing during the method is performed using a 96-well pipetting head with 200µl tips. A "high" step is about 2mm below the top of the liquid level, a "medium" step is in the middle of the liquid volume, and a "low" step is about 3.5mm above the bottom of the well.
- Shaking speeds should be optimized based on the shaking hardware available. Shaking should be sufficient to mix the liquid within the confines of the well, and when magnetic Resin is present, should be sufficient to keep the resin off the bottom of the plate. Starting points for shaking speeds are listed below:

  3mm Orbit Shaker Speeds

  - Proteinase K and Lysis steps: 1,300rpm
  - Binding step: 1,000rpm
  - Washing and Elution steps: 1,300rpm

  2mm Orbit Shaker Speeds

  - Proteinase K and Lysis steps: 2,000rpm
  - Shake during Lysis Incubation: 1,700rpm
  - Binding step: 1,300rpm
  - Wash 1 and 2 steps: 1,800rpm
  - Wash 3 step: 1,600rpm
  - Elution step: 1,800rpm

5.A. Automated Genomic DNA Purification from Anticoagulated Whole Blood

1. **Sample Preparation.** The following protocol describes the chemistry for purifying gDNA from 350µl of input sample. The sample must be dispensed into a 2.2ml deep-well plate. Volumes of anticoagulated whole blood from 100–350µl can be processed with this chemistry.

   Note: The heat block temperature must be set to 85°C before starting the method and should remain at that temperature for the duration of the method.

2. **Proteinase K Addition.** The liquid-handling robot adds 35µl of Proteinase K to each sample in the sample plate*. The sample plate is shaken for 15 seconds after addition.

3. **Lysis Buffer Addition.** The liquid-handling robot adds 350µl of Cell Lysis Buffer to each sample in the sample plate*. The lysis buffer is dispensed in 120µl increments with a 10-second shake after every addition. The sample is then tip mixed to fully incorporate the Proteinase K, lysis buffer and sample. The tip mixing routine for a homogenous mixture consists of the following 100µl mixing height sequence: High aspirate, low dispense, high aspirate, medium dispense, medium aspirate, low dispense, medium aspirate, high dispense, low aspirate, high dispense, high aspirate, low dispense. The samples are shaken for 15 seconds and then the homogenous lysis mixture is transferred to a 1.2ml plate for lysis incubation.

   Note: Lysis buffer is denser than the blood and requires fairly extensive mixing to make sure the blood and lysis buffer form a homogeneous mixture. This mixing routine is critical for good lysis.

4. **Lysis Incubation.** The samples are incubated and mixed as follows: 10-minute heated incubation, 30 seconds shaking with 10 rounds of 75µl tip mix (at liquid level + 4mm) off of the heater and another 10-minute heated incubation.

5. **Binding Buffer Addition.** The lysate is transferred into a 2.2ml binding plate and the liquid-handling robot adds 385µl of Binding Buffer to each sample in the sample plate*. The Binding Buffer is dispensed in 100µl increments with a 15-second shake between every addition.

   Note: If RNase treatment is desired, prepare a 0.1mg/ml solution of RNase A (Cat.# A7974) in Binding Buffer when preparing and dispensing reagents. Add RNase A to the reservoir before adding Binding Buffer. Mix the RNase A and Binding Buffer by pipetting using a serological pipette.

6. **Resin Addition.** The liquid-handling robot then adds 70µl of Resin mixed 1:1 with Binding Buffer to each sample in the sample plate*. After adding Resin, 80µl of sample is transferred back to the predispensed Resin plate, 50µl is tip mixed for 5 cycles, then the whole volume is transferred back to the sample plate to remove the Resin from the predispensed plate. Then Resin is removed from the tips with 10 cycles of 50µl tip mix in the binding plate.

   Note: The 1:1 Resin and Binding Buffer mixture is extensively tip mixed in the trough to create a homogeneous mixture for dispensing.

7. **DNA Binding.** The sample plate is incubated for 20 minutes with shaking. At the beginning and end of binding and two times during the binding, the shaking stops and the sample is tip mixed. The tip mixing routine to create a homogenous mixture consists of the following 100µl mixing height sequence: Aspirate high, dispense low, aspirate low, dispense high, aspirate high, dispense medium, aspirate medium, dispense low.

   Note: Binding Buffer is denser than the blood lysate and the Resin is less dense than the blood lysate. Because of this, the reagents and blood lysate will form layers that requires fairly extensive mixing to make sure the lysate, Binding Buffer and Resin form a homogeneous mixture. This mixing routine is critical for good binding.

8. **Cleared Lysate Removal.** Half of the sample volume from the bind plate is moved into a 1.2ml plate and transferred to the magnetic separation device and magnetized for 50 seconds to capture the Resin. The liquid-handling robot removes 125µl increments of the cleared lysate to the lysate waste plate. This process is repeated for the second half of the sample volume.

5.A. Automated Genomic DNA Purification from Anticoagulated Whole Blood (continued)

9. **Wash Buffer Addition #1.** The liquid-handling robot adds 50µl of 47.5–50% ethanol to each sample in the sample plate. The sample plate is shaken for 40 seconds. The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 30 seconds. The wash is tip mixed using 120µl for 20 cycles (at 2.5mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 20 seconds. The wash is tip mixed using 120µl for 20 cycles (at 3mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 10 seconds. The tip mixing routine to break up the resin consists of the following 100µl mixing height sequence: Aspirate low, dispense high, aspirate high, dispense low, aspirate high, dispense medium, aspirate medium, dispense low, aspirate low, dispense high, aspirate low, dispense high, aspirate high, dispense low.

10. **Wash Buffer Removal #1.** The sample plate is moved onto the magnetic separation device to capture the Resin for 25 seconds. The liquid-handling robot removes 125µl increments of the Wash Buffer waste to the wash waste plate.

11. **Wash Buffer Addition #2.** The liquid-handling robot adds 50µl of 47.5–50% ethanol to each sample in the sample plate. The sample plate is shaken for 30 seconds. The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 20 seconds. The wash is tip mixed using 120µl for 20 cycles (at 3mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The liquid-handling robot adds the last 125µl of wash buffer to each sample in the sample plate. The sample plate is shaken for 10 seconds. The tip mixing routine to break up the resin consists of the following 100µl mixing height sequence: Aspirate low, dispense high, aspirate high, dispense low, aspirate high, dispense medium, aspirate medium, dispense low, aspirate low, dispense high, aspirate low, dispense high, aspirate high, dispense low.

12. **Wash Buffer Removal #2.** The sample plate is moved onto the magnetic separation device for 25 seconds to capture the Resin. The liquid-handling robot removes 125µl increments of the Wash Buffer waste to the wash waste plate.

13. **Ethanol Wash Addition.** The liquid-handling robot adds 225µl of ethanol to each sample in the sample plate. The sample plate is shaken for 15 seconds. The liquid-handling robot adds 225µl of ethanol to each sample in the sample plate. The sample is shaken for 30 seconds.

14. **Ethanol Wash Removal.** The sample plate is moved onto the magnetic separation device to capture the Resin for 25 seconds. The liquid-handling robot removes 125µl increments of the ethanol wash waste to the wash waste plate. After the last removal of waste, the system pauses for 10 seconds with the plate still on the magnet and a final 20µl of ethanol waste is removed from the plate to the wash waste plate.

15. **Heat Block Drying.** The sample plate is moved onto the heat block for 90 seconds.

16. **Tris Buffer Addition.** The liquid-handling robot adds 110µl of 25mM Tris-HCl (pH 8.0) to each sample in the sample plate. The samples undergo a series of shaking and incubations (30-second shaking, 3-minute incubation, 30-second shaking, 3-minute incubation and 30-second shaking) to elute DNA from the Resin into the Tris Buffer.

17. **Elution.** The Sample Plate is moved onto the magnetic separation device, Resin is captured for 2 minutes, and the supernatant is removed to an intermediate elution plate. The intermediate plate is moved onto the magnetic separation device, Resin is captured for 5 minutes, and the supernatant is removed to the final elution plate.

18. **Method Ends.** The automated Maxwell® HT 96 gDNA Blood Isolation System method is now complete. The purified DNA samples in the elution plate may be processed immediately or stored at 4°C. For prolonged stability, add 10µl of 10mM EDTA (pH 8.0) included with the Maxwell® HT 96 gDNA Blood Isolation System. The EDTA should only be added after completing absorbance readings.

5.B. Automated Genomic DNA Purification from Saliva Samples in Oragene®•Discover Devices

1. **Sample Preparation.** The following protocol describes the chemistry for purifying gDNA from 350µl or 700µl of input sample.

   Note: The heat block temperature must be set to 85°C before starting the method and should remain at that temperature for the duration of the method.

2. **Binding Buffer Addition.** The sample is transferred into a 2.2ml binding plate and the liquid-handling robot adds 385µl of Binding Buffer to each sample in the sample plate*. The Binding Buffer is dispensed in 100µl increments with a 15 second shake between every addition.

   Note: If RNase treatment is desired, prepare a 0.1mg/ml solution of RNase A (Cat.# A7974) in Binding Buffer when preparing and dispensing reagents. Add RNase A to the reservoir before adding Binding Buffer. Mix the RNase A and Binding Buffer by pipetting using a serological pipette.

3. **Resin Addition.** The liquid-handling robot then adds 70µl of Resin mixed 1:1 with Binding Buffer to each sample in the sample plate*. After adding Resin, 80µl of sample is transferred back to the predispensed Resin plate, 50µl is tip mixed for 5 cycles, then the whole volume is transferred back to the sample plate to remove the Resin from the predispensed plate. Then Resin is removed from the tips with 10 cycles of 50µl tip mix in the binding plate.

   Note: The 1:1 Resin and Binding Buffer mixture is extensively tip mixed in the trough to create a homogeneous mixture for dispensing.

4. **DNA Binding.** The sample plate is incubated for 20 minutes with shaking. At the beginning and end of binding and two times during the binding, the shaking stops and the sample is tip mixed. The tip mixing routine to create a homogenous mixture consists of the following 100µl mixing height sequence: Aspirate high, dispense low, aspirate low, dispense high, aspirate high, dispense medium, aspirate medium, dispense low.

   Note: Binding Buffer is denser than the saliva sample and the Resin is less dense than the saliva sample . Because of this, the reagents and saliva sample will form layers that requires fairly extensive mixing to make sure the saliva sample, Binding Buffer and Resin form a homogeneous mixture. This mixing routine is critical for good binding.

5. **Cleared Lysate Removal.** Half of the sample volume from the bind plate is moved into a 1.2ml plate, transferred to the magnetic separation device and magnetized for 50 seconds to capture the Resin. The liquid-handling robot removes 125µl increments of the cleared lysate to the lysate waste plate. This process is repeated for the second half of the sample volume.

5.B. Automated Genomic DNA Purification from Saliva Samples in Oragene®•Discover Devices (continued)

6. **Wash Buffer Addition #1.** The liquid-handling robot adds 50µl of 47.5–50% ethanol to each sample in the sample plate. The sample plate is shaken for 40 seconds. The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 30 seconds. The wash is tip mixed using 120µl for 20 cycles (at 2.5mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 20 seconds. The wash is tip mixed using 120µl for 20 cycles (at 3mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 10 seconds. The tip mixing routine to break up the resin consists of the following 100µl mixing height sequence: Aspirate low, dispense high, aspirate high, dispense low, aspirate high, dispense medium, aspirate medium, dispense low, aspirate low, dispense high, aspirate low, dispense high, aspirate high, dispense low.
7. **Wash Buffer Removal #1.** The sample plate is moved onto the magnetic separation device to capture the Resin for 25 seconds. The liquid-handling robot removes 125µl increments of the Wash Buffer waste to the wash waste plate.
8. **Wash Buffer Addition #2.** The liquid-handling robot adds 50µl of 47.5–50% ethanol to each sample in the sample plate. The sample plate is shaken for 30 seconds. The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The sample plate is shaken for 20 seconds. The wash is tip mixed for 20 cycles at 120 µl volume(at 3mm above the bottom of the well). The liquid-handling robot adds 125µl of Wash Buffer to each sample in the sample plate. The liquid-handling robot adds the last 125µl of wash buffer to each sample in the sample plate. The sample plate is shaken for 10 seconds. The tip mixing routine to break up the resin consists of the following 100µl mixing height sequence: Aspirate low, dispense high, aspirate high, dispense low, aspirate high, dispense medium, aspirate medium, dispense low, aspirate low, dispense high, aspirate low, dispense high, aspirate high, dispense low.
9. **Wash Buffer Removal #2.** The sample plate is moved onto the magnetic separation device for 25 seconds to capture the Resin. The liquid-handling robot removes 125µl increments of the Wash Buffer waste to the wash waste plate.
10. **Ethanol Wash Addition.** The liquid-handling robot adds 225µl of ethanol to each sample in the sample plate. The sample plate is shaken for 15 seconds. The liquid-handling robot adds 225µl of ethanol to each sample in the sample plate. The sample is shaken for 30 seconds.
11. **Ethanol Wash Removal.** The sample plate is moved onto the magnetic separation device to capture the Resin for 25 seconds. The liquid-handling robot removes 125µl increments of the ethanol wash waste to the wash waste plate. After the last removal of waste, the system pauses for 10 seconds with the plate still on the magnet and a final 20µl of ethanol waste is removed from the plate to the wash waste plate.
12. **Heat Block Drying.** The sample plate is moved onto the heat block for 90 seconds.
13. **Tris Buffer Addition.** The liquid-handling robot adds 110µl of 25mM Tris-HCl (pH 8.0) to each sample in the sample plate. The samples undergo a series of shaking and incubations (30-second shaking, 3-minute incubation, 30-second shaking, 3-minute incubation and 30-second shaking) to elute DNA from the Resin into the Tris Buffer.

14. **Elution.** The Sample Plate is moved onto the magnetic separation device, Resin is captured for 2 minutes, and the supernatant is removed to an intermediate elution plate. The intermediate plate is moved onto the magnetic separation device, Resin is captured for 5 minutes, and the supernatant is removed to the final elution plate.

15. **Method Ends.** The automated Maxwell® HT 96 gDNA Blood Isolation System method is now complete. The purified DNA samples in the elution plate may be processed immediately or stored at 4°C. For prolonged stability add 10µl of 10mM EDTA (pH 8.0) included with the Maxwell® HT 96 gDNA Blood Isolation System. The EDTA should only be added after completion of absorbance readings.

6. Quantitation and Analysis of Isolated Genomic DNA

Concentration and purity of eluted DNA can be analyzed directly using a spectrophotometer. The molecular weight of eluted DNA can be analyzed by agarose gel electrophoresis.

A_{260}/A_{280} ratio can be used to determine DNA purity (with a number of important limitations [1–3]). An A_{260}/A_{280} ratio between 1.7 and 2.0 is generally accepted as representative of a high-quality DNA sample. The ratio can be calculated after subtracting the non-nucleic acid absorbance at A_{320}.

$$\text{DNA purity } (A_{260}/A_{280}) = \frac{(A_{260} \text{ reading} - A_{320} \text{ reading})}{(A_{280} \text{ reading} - A_{320} \text{ reading})}$$

Note: Many spectrophotometers automatically subtract the absorbance at a reference value around 340nm from the absorbance at 230nm, 260nm and 280nm before reporting these values and ratios. Consult your spectrophotometer user manual to determine whether this calculation is performed.

Other techniques such as gel analysis also may be valuable when assessing the relative quality of isolated genomic DNA. We consistently observe relatively poor agreement between concentration values obtained from the same sample using different quantitation methodologies. This has been observed using a variety of commercially available purification systems, and therefore, we do not recommend comparing yields obtained using different quantitation methods. In our experience, spectrophotometric analysis coupled with functional testing in downstream applications is typically the most representative assessment of sample quantity and quality.

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7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Possible Causes and Comments
Low DNA yield	Insufficient mixing of sample during processing. Vigorous mixing during lysis, binding and elution is essential to ensure efficient capture of DNA from the sample. Check both tip mixing and orbital shaking or vortexing to verify that they are effective.
	Insufficient resuspension of Resin before use. Thoroughly mix the Resin reagent bottle, including inverting the bottle to confirm that no Resin adheres to the bottle.
	Sample contained too few white cells per milliliter of blood. DNA yield depends on the amount of starting material. Blood samples with low white cell counts will have reduced yields because of the low sample input.
	Insufficient elution volume. If the volume of 25mM Tris-HCl (pH 8.0) is reduced to try to improve eluate concentration, it may result in lower yield.
	For automated protocol, ensure reagents are in the correct deck locations. Check to ensure that all protocol steps were followed correctly and that the correct reagents were used at each step. This ensures optimal purification of genomic DNA.
Eluted DNA looks brown or red	Incomplete lysis of blood cells. Failure to add sufficient volume of Proteinase K or Cell Lysis Buffer will result in incomplete lysis and digestion of cells and proteins.
Degraded DNA	Nucleases were introduced during purification. Use nuclease-free plasticware or glassware. Use filter tips during all pipetting steps. Wear gloves at all times. Nucleases introduced after elution will degrade DNA. Add EDTA to a final concentration of 0.1–1mM to protect eluted samples from nucleases.
	Old samples or samples that have been handled or stored improperly may contain DNA that is already degraded.
	DNA was eluted with water rather than 25mM Tris buffer.
Resin in final eluate	Concentrated DNA solutions can be viscous. Additional time may be required to capture Resin from such viscous solutions. The eluted DNA should be collected slowly and any residual Resin removed from the eluted DNA by performing additional magnetic captures or a centrifugation.

8. References

1. Wilfinger, W.W., Mackey, M. and Chanczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques* **22**, 474–81.
2. Glasel, J.A. (1995) Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *BioTechniques* **18**, 62–3.
3. Manchester, K.L. (1995) Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. *BioTechniques* **19**, 208–10.

9. Related Products

Buffers Available Separately

Product	Size	Cat.#
Wash Buffer (WBA)	500ml	A1761
10 mM EDTA, pH 8.0	10ml	A2631
ReliaPrep™ Resin	5.5ml	A1753
Binding Buffer (BBA)	200ml	A1742
Cell Lysis Buffer (CLD)	160ml	A1761
Proteinase K (PK) Solution	4ml	MC5005
	16ml	MC5008
	23ml	A5051
RNase A Solution	1ml	A7973
	5ml	A7974

High-Throughput Genomic DNA Isolation

Product	Size	Cat.#
ReliaPrep™ Large Volume HT gDNA Isolation System	1 each	A2751
HSM 2.0 Instrument	1 each	A2715

Low-Throughput Nucleic Acid Isolation

Product	Size	Cat.#
ReliaPrep™ gDNA Tissue Miniprep System	250 preps	A2052
ReliaPrep™ Blood gDNA Miniprep System	250 preps	A5082
ReliaPrep™ FFPE Total RNA Miniprep System	100 reactions	Z1002
ReliaPrep™ FFPE gDNA Miniprep System	100 reactions	A2352

Additional sizes available.

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10. Summary of Changes

The following changes were made to the 4/21 revision of this document:

1. Added Cat.# AS9309 to Section 3.
2. Updated the cover page.