Technical Bulletin

MagneSil® Genomic, Large Volume System

INSTRUCTIONS FOR USE OF PRODUCTS A4080, A4082 AND A4085.

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MagneSil® Genomic, Large Volume System

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

1. Description

The MagneSil® Genomic, Large Volume System[a] is designed for scalable, automated genomic DNA isolation from large-volume samples, eliminating laborious centrifugation steps and the use of hazardous organic solvents. The System has been automated on the Tecan Freedom EVO® liquid-handler, providing walkaway purification of genomic DNA from a variety of starting materials, including 1–10ml whole blood samples, regardless of sample storage or shipping conditions. The system uses only the amount of reagents required to process each sample, maximizing efficiency and value per prep.

The MagneSil® Genomic, Large Volume System uses a robust non-centrifugation-based automated method to purify genomic DNA from fresh, frozen or mishandled blood and other samples with similar yields and quality.

The system bypasses many of the challenges of traditional centrifugation-based methods by lysing the entire whole blood sample and then directly capturing total genomic DNA from the lysed sample using MagneSil® paramagnetic particles (PMPs). The genomic DNA bound to the MagneSil® PMPs is washed to remove contaminants such as heme and cellular proteins and then eluted into an aqueous solution ready for use in downstream applications (Figure 1). There is no need for tedious and lengthy DNA rehydration. The purified genomic DNA is suitable for a variety of downstream applications such as single and multiplex PCR, restriction digestion and real-time PCR.

Hardware Requirements

The hardware listed below is required for use of the MagneSil® Genomic, Large Volume System. All of the products listed below are provided with purchase of specifically configured Tecan Freedom EVO® liquid-handling systems (Freedom EVO®/gDNA XL). If you are performing the manual procedure, these products must be purchased separately.

For system evaluation, an e-protocol—a paperless, self-guided protocol that prompts you through each step of the manual purification method and automatically sets and drives all the shaker steps—is available upon request from Promega Technical Services (Email: **techserv@promega.com**).
The e-protocol requires a PC with a Windows® 2000 or XP operating system and a cable to connect the IKA KS130 Control Shaker to the computer (PC 2.1 cable, IKA Works Cat.# 2700700).

Product	Vendor	Quantity*	Cat.#
MagnaBot® Large Volume Magnetic Separation Device	Promega	1	V3471
Tube Holder, 50ml Tubes (required if processing >8 samples per run)	Promega	4	Z3631
Heat Block Insert	Promega	1	Z3651
Heat Block Adapter, 50ml Tubes	Promega	1	Z3661
Shaker Top Adapter	Promega	1	Z3671
Heat Block (set to 90–95°C)	Fisher VWR		11-718-2, 11-715-125D 13259-032, 13259-052
IKA KS130 Control Orbital Shaker	IKA Works		2980100
50ml Conical Tubes	Corning Fisher		430291 05-538-55A

*Quantities vary depending on the number of samples processed. Quantities given are for low-throughput systems (24 samples). Please inquire for quantities required for mid- and high-throughput systems (48–96 samples).

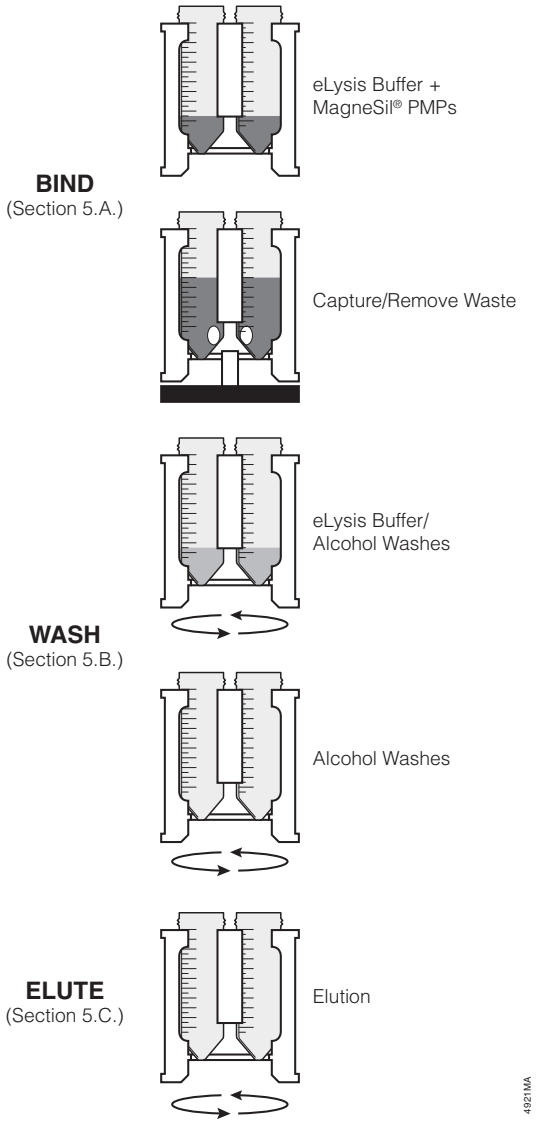


Figure 1. MagneSil® Genomic, Large Volume System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneSil® Genomic, Large Volume System	8 isolations	A4080

Each system contains sufficient reagents for 8 purifications of up to 10ml each. Includes:

- 200ml eLysis Buffer
- 2 × 19ml MagneSil® Paramagnetic Particles (PMPs)
- 200ml Alcohol Wash (concentrated)
- 45ml Elution Buffer

Product	Size	Cat.#
MagneSil® Genomic, Large Volume System	48 isolations	A4082

Each system contains sufficient reagents for 48 purifications of up to 10ml each. Includes:

- 1L eLysis Buffer
- 2 × 94ml MagneSil® Paramagnetic Particles (PMPs)
- 2 × 500ml Alcohol Wash (concentrated)
- 300ml Elution Buffer

Product	Size	Cat.#
MagneSil® Genomic, Large Volume System	96 isolations	A4085

Each system contains sufficient reagents for 96 purifications of up to 10ml each. Includes:

- 2 × 1L eLysis Buffer
- 4 × 94ml MagneSil® Paramagnetic Particles (PMPs)
- 4 × 500ml Alcohol Wash
- 2 × 300ml Elution Buffer

Storage Conditions: Store the MagneSil® Genomic, Large Volume System at room temperature (22–25°C). Do not refrigerate or freeze the MagneSil® Paramagnetic Particles.

3. General Considerations

3.A. Comparison to Centrifugation-Based Methods

The MagneSil® Genomic, Large Volume System is designed to address the challenges of traditional genomic DNA purification methods that rely on centrifugation-based organic or differential extraction chemistries. Table 1 outlines how the MagneSil® Genomic DNA purification strategy overcomes the challenges of typical centrifugation-based methods. The MagneSil® Genomic, Large Volume System technology also efficiently purifies genomic DNA regardless of how the blood samples have been stored or handled (Figure 2).

Table 1. Comparison of MagneSil® Genomic, Large Volume System and Centrifugation-Based Genomic DNA Purification Methods.

Centrifugation-Based Method Steps	Centrifugation-Based Method Challenges	MagneSil® Genomic DNA Purification Solution
Differential separation, centrifugation of intact white blood cells.	Shipment and storage of whole blood samples may result in white blood cell lysis.	Binding from directly lysed whole blood allows genomic DNA purification regardless of sample storage/shipment conditions. Special sample collection and storage tubes (e.g., PAXgene® tubes) are not required.
Complete resuspension of cell pellet is required for good lysis.	Loss of white blood cells during decanting and cell resuspension.	Genomic DNA is directly captured from the whole blood sample. No centrifugation required.
Genomic DNA precipitation by centrifugation.	Loss of DNA pellet during multistep process for DNA precipitation and washes.	No DNA precipitation or centrifugation required. DNA is bound to easily visible MagneSil® PMPs.
Resuspension of genomic DNA pellet in aqueous solution.	Not automatable. Lengthy incubation time required for rehydration of precipitated, dried DNA pellet.	Genomic DNA is eluted directly from MagneSil® PMPs into an aqueous solution.

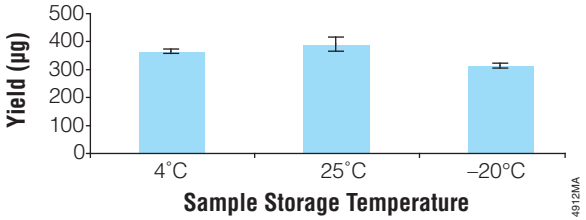


Figure 2. Effect of sample storage temperature on DNA yield. Using the MagneSil® Genomic, Large Volume System, genomic DNA was purified from 10ml whole blood (white cell count: 9×10^6 cells/ml) that had been stored at 4°C, at room temperature (25°C), or frozen (–20°C) for seven days. Yield was determined by absorbance measurement (A_{260}). Average yield from three purifications per storage condition is presented.

3.B. Sample Processing Capacity

Total yield of genomic DNA depends on 1) volume of whole blood sample processed and 2) number of white blood cells/ml of whole blood. The MagneSil® Genomic, Large Volume System is designed for purification of genomic DNA from up to 10ml of whole blood in one purification procedure. This input volume limitation assumes that the average number of white blood cells/ml whole blood sample from a normal healthy adult will range between 4.5×10^6 and 1.1×10^7 (1). Therefore, the cell number input limitation of the MagneSil® Genomic, Large Volume System is approximately 1×10^8 leukocytes in a sample volume. Exceeding the recommended volume or cell number may result in MagneSil® PMPs clumping and may adversely affect yield and quality of the purified genomic DNA.

3.C. Elution

The MagneSil® Genomic, Large Volume System protocol is designed for optimal yield of purified genomic DNA. However, the recommended elution volumes may be adapted to accommodate downstream applications that have specific DNA concentration requirements. Decreasing the elution volume will increase the concentration of the purified DNA but will also result in lower total yield. Figure 3 shows the effect of varying elution volumes on DNA concentration and yield.

For optimal elution of genomic DNA from the MagneSil® PMPs, room temperature Elution Buffer is added to the MagneSil® PMPs, and after vigorous vortexing the sample is placed on a heat block set at 90–95°C. Use of preheated Elution Buffer will significantly decrease yield and concentration. Additionally, failure to heat after addition of the room temperature Elution Buffer to the MagneSil® PMPs will significantly decrease yield and concentration. For optimal elution conditions, the MagneSil® PMPs must be heated gradually and vortexed vigorously during mix steps to efficiently release the genomic DNA.

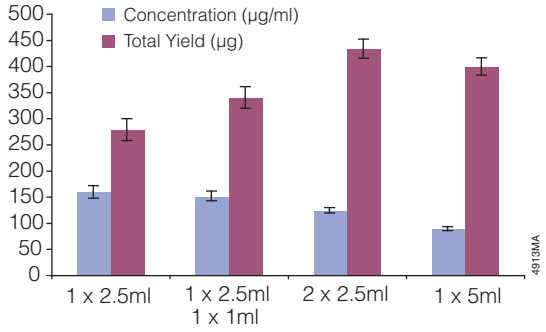


Figure 3. Effect of elution volume on genomic DNA yield and concentration. MagneSil® Genomic, Large Volume System was used to purify genomic DNA from 10ml whole blood. DNA was eluted in various volumes as shown. DNA concentration and yield were determined by absorbance measurement (A_{260}).

3.D. Manual vs. Automated Processing

Manual Processing

An e-protocol for manual processing is available upon request from Promega Technical Services (Email: **techserv@promega.com**)
The e-protocol is a paperless, self-guided protocol that prompts you through each step of the purification and automatically controls all the shaker steps. The e-protocol is an adaptation of the automated method for simple evaluation of the protocol performed by the Tecan Freedom EVO® instrument. By inputting the volume for each blood tube, the e-protocol provides the scalability function of the automated method using identical hardware. When using the automated method, the volume in each tube is automatically detected, and the chemistry is scaled accordingly on a per-tube basis. The manual protocol gives equivalent performance to the automated method but does not require the higher throughput instrumentation. The manual protocol is designed for users performing multiple small-batch-size purifications, typically during system evaluation.

e-Protocol Download and Installation Instructions

1. Request the e-protocol from: **techserv@promega.com**
2. Install the MagneSil® Genomic, Large Volume System e-protocol as follows:
   a. Use Windows® Explorer to browse to the location where you saved the e-protocol.
   b. Double-click on **MagGenomicLVdwnld.exe** to unzip the contents of the downloaded file. Save these files.
   c. Use Windows® Explorer to browse to the location where you saved the unzipped files.
   d. Double-click on the **MagGenomicLVsetup.exe file**.
   e. An installation window will appear. Follow the directions provided to install the manual e-protocol on your computer.
   f. Upon successful installation, the manual e-protocol program should be located at: **C:\Program Files\MagneSil Genomic LV**
   g. Double-click on the **MagneSil Genomic LV eprotocol.exe** file to start the e-protocol.

Automated Processing

Automated processing is available using the Tecan Freedom EVO® liquid-handling robot. We have worked with Tecan to provide fully integrated chemistry and instrumentation solutions for a variety of throughput requirements. Three instrument configurations have been designed to meet various throughput needs while maintaining broad functionality using a single validated method. Each system comes with all the hardware required and a validated automated method. Automated processing provides scalability to easily adapt to daily needs; the instrument senses the volume of sample in each tube and scales the reagent volumes per tube appropriately without user intervention. The method will process variable sample volumes within a single run as well as variable batch sizes between runs. Genomic DNA can be purified directly from common sample collection tubes such as VACUTAINER® tubes.

Product	Instrument	Part#
Low-throughput system (up to 24 samples/day)	Tecan Freedom EVO® 150 LT	Tecan 10760420
Mid-throughput system (up to 48 samples/day)	Tecan Freedom EVO® 150 MT	Please Contact Tecan
High-throughput system (up to 96 samples/day)	Tecan Freedom EVO® 200 HT	Please Contact Tecan

4. Before You Begin

Materials to Be Supplied by the User

- ethanol (95%)
- isopropanol (99%)
- 50ml tubes (1 per purification; Corning 50ml orange-cap tubes recommended)
- MagnaBot® Large Volume Magnetic Separation Device (Cat.# V3471)
- Tube Holder, 50ml tubes (Cat.# Z3631; required if processing more than 8 samples/run)
- Heat Block Insert (Cat.# Z3651)
- Heat Block Adapter, 50ml Tubes (Cat.# Z3661)
- Shaker Top Adapter (Cat.# Z3671)
- Heat Block set to 90–95°C (Fisher Cat.# 11-718-2 or 11-715-125D, VWR Cat.# 13259-032 or 13259-052)
- IKA KS130 Control Orbital Shaker (IKA Works Cat.# 2980100)

Equipment Requirements for e-Protocol:

- PC for remote control of IKA orbital shaker
  Minimum Windows® 2000 or XP operating system
- PC 2.1 cable to connect PC to the IKA orbital shaker (IKA Works Cat.# 2700700)
- If connecting the IKA orbital shaker to computer through a USB port, you will require a USB/serial adapter.
- A common nonslip mat. Place the IKA orbital shaker on a nonslip surface to keep the shaker in place during all shaking steps.

4.A. Preparation of Alcohol Wash Solution

Add the indicated volume (see bottle label) of ethanol and isopropanol to the bottle of Alcohol Wash. Mark the bottle label to record that these additions have been made. Failure to add both ethanol and isopropanol to the Alcohol Wash Solution will result in poor system performance.

4.B. Preparation of Computer and Shaker for Manual e-Protocol

1. Turn on your computer.

Do not connect the shaker to the computer or turn on the shaker until directed. Steps must be performed in a specific order to ensure successful communication between the computer and the shaker.

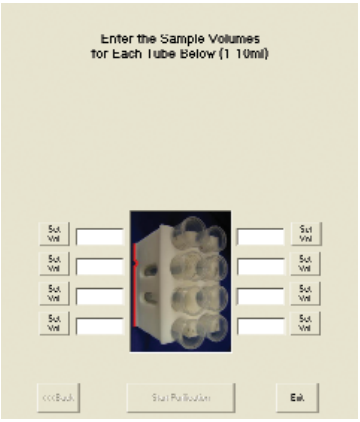
2. Ensure that the **MagneSil Genomic LV eprotocol.exe** program has been installed.

   Note: If you are using a USB/serial converter, make sure that the USB-to-serial conversion cable driver has been installed. This driver should be provided with your USB/serial converter.

3. Plug the serial connection side of the cable into the shaker.

4. Connect the cable from the shaker to the computer either through a serial port or into a USB port using a USB/serial converter.
5. Turn on the shaker.
6. Wait approximately one minute for the shaker to complete its self-test.
7. Open the **MagneSil Genomic LV eprotocol.exe** program.
8. The program will then proceed to the first screen of the manual e-protocol (Figure 4).
9. Proceed with genomic DNA purification following the program instructions. The computer will drive all shaker settings.

Figure 4. First screen of manual e-protocol.



4.C. Troubleshooting e-Protocol Shaker Setup

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	Comments
Error: Shaker not detected	Verify that the shaker is connected to the computer and that it is plugged in and turned on. To ensure successful communication between the computer and the shaker, they must be turned on in the order recommended.
	Ensure that the USB to Serial converter driver is installed.
	Shaker may have been turned on before the computer. Be sure to turn on the computer first.
	Shaker may not have finished its self-test before the purification method was started. Wait at least one minute after switching on the shaker before starting the purification method.

5. Genomic DNA Purification Procedure

This overview describes the automated liquid-handling and purification steps required for genomic DNA isolation using the MagneSil® Genomic, Large Volume System. This protocol can be performed either manually using the e-protocol or on an automated liquid-handling workstation. Shaker speeds and times will be set automatically by the automated method or the e-protocol.

The protocol below describes use of an IKA Works KS 130 Control Shaker. This is the only shaker currently validated for use with the MagneSil® Genomic, Large Volume System. For optimal mixing performance, the shaker must have a 4mm orbit. Use of a shaker other than the IKA Works KS 130 Control Shaker will result in inefficient MagneSil® PMP washing and poor genomic DNA purification.

During all mixing steps, the shaker should generate a vortex to ensure efficient mixing and washing of the MagneSil® PMPs.

5.A. Sample Lysis and DNA Binding

1. Place the empty, uncapped, 50ml conical tubes into the 50ml tube holder.
2. Add 1–10ml of sample to the 50ml conical tubes in the tube holder and place the tube holder onto the shaker.
3. Set shaker speed at 600rpm.
4. The indicated volume of eLysis Buffer is added to the sample depending on the sample starting volume (Table 2).
5. Shake at 600rpm for 1.5 minutes to lyse the samples.
6. Stop the shaker.
7. Set the shaker speed at 600rpm.

Table 2. Volume of eLysis Buffer and MagneSil® PMPs to Add to Various Volumes of Whole Blood Sample.

Sample Volume	Volume of eLysis Buffer	Volume of MagneSil® PMPs
1ml	0.9ml	0.35ml
2ml	1.8ml	0.7ml
3ml	2.7ml	1.05ml
4ml	3.6ml	1.4ml
5ml	4.5ml	1.75ml
6ml	5.4ml	2.1ml
7ml	6.3ml	2.45ml
8ml	7.2ml	2.8ml
9ml	8.1ml	3.15ml
10ml	9ml	3.5ml

8. Thoroughly resuspend the MagneSil® PMPs by vigorously shaking the bottle by hand. Hold the bottle upside down to ensure that the MagneSil® PMPs are not adhering to the bottom of the bottle.

Shake the reagent bottle vigorously to ensure thorough resuspension of the MagneSil® PMPs before use. Incomplete resuspension of the MagneSil® PMPs in the reagent bottle will result in variable yield and purity of the isolated DNA.

9. Add the indicated volume of thoroughly resuspended MagneSil® PMPs (Table 2) to the lysed sample.

10. Start the shaker and shake at 600rpm for 30 seconds. After 30 seconds, reduce the shaker speed to 400rpm. Shake at 400rpm for 4 minutes.

11. Stop the shaker. Remove the 50ml tube holder containing the sample lysate and MagneSil® PMPs and place onto the magnetic base.

12. Wait for 4 minutes to capture the MagneSil® PMPs to the side of the tubes. Remove supernatant and discard to waste.

5.B. Sample Washing

Combined eLysis Buffer/Alcohol Wash #1

13. Remove the 50ml tube holder from the magnetic base and place on the shaker. Set the shaker speed at 700rpm.

14. Add 4.5ml of eLysis Buffer to each 50ml conical tube containing the MagneSil® PMPs.

15. Add 1.5ml of Alcohol Wash Solution (ethanol and isopropanol added) to each 50ml conical tube containing MagneSil® PMPs and eLysis Buffer.

16. Shake at 700rpm for 30 seconds.

17. Stop the shaker. Remove the 50ml tube holder containing the MagneSil® PMPs and wash solutions and place onto the magnetic base.

18. Wait for 1 minute to capture the MagneSil® PMPs to the side of the tubes. Remove supernatant and discard to waste.

Combined eLysis Buffer/Alcohol Wash #2

19. Remove the 50ml tube holder from the magnetic base and place on the shaker.

20. Set the shaker speed at 700rpm.

21. Add 3ml of eLysis Buffer to each 50ml conical tube containing the MagneSil® PMPs.

22. Add 3ml of Alcohol Wash Solution (ethanol and isopropanol added) to each 50ml conical tube containing MagneSil® PMPs and eLysis Buffer.

23. Shake at 700rpm for 30 seconds.

24. Stop the shaker. Remove the 50ml tube holder containing the MagneSil® PMPs and wash solutions and place onto the magnetic base.

25. Wait for 1 minute to capture the MagneSil® PMPs to the side of the tubes. Remove supernatant and discard to waste.

Combined eLysis Buffer/Alcohol Wash #3

26. Repeat the combined eLysis Buffer/Alcohol Wash #2 for a total of 3 combined eLysis Buffer/Alcohol Wash steps.

Alcohol Washes (Repeat for a total of 3 alcohol washes)

27. Remove the 50ml tube holder from the magnetic base and place on the shaker.

28. Set the shaker speed to 750rpm.

29. Add 9ml of Alcohol Wash Solution (ethanol and isopropanol added) to each 50ml conical tube containing MagneSil® PMPs.

30. Shake at 750rpm for 30 seconds.

31. Stop the shaker. Remove the 50ml tube holder containing the MagneSil® PMPs and wash solutions and place onto the magnetic base.

32. Wait for 1 minute to capture the MagneSil® PMPs to the side of the tubes. Remove supernatant and discard to waste.

33. Repeat for a total of 3 alcohol washes.

5.C. Elution of Purified Genomic DNA

34. Ensure that all the Alcohol Wash Solution has been aspirated away from the MagneSil® PMPs. Do not dry the MagneSil® PMPs.

35. Remove the 50ml tube holder from the magnetic base and place on the shaker. Set the shaker speed at 800rpm.

36. Add 2.5ml room temperature Elution Buffer to the tubes containing the MagneSil® PMPs. Shake at 800rpm for 2 minutes. Elution volume may be adapted to meet your requirements (see Section 3.C).

(!) Use of preheated Elution Buffer will significantly decrease yield and concentration.

37. Stop the shaker. Remove the 50ml tube holder containing the MagneSil® PMPs and Elution Buffer to a heat block (with heat block adapter) set to 90–95°C. Heat the sample on the heat block for 15 minutes.

(!) **Note:** The heat block must be set at 90–95°C to ensure a sample elution temperature of at least 55°C. Heated elution must be performed using a heat block. **Do not use a water bath**. Use of a water bath will result in decreased yield.

38. Remove the 50ml tube holder containing the MagneSil® PMPs and Elution Buffer from the heat block and place back onto the shaker.

39. Shake at 800rpm for 2 minutes.

40. Stop the shaker. Remove the 50ml tube holder containing the MagneSil® PMPs and Elution Buffer from the shaker back onto the heat block.

41. Heat the sample for 5 minutes.

42. Repeat Steps 38 through 41 for a total of two cycles of 5 minutes heating followed by 2 minutes shaking.

43. After the final shaking step, remove the 50ml tube holder containing the MagneSil® PMPs and Elution Buffer from the shaker and place onto the magnetic base.

44. Wait for 5 minutes or until all the MagneSil® PMPs are captured to the side of the tubes.

45. Slowly aspirate supernatant containing purified genomic DNA to a new tube.

    Note: For samples containing large amounts of DNA (e.g., 10ml sample volumes) the supernatant will be viscous. Aspirate the supernatant slowly. If MagneSil® PMPs are aspirated with the purified sample, they should be removed by placing the tube on the magnet a second time. After capture of the PMPs, remove the supernatant to a new tube.

46. Repeat the elution procedure (Steps 34–45) for maximal yield.

6. Quantitation and Analysis of Isolated Genomic DNA

Using the recommended reagent and elution volumes, we have found that many downstream applications can routinely be performed using a fixed volume (e.g., 1µl) of isolated genomic DNA without the need for quantitation. However, careful quantitation may be required to optimize performance in specific applications, maximize reproducibility, or provide documentation of sample quality and yield. Spectrophotometric analysis is widely accepted as the quantitation method of choice because of its relative ease, efficiency, and cost-effectiveness. The technique is simple to perform and can be scaled to fit virtually any throughput need without the need for additional reagents or labor-intensive manipulation.

Spectrophotometric measurement of genomic DNA concentration is based on the Beer-Lambert equation, which defines the linear relationship between absorbance and concentration. Samples are diluted appropriately, read in the spectrophotometer using a quartz cuvette or 96-well plate, and the absorbance reading is used to calculate the sample concentration. A protocol for spectrophotometric genomic DNA quantitation is provided.

1. Dilute samples before measurement (if required). Samples must be diluted to give a reading within the linear range of the instrument used. The linear range of most spectrophotometers is between 0.1 and 1 O.D. units.
   - Usually a 1:10 dilution is appropriate. (e.g., 10µl of sample added to 90µl solvent).
   - The volumes measured in the spectrophotometer will depend on the format and minimum volume requirements of your specific instrument.
   - Quantitation of genomic DNA can be affected by the solvent used to dilute the genomic DNA before measurement (1). We recommend use of Elution Buffer or TE Buffer (10mM Tris [pH 7.5], 1mM EDTA [pH 8.0]).
2. Mix diluted samples by vortexing or pipetting several times.
3. Prepare the spectrophotometer to measure absorbance at 260nm, 280nm, and 320nm.
4. Rinse a clean quartz cuvette with ultrapure water and blot on a Kimwipes® tissue to remove excess liquid.
5. Add TE (or other solvent) to the cuvette and either blank the spectrophotometer or read absorbance for autobackground correction.
6. Rinse cuvette 2–3 times with ultrapure water and blot to dry. Read all samples, rinsing the cuvette between readings.

   If a 96-well plate reader is used, diluted samples and appropriate blanks can be added to the plate and read directly at 260nm, 280nm and 320nm.

Calculation of DNA Concentration

The DNA concentration of each sample is determined by adjusting the A_{260} measurement for turbidity (measured by absorbance at A_{320}), multiplying by the dilution factor, and using the relationship that an A_{260} of 1.0 = 50µg/ml genomic DNA.

Concentration (µg/ml) = (A_{260} reading – A_{320} reading) × dilution factor × 50

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume. The standard MagneSil® Genomic, Large Volume System genomic DNA purification procedure with a double elution generates a final total sample volume of approximately 4ml.

DNA Yield (µg) = DNA concentration × Total Sample Volume (ml)

A_{260}/A_{280} ratio can be used to determine DNA purity (with a number of important limitations [2–4]). 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} to remain consistent.

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

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

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	Causes and Comments
Low DNA yield	All performance testing of the MagneSil® Genomic, Large Volume System is based on quantitation by spectrophotometry. In our experience, quantitation by other methods often indicates yields that are different than those obtained by spectrophotometry.
	Sample size too large. The MagneSil® Genomic, Large Volume System is optimized for purification from 1–10ml samples. Exceeding the 10ml limit will result in a reduction in yield and concentration of the isolated DNA and will cause excessive clumping of the MagneSil® PMPs, making handling difficult.
	Failure to add both ethanol and isopropanol to the Alcohol Wash Solution concentrate. Ensure that both additions were made.
	Whole blood samples may contain excessive white blood cells/ml. The MagneSil® Genomic, Large Volume System protocol is optimized for normal healthy adult whole blood samples, which generally contain between 4.5×10^6 and 1×10^7 white cells/ml (1). Whole blood samples containing more white cells/ml may cause particle clumping during the purification process, causing reduced yield.
	Whole blood samples may contain too few white cells/ml. DNA yield is dependent on the amount of starting material. Blood samples with low white cell counts will give reduced yields because of the low sample input.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Low DNA yield (continued)	Combined eLysis Buffer/Alcohol Wash steps performed incorrectly. Ensure that the eLysis Buffer is added to the MagneSil® PMPs first, followed by the Alcohol Wash Solution (with ethanol and isopropanol added). Adding Alcohol Wash Solution before the eLysis Buffer during the combined eLysis Buffer/Alcohol Wash steps will result in decreased DNA yield.
	Samples mixed too vigorously during wash steps. Use the recommended mixing speeds and times during the wash steps. During all mixing steps, the solution should be vortexed to ensure efficient mixing and washing of the MagneSil® PMPs.
	Heated elution buffer was added to the MagneSil® PMPs. The elution buffer should be at room temperature when added to the MagneSil® PMPs containing genomic DNA. Adding pre-heated Elution Buffer will result in suboptimal elution of purified DNA.
	Elution Buffer and MagneSil® PMPs not heated effectively. After room temperature Elution Buffer is added to the MagneSil® PMPs, the MagneSil® PMP/Elution Buffer solution is heated. Heating at lower temperatures than recommended (i.e., sample temperature <55°C) will result in lower yield and increased alcohol carryover. The heat block must be set at 90–95°C to ensure a sample elution temperature of at least 55°C. Heated elution must be performed using a dry heat block. **Do not** use a water bath.
	Elution Buffer and MagneSil® PMPs heated too much. Do not set the heat block temperature above 95°C. Doing so will cause rapid evaporation of Elution Buffer, preventing DNA rehydration and elution.
	Modification of elution volumes. Although reducing elution volume will increase purified DNA concentration, it will decrease total yield. See Section 3.C.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Low DNA yield (continued)	Insufficient mixing during elution. Vigorously mix the Elution Buffer and MagneSil® PMPs. During **successful elution,** the MagneSil® PMPs are thoroughly broken apart during the mixing step. During shaking steps, a constant "line" of liquid around the 7ml mark on the conical tube is observed and the elution solution is a homogeneous dull brown color. During **poor elution,** MagneSil® PMPs remain in a loose clump swirling at the bottom of the tubes during shaking steps. Instead of a line around the 7ml mark, "wobbling" of the solution between the 5 and 10ml marks is observed during shaking steps. The solution is a nonhomogeneous shiny brown color during shaking steps.
	Failure to resuspend the MagneSil® PMPs adequately before use. Thoroughly resuspend MagneSil® PMPs in the reagent bottle before dispensing for sample purification.
	MagneSil® PMPs cannot be stored in 50ml conical tubes between purification runs. The 50 ml tubes do not prevent evaporation, therefore the composition of MagneSil® PMPs stored in these tubes changes over time. MagneSil® PMPs should be stored in the reagent bottle provided.
	Check to ensure that all protocol steps were followed correctly, and that the correct reagents were used at each step. Successful use of the MagneSil® Genomic, Large Volume System is dependent on use of the correct reagents in the correct order. This ensures optimal purification of genomic DNA away from sample contaminants.
Purified DNA looks red or low A_{260}/A_{280} ratio	During the first three wash steps, eLysis Buffer may have been used alone instead of mixed with Alcohol Wash. Ensure that the first three wash steps are followed correctly.
	eLysis Buffer may have been used for the last three wash steps. Ensure that the last three wash steps are performed using Alcohol Wash Solution alone.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Purified DNA looks red or low A_{260}/A_{280} ratio (continued)	Mixing not vigorous enough. A vortex should be produced and the MagneSil® PMPs should form a uniform suspension during mixing.
	Failure to resuspend the MagneSil® PMPs adequately before use. Thoroughly resuspend the MagneSil® PMPs in the reagent bottle before dispensing so that the correct amount of particles are used for capture of genomic DNA from the sample lysate. Inefficient mixing will result in a gradient of MagneSil® PMPs and cause some samples to receive more PMPs than others. This will result in a less clean prep and variable yield.
MagneSil® PMPs clumped	Too much sample. Increasing sample size above 10ml may not result in increased yield. Also, whole blood samples with higher than average white cell counts/ml will result in particle clumping. Too much particle clumping may result in lower yields.
	Insufficient mixing. Vigorously mix during binding, wash and incubation steps to resuspend MagneSil® PMPs. Optimal performance is achieved when the particles are mixed well during all these steps.
	The MagneSil® Genomic, Large Volume System is optimized for use with the IKA Works KS130 Control Shaker. This shaker has the combination of a 4mm orbit coupled with high rpm settings to enable mixing of 50ml conical tubes in the tube holder and production of a vortex in the tubes during mixing steps. Formation of a vortex during all shaking steps is critical for optimal performance.
DNA degradation	Nucleases introduced during purification and by handling. Use nuclease-free plastic- or glassware. Use filter tips during all pipetting steps. Wear gloves at all times. Nucleases introduced after elution will degrade DNA.
	Degradation occurred before the purification process. DNA sample may have been degraded before the purification process. If sample DNA was degraded before purification, the resulting purified DNA will also be degraded.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
MagneSil® PMPs visible in eluted DNA	Concentrated solutions of eluted DNA can become viscous. Additional time may be required to capture MagneSil® PMPs from such viscous solutions. Small amounts of PMPs may also be transferred during manual purification. The eluted DNA should be collected slowly and residual PMPs removed from the eluted DNA by performing a second magnetic capture. Small amounts of residual PMPs will not affect downstream applications.

8. References

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