



Promega

Technical Bulletin

MagneSil[®] Blood Genomic, Max Yield System

INSTRUCTIONS FOR USE OF PRODUCT MD1360.



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MagneSil® Blood Genomic, Max Yield System

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 of this system. E-mail techserv@promega.com.

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1. Description

Traditional procedures for isolating genomic DNA from whole blood can be lengthy and potentially hazardous. Furthermore, many procedures can have substantial carryover of inhibitors, limiting their usefulness. The MagneSil® Blood Genomic, Max Yield System^(a) is designed for the purification of DNA from 200µl of whole blood and is used in conjunction with automated purification protocols on robotic workstations such as the Beckman Coulter Biomek® FX platform using 96-well formats. Specific instructions are provided for the Biomek® FX automated liquid handling workstation. A validated method for this automated liquid handling workstation and general automation guidelines for adaptation to other liquid handling platforms can be requested at: www.promega.com/automethods

The Biomek® FX can process 96 samples in about 90 minutes and requires no hands-on time once the samples are placed on the robot. Depending on the equipment being used, turnaround time between consecutive runs can be less

1. Description (continued)

than 5 minutes. The MagneSil® Blood Genomic System can also be scaled down to process smaller amounts of whole blood, which can reduce the per prep cost of reagents and plasticware.

DNA purified with the MagneSil® Blood Genomic System can be used in STR and PCR^(b) applications as well as more stringent applications such as multiplexed PCR (e.g., the Y Chromosome Deletion Detection System, Version 2.0, Cat.# MD1531) or the READIT® SNP Genotyping System, Cat.# MD1290.

The MagneSil® Blood Genomic System uses MagneSil® Paramagnetic Particles (PMPs)^(a), which can be considered a “mobile solid phase”. Unlike column-based systems, the binding of nucleic acids to magnetic particles can occur in solution, resulting in increased binding kinetics and binding efficiency. Particles can also be completely resuspended during the wash steps of a purification protocol, thus enhancing the contact with, and removal of, contaminants, increasing nucleic acid purity.

Selected Citations Using the MagneSil® Blood Genomic, Max Yield System:

- Bailey, A.M. *et al.* (2003) Robotic nucleic acid isolation using a magnetic bead resin and an automated liquid handler for biological agent simulants. *J. Assoc. Lab. Automation* **8**, 113–20.

This study describes the development of a system that can rapidly and accurately detect traces of biological agents from environmental samples. Using *Erwinia herbicola* and *Bacillus subtilis* var. *niger* as models for potential biological warfare agents, a method for DNA extraction using the MagneSil® Blood Genomic, Max Yield System, alone and in combination with the Wizard® Magnetic DNA Purification System for Food, was automated on a Beckman Coulter Biomek® FX robotic liquid handling system. The isolated DNA was used in a TaqMan® real-time PCR assay that specifically amplified and identified DNA species. In addition, the study evaluated the ability of the MagneSil®-based DNA purification technology to eliminate PCR inhibitors. Various soil samples, surface swabs and air samples were mixed with bacterial cultures to see if any contaminants present in the samples inhibited PCR. It was found that the modified MagneSil® method described by the authors eliminated many PCR inhibitors.

For additional peer-reviewed articles that cite use of the MagneSil® Blood Genomic, Max Yield System, visit: www.promega.com/citations

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneSil® Blood Genomic, Max Yield System	1 × 96 preps	MD1360

For Laboratory Use. Each system contains sufficient reagents to perform 1 × 96-well plate preparations. The MagneSil® Blood Genomic, Max Yield System, includes:

- 160ml Lysis Buffer, Blood
- 90ml Salt Wash, Blood
- 25ml MagneSil® PMPs
- 70ml Alcohol Wash, Blood
- 45ml Elution Buffer, Blood
- 300µl Anti-Foam Reagent

Storage Conditions: All components should be stored at room temperature (20–25°C). Do not freeze the MagneSil® PMPs.

3. Before You Begin

Materials to Be Supplied by the User

- Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
- MagnaBot® Spacer, 1/8 Inch (Cat.# V8581)
- deep-well microplates (Marsh # AB-0932 [2.2ml] and AB-0787 [1.2ml] or comparable)
- pyramid bottom 96-well reservoirs (Innovative Microplates Cat.# S30014 or comparable)
- Heat Transfer Block (Cat.# Z3271 or comparable)
- 96-Well Collection Plate (Cat.# A9161 or comparable)
- Biomek® FX instrument (Beckman Coulter Instruments)

3.A. Preparation of Solutions

1. Prepare the Lysis Buffer, Blood, solution by adding 200µl of the Anti-Foam Reagent to the bottle of Lysis Buffer, Blood. Mix well.
2. Prepare the Alcohol Wash, Blood, solution by adding 95–100% ethanol and isopropyl alcohol (IPA; amount indicated on each bottle label) to the Alcohol Wash bottle and mix well.

3.B. Sample Preparation

Blood samples must be dispensed to the 2.2ml deep-well microplate manually before DNA isolation. We recommend using whole blood samples that are fresh or have been stored at 4°C for less than 2 weeks.

4. Automated Processing Requirements for the Biomek® FX

4.A. Instrument Requirements for the Biomek® FX

The following is a list of Beckman Coulter parts that are required for automation of the MagneSil® Blood Genomic, Max Yield System, on the Biomek® FX liquid handling workstation.

Part Description	Quantity	Beckman Coulter Ordering Information
Biomek® FX Software version 2.1 (minimum)		Contact Beckman
96-channel POD	1	Contact Beckman
Minimum number of Labware Positions by 1 POD	16	Contact Beckman
Tip Loader ALP	1	Cat.# 719356
Heating/Cooling ALP, Single Position	1	Cat.# 719361
Orbital Shaker ALP (optional)	1	Cat.# 379448
Tip Wash Station (96-channel) (optional)	1	Cat.# 719363
Recirculating Waterbath (120v)	1	VWR Cat.# 13270-105
	or	
Recirculating Waterbath (240v)	1	VWR Cat.# 13270-106

4.B. Labware Requirements for the Biomek® FX

The following is a list of labware that is required for automation of the MagneSil® Blood Genomic, Max Yield System, on the Biomek® FX liquid handling workstation.

Part Description	Quantity	Ordering Information
Deep Well MagnaBot® 96 Magnetic Separation Device	1	Promega Cat.# V3031
MagnaBot® Spacer, 1/8 Inch	1	Promega Cat.# V8581
Heat Transfer Block	1	Promega Cat.# Z3271
2ml Deep-well plates (or comparable)	4	Marsh Cat.# AB-0932
1.2ml Deep-well plates (or comparable)		Marsh Cat.# AB-0564/BP
Pyramid bottom 96-well reservoir (or comparable)	3	Innovative Microplate Cat.# S30014
Polystyrene U-bottom multiwell plate (or comparable)	2	Greiner America Cat.# 650101
Biomek® FX Standard 250 tips-Barrier (rack)	4	Beckman Coulter Cat.# 717253
Biomek® FX Standard 250 tips (rack) (optional)	4	Beckman Coulter Cat.# 717251

4.C. Biomek® FX Deck Setup

This is an example of the MagneSil® Blood Genomic, Max Yield System deck layout on a Biomek® FX instrument. Your specific deck layout may be different depending on your Biomek® FX configuration.

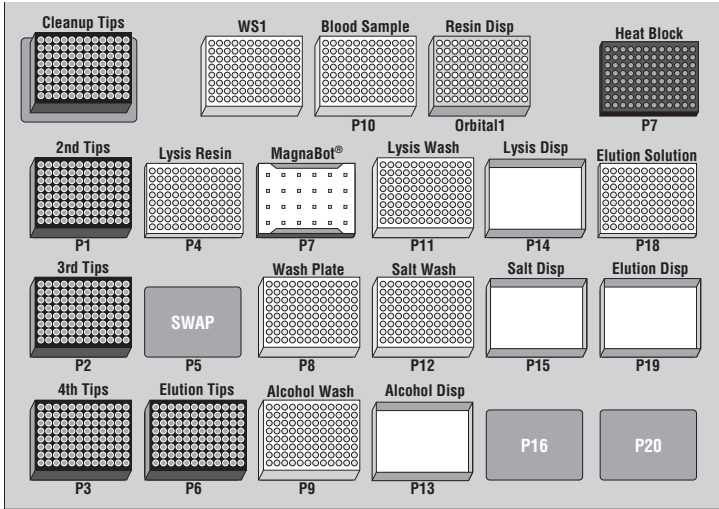


Figure 1. Deck layout of the Biomek® FX instrument for the MagneSil® Blood Genomic, Max Yield System.

ALP Name	Equipment
Tip Loader	200µl ART Biomek® FX tips
P1	200µl ART Biomek® FX tips
P2	200µl ART Biomek® FX tips
P3	200µl ART Biomek® FX tips
P4	Empty Greiner 96-well, U-bottom plate ("Lysis Resin")
P5	Empty: Swap spot
P6	200µl ART Biomek® FX tips
P7	Deep Well MagnaBot® with a MagnaBot® Spacer 1/8 Inch
P8	Empty 1.2ml deep-well round bottom plate ("wash plate")
P9	Empty 2.2ml deep-well square bottom plate ("Alcohol Wash")
P10	2.2ml deep-well square bottom plate containing 200µl whole blood samples
P11	Empty 2.2ml deep-well square bottom plate ("Lysis Wash")
P12	Empty 2.2ml deep-well square bottom plate ("Salt Wash")
P13	Pyramid Bottom reservoir plate containing 140ml Alcohol Wash ("Alcohol Disp")
P14	Pyramid Bottom reservoir plate containing 160ml Lysis Wash ("Lysis Disp")

4.C. Biomek® FX Deck Setup (continued)

ALP Name	Equipment
P15	Upside down tip box lid containing 90ml Salt Wash (“Salt Disp”)
P16	Empty
P17	Heating/Cooling ALP with a Promega Heat Transfer Block connected to a recirculating water bath (set at 80°C)
P18	Empty Greiner 96-well, U-bottom plate (“Elution Sln”)
P19	Upside down tip box lid containing 45ml Elution Buffer (“Elution Disp”)
P20	Empty
WS1	Tip Wash Station (96 channel)
Orbital1	Pyramid Bottom reservoir plate containing 25ml MagneSil® PMPs

4.D. Biomek® FX-Specific Pre-Run Recommendations

The Biomek® FX automated platform allows users the flexibility to configure the robot’s deck configuration according to need. Because of this flexibility in deck configuration, it is likely that the deck used for writing a Biomek® FX method will differ from an end-user’s deck. Therefore, it will be generally necessary to map an imported method onto an end-user’s deck configuration. To map an imported method onto your deck, please follow the instructions provided in the document *Biomek® FX Deck Mapping* (www.promega.com/automethods/beckman/biomekfx/default.asp).

5. Description of Automated MagneSil® Blood Genomic, Max Yield System

This overview describes general liquid handling steps required for automating the MagneSil® Blood Genomic, Max Yield System, and can be adapted to a variety of automated liquid handling robots. For additional information for adaptation to liquid handling robots other than those referenced above, please see Section 6 “General Guidelines for Adaptation to Alternative Robotic Platforms”.

Note: Blood samples must be dispensed to the deep-well microplate manually before DNA isolation.

- 1. Deck Preparation.** Automated dispensing of all reagents into appropriate plates. The use of plates instead of open reservoirs for reagent dispensing during the method decreases the possibility of sample cross-contamination. The following reagent volumes are dispensed into each well of the appropriate plates: 210µl of Elution Buffer, Blood; 1,500µl of Lysis Buffer, Blood (with Anti-Foam Reagent added); 100µl of MagneSil® PMPs (resuspended on Orbital Shaker ALP for approximately 2 minutes); 800µl of Salt Wash, Blood; and 1,220µl of Alcohol Wash, Blood (with ethanol and isopropyl alcohol added). **Note:** This deck preparation step can be separated from the rest of the purification method if desired. Plates could be predispensed, covered and used at a later time.
- 2. Cell Lysis and DNA Binding.** Four hundred and seventy microliters of Lysis Buffer, Blood, is added to the 2ml plate containing 200µl whole blood samples and mixed well by pipetting. Next, 130µl of Lysis Buffer, Blood, is premixed with 100µl of MagneSil® particles, then transferred to the samples. The samples are mixed well by pipetting. An additional 120µl of Lysis Buffer, Blood, is added to the samples and mixed well by pipetting. This step lyses the blood cells and denatures protein and heme, freeing the DNA so it can bind to the MagneSil® PMPs.
- 3. Volume Reduction.** The Cell Lysis and DNA Binding steps are performed in a 2ml deep-well, square-well plate due to reagent volumes. All remaining purification steps are performed in a 1.2ml deep-well, round-well plate, which is compatible with the Deep Well MagnaBot® Device. Following the Cell Lysis and DNA Binding steps, the instrument performs a volume reduction step by transferring the entire contents from the 2ml deep-well sample plate into a 1.2ml deep-well, round-well plate. During this transfer, the 1.2ml deep-well plate is placed on the Deep Well MagnaBot® device with 1/8 Inch Spacer to capture of the MagneSil® PMPs containing bound genomic DNA. This volume reduction step is performed by transferring the lysate in three separate pipetting steps to promote efficient capture of the MagneSil® PMPs containing bound genomic DNA. Samples are initially mixed on the Deep Well MagnaBot® Device to promote capture of any MagneSil® PMPs that have settled to the bottom of the well. After an additional 30 seconds of capture, the supernatant is discarded to waste and the 1.2ml sample plate is removed from the Deep Well MagnaBot® Device to proceed to the remaining wash and elution steps.
- 4. Two Lysis Washes.** One hundred and eighty microliters of Lysis Buffer, Blood, is added to the samples and mixed well by pipetting. This is repeated for a total of 360µl of Lysis Buffer, Blood, addition/mixing. The sample plate is then placed onto the Deep Well MagnaBot® Device for 30 seconds to capture the MagneSil® PMPs. The supernatant is discarded and the sample plate is then removed from the Deep Well MagnaBot® Device. This wash step is repeated for a total of two 360µl washes. **Note:** During mixing steps, some samples may contain particle clumps. This clumping effect is due to sample-to-sample variation in the quantity of protein and/or DNA in the whole blood sample. This will not affect downstream DNA yield or quality.

5. **Two Salt Washes.** One hundred and eighty microliters of Salt Wash, Blood, is added to the samples and mixed well by pipetting. This is repeated for a total of 360µl of Salt Wash, Blood, addition/mixing. The sample plate is then placed onto the Deep Well MagnaBot® Device for 30 seconds to capture the MagneSil® particles. The supernatant is discarded and the sample plate is then moved off of the Deep Well MagnaBot® Device. This wash step is repeated for a total of two 360µl washes. **Note:** During mixing steps, some samples may contain particle clumps. This clumping effect is due to sample-to-sample variation in the quantity of protein and/or DNA in the whole blood sample. This will not affect downstream DNA yield or quality.
6. **Three Alcohol Washes.** One hundred and eighty microliters of Alcohol Wash, Blood, is added to the samples and mixed well by pipetting. This is repeated for a total of 360µl of Alcohol Wash, Blood, addition/mixing. The sample plate is then placed onto the Deep Well MagnaBot® Device for 30 seconds to capture the MagneSil® PMPs. The supernatant is discarded and the sample plate is then moved off of the Deep Well MagnaBot® Device. This wash step is repeated for a total of three 360µl washes. **Note:** During mixing steps, some samples may contain particle clumps. This clumping effect is due to sample-to-sample variation in the quantity of protein and/or DNA in the whole blood sample. This will not affect downstream DNA yield or quality.
7. **Drying/Removal of Residual Alcohol.** Following the last alcohol wash, the samples are air-dried for 2 minutes. Any residual alcohol left in the wells is then removed. The sample plate is then moved to the Heating/Cooling (H/C) ALP for 5 minutes. The H/C ALP, with recirculating water bath previously set at 80°C, is necessary for efficient sample drying (and elution).
8. **Elution of Purified Genomic DNA.** Two hundred and ten microliters of Elution Buffer, Blood, is added to the samples (still on the H/C ALP). After 80 seconds, the samples are mixed well by pipetting. Five 1-minute pauses and mixes are then performed to promote elution of the genomic DNA from the MagneSil® PMPs. The sample plate is then placed onto the Deep Well MagnaBot® Device for 50 seconds to capture the MagneSil® PMPs. The supernatant is collected and saved in the 96-well, round-bottom plate labeled "Elution Sln".
9. **Method Ends.** Purified genomic DNA has been eluted into the 96-well, round-bottom plate labeled "Elution Sln".

6. General Guidelines for Adaptation to Alternative Robotic Platforms

Due to the variability of whole blood samples and storage conditions used, blood samples may clump throughout this purification procedure. Despite this clumping, the final purified samples are of high quality and are functional in downstream amplifications. However, due to clumping variability, we recommend that aerosol-resistant tips (barrier tips) be used for this method to decrease the chance for contamination of the instrumentation pipetting device. If your robotic platform uses fixed tips, be sure that the tips are washed thoroughly with bleach, hydrogen peroxide, or other appropriate wash solutions between pipetting steps to avoid sample cross-contamination. Also, if your robotic platform uses system liquid to perform pipetting steps, be sure to limit the exposure of samples to system liquid during all pipetting steps by increasing the volume of leading air gaps that are used for pipetting.

The MagneSil® PMPs used for this purification process settle over time. We recommend thoroughly mixing the MagneSil® PMPs on the automated platform prior to dispensing. Resuspension of the MagneSil® PMPs can be accomplished by thorough mixing or shaking.

To efficiently elute the final purified samples from the MagneSil® PMPs, it is important to include a heating step. This heating step ensures the samples are thoroughly dried following the last alcohol wash step and also helps to efficiently elute the final product from the MagneSil® PMPs.

7. Related Products

Product	Size	Cat.#
Deep Well MagnaBot® 96 Magnetic Separation Device*	1 each	V3031
MagnaBot® Spacer, 1/8 Inch	1 each	V8581
Heat Transfer Block	1 each	Z3271
Collection Plates (4-pack)	1 each	A9161

* For Laboratory Use.

^(a)U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, Japanese Pat. No. 3253638, European Pat. No. 0 895 546 and Mexican Pat. No. 209436 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles. Other patents are pending.

^(b)The PCR process is covered by patents issued and applicable in certain countries*. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

*In Europe, effective March 28, 2006, European Pat. Nos. 201,184 and 200,362, will expire. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.