Technical Bulletin

MagneSil® ONE, Fixed Yield Blood Genomic System

INSTRUCTIONS FOR USE OF PRODUCT MD1370.

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MagneSil® ONE, Fixed Yield Blood Genomic System

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1. Description

Access to the human genome and other complete genome sequences is playing an increasingly important role in many research fields. To this end, many novel genotyping methods have been developed in addition to single-locus amplification. These systems often rely on analysis of small amounts of DNA but may be limited to a defined range of input DNA for maximum reproducibility. Currently, the downstream application requirement for a narrow range of input DNA adds time and labor to DNA quantitation and normalization.

The MagneSil® ONE, Fixed Yield Blood Genomic System[a] is designed for the purification of a "fixed yield" of DNA ranging from 500–1500ng with an average yield of 1µg from 60µl of anti-coagulated whole blood, thus eliminating the need to quantitate the yield and normalize post-purification. Specific instructions are provided for the Beckman Coulter Biomek® FX automated

1. Description (continued)

liquid handling workstation. A validated method for this automated liquid handling workstation can be requested at: **www.promega.com/automethods/**

General automation guidelines are provided for adaptation to other liquid handling platforms. The Biomek® FX can process 96 samples in approximately 1 hour and requires no hands-on time once the samples are placed on the robot.

The DNA purified from these samples can be used in STR and PCR[(b)] analysis as well as more stringent applications such as multiplexed PCR (e.g., Promega's Y Chromosome Deletion Detection System) or the READIT® Assay, Cat.# MD1290.

The MagneSil® ONE System uses MagneSil® Paramagnetic Particles (PMPs)[(a)]—Fixed Yield, 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.

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneSil® ONE, Fixed Yield Blood Genomic System	1 × 96 preps	MD1370

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

- 160ml Lysis Buffer, Blood
- 25ml MagneSil® PMPs—Fixed Yield
- 120ml 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 microwell plates (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 of each indicated on the bottle label) to the Alcohol Wash bottle and mix well.

3.B. Sample Preparation

Blood samples must be dispensed to the 1.2ml deepwell microplate manually before DNA isolation. It is recommended that whole blood samples be stored at 4°C for less than 2 weeks.

4. Automated Processing Requirements for the Beckman Coulter Biomek® FX

4.A. Instrumentation Requirements for the Biomek® FX

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

Description	Quantity	Beckman Coulter Part Number
Biomek® FX Software version 2.1 (minimum)		Contact Beckman Coulter
96-channel POD	1	Contact Beckman Coulter
Minimum number of Labware Positions by 1 POD	16	Contact Beckman Coulter
Tip Loader ALP	1	Beckman Coulter Cat.# 719356
Heating/Cooling ALP, Single Position	1	Beckman Coulter Cat.# 719361
Orbital Shaker ALP (optional)	1	Contact Beckman Coulter
Tip Wash Station (96-channel) (optional)	1	Beckman Coulter Cat.# 719363
Recirculating Waterbath	1	VWR Cat. #13272-200

4.B. Labware Requirements for the Biomek® FX

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 Deepwell plates (or comparable)	2	Marsh Cat.# AB-0932
1.2ml Deepwell plates (or comparable)	1	Marsh Cat.# AB-0564/BP
Pyramid-bottom 96-well reservoir (or comparable)	1	Innovative Microplate Cat.# S30014
Polystyrene U-bottom multiwell plate (or comparable)	2	Greiner America Cat.# 650101
Biomek® AP96 P250 tips, sterile barrier	3	Beckman Coulter Cat.# 717253

4.C. Biomek® FX Deck Setup

Figure 1 is an example of the MagneSil® ONE, Fixed Yield Blood Genomic System deck setup on the Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

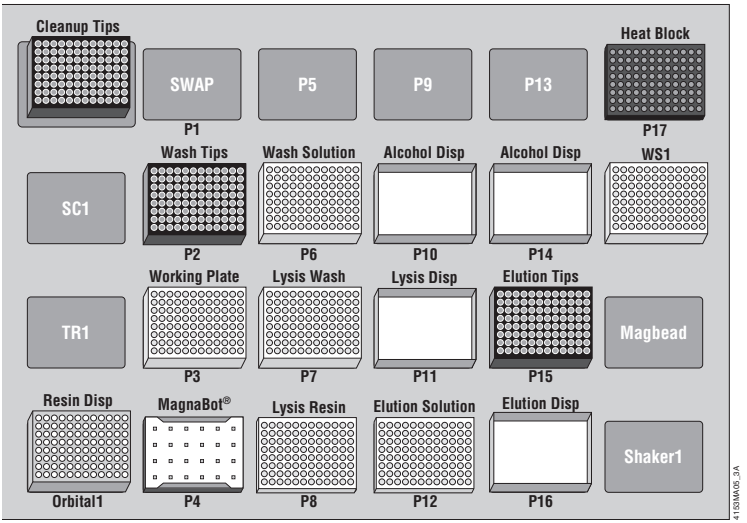

Figure 1. Deck layout of the Biomek® FX instrument for the MagneSil® ONE, Fixed Yield Blood Genomic System.

ALP Name	Equipment
Tip Loader	200µl ART Biomek® FX tips
SC1	Empty
TR1	Empty
Orbital 1	Pyramid Bottom reservoir plate containing 25ml MagneSil® PMPs
P1	Empty: Swap spot
P2	200µl ART Biomek® FX tips
P3	1.2ml deepwell round bottom plate containing 60µl whole blood samples
P4	Deep Well MagnaBot® Device with MagnaBot® spacer 1/8 inch
P5	Empty
P6	Empty 2.2ml deepwell square bottom plate (“Wash Solution”)
P7	Empty 2.2ml deepwell square bottom plate (“Lysis Wash”)
P8	Empty Greiner 96-well round bottom plate (“Lysis Resin”)
P9	Empty
P10	Upside down tip box lid containing 95ml Alcohol Wash, blood (“Alcohol Disp”; ethanol and isopropyl alcohol added)

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

ALP Name	Equipment
P11	Upside down tip box lid containing 160ml Lysis Wash, Blood ("Lysis Disp")
P12	Empty 96-well round bottom plate ("Elution Sln")
P13	Empty
P14	Upside down tip box lid containing 95ml Alcohol Wash, Blood ("Alcohol Disp"; ethanol and isopropyl alcohol added)
P15	200µl ART Biomek® FX tips
P16	Upside down tip box lid containing 45ml Elution Buffer, Blood ("Elution Disp")
P17	Heating/Cooling ALP with a Promega Heat Transfer Block connected to a recirculating water bath (80°C)
WS1	Tip Wash Station (96 channels)

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/biomek/default.asp).

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

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

1. **Deck Preparation.** Automated dispensing of all reagents into the appropriate plates. The use of plates instead of open reservoirs for reagent dispensing during the method decreases the probability of sample cross-contamination. Two hundred and ten microliters of Elution Buffer, Blood, 1,400µl of Lysis Buffer, Blood (with Anti-Foam added), 15µl of MagneSil® PMPs—Fixed Yield (after being resuspended on an orbital shaker for approximately 2 minutes), and 2,100µl of Alcohol Wash, Blood, are dispensed into seperate plates. **Note:** This deck preparation step can be separated from the rest of the purification method if desired. Plates can be predispensed, covered, and used at a later time.

2. **Cell Lysis and DNA Binding.** One hundred and ten microliters of Lysis Buffer, Blood, and 15µl of MagneSil® PMPs—Fixed Yield are premixed, then added to 60µl whole blood samples (stored in a 1.2ml deepwell round-bottom plate) and mixed well by pipetting. This step facilitates lysis of the blood cells and denatures protein and heme. An additional 430µl of Lysis Buffer, Blood, is added and mixed well by pipetting to promote efficient binding of the genomic DNA to the MagneSil® PMPs—Fixed Yield. The sample plate is then placed onto the Deep Well MagnaBot® Device with the MagnaBot® 1/8 inch spacer. Samples are initially mixed on the Deep Well MagnaBot® Device to promote capture of any MagneSil® PMPs—Fixed Yield that have settled to the bottom of the well. After an additional 30 seconds of capture, the supernatant is discarded and the sample plate is moved off of the Deep Well MagnaBot® Device.

3. **Two Lysis Washes.** Three hundred and sixty microliters of Lysis Buffer, Blood, is added to the samples and mixed well by pipetting. The sample plate is then placed onto the Deep Well MagnaBot® Device for 30 seconds to capture the MagneSil® PMPs—Fixed Yield. The supernatant is discarded and the sample plate is then moved off of the Deep Well MagnaBot® Device. This wash step is then repeated.

4. **Three Alcohol Washes.** Six hundred and eighty microliters of Alcohol Wash, Blood, is added to the samples and mixed well by pipetting. The sample plate is then placed onto the Deep Well MagnaBot® Device for 30 seconds to capture the MagneSil® PMPs—Fixed Yield. The supernatant is discarded and the sample plate is then moved off of the Deep Well MagnaBot® Device. This wash step is then repeated twice.

5. **Drying/Removal of Residual Alcohol.** The sample plate is then moved to the Heating/Cooling (H/C) ALP for 5 minutes. The Heating/Cooling ALP, with a recirculating water bath previously set at 80°C, is necessary for efficient sample drying (and elution).

6. **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). The samples are incubated on the heating device with tip mixing intermittently for 8–10 minutes. The sample plate is then placed onto the Deep Well MagnaBot® Device for 1 minute to capture the MagneSil® PMPs—Fixed Yield. The supernatant is collected and saved in a clean 96-well round bottom plate.

7. **Method Ends.** Purified genomic DNA has been eluted into the 96-well round bottom plate.

6. General Guidelines for Adaptation to Alternative Robotic Platforms

We recommend the use of aerosol-resistant tips (barrier tips) for this method to decrease the chance of contaminating the instrument's 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 system liquid is used 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—Fixed Yield used for this purification process settle quickily. We recommend thoroughly mixing the MagneSil® PMPs—Fixed Yield on the automated platform prior to dispensing to samples. Resuspension of the MagneSil® PMPs—Fixed Yield can be accomplished by thorough mixing or shaking.

To efficiently elute the final purified samples from the MagneSil® PMPs—Fixed Yield, 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—Fixed Yield.

7. Related Products

Product	Size	Cat#
Deep Well MagnaBot® 96 Magnetic Separation Device*	each	V3031
MagnaBot® Spacer 1/8 inch	each	V8581

* For Laboratory Use.

[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, Japanese Pat. No. 3253638 and European Pat. Nos. 0 895 546 and 1 204 741 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles and simultaneous isolation and quantitation of DNA. Other patents are pending.

[b]The PCR process, which is the subject of European Pat. Nos. 201,184 and 200,362 owned by Hoffmann-LaRoche*, 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. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

*The above primary European Pat. Nos. 201,184 and 200,362 will expire on March 28, 2006. 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.

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