

Automated Wizard® MagneSil™ 384-Well Sequencing Reaction Clean-Up System

Automated Protocol #EP010

DESCRIPTION OF THE BECKMAN BIOMEK® FX METHOD WITH PRODUCT A8231.

Please visit the web site to verify that you are using the most current version of this Automated Protocol.

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I. Description

This describes automation of the Wizard® MagneSil™ 384-Well Sequencing Reaction Clean-Up System^(a). Specific instructions are provided for the Beckman Biomek® FX Workstation. For information about obtaining the validated method, please see: www.promega.com/automethods/

Please refer to the *Wizard® MagneSil™ Sequencing Reaction Clean-Up System Technical Bulletin #TB287* to troubleshoot chemistry issues.

II. Product Components

Product	Size	Cat.#
MagneSil™ GREEN(a) For Laboratory Use.	100ml	A8231

A maximum of five 384-well plates can be processed with one 100ml bottle of MagneSil™ GREEN Paramagnetic Particles (PMPs). This capacity was determined using the Turbulator2™ reservoir to resuspend the PMPs and includes a 30ml dead volume when using this reservoir.

Storage Conditions: Store all components at room temperature. **Do not freeze.**

III. Materials to Be Supplied by the User

- ethanol, 90%, 60ml per 384-well plate
- nuclease-free water, 35ml per 384-well plate
- Microseal® 384-well, polypropylene, Microplate V.2.0 (MJ Research, Inc., Cat.# MSP-3842)
- 384-well Reaction Plate, polypropylene (Applied BioSystems, Cat.# 4309849)
Note: This method was written and tested using an MJ Research, Inc., 384-well PCR plate for the sample plate, and an Applied BioSystems 384-well reaction plate as the final elution plate. Other 384-well PCR plates may be substituted, with the understanding that modifications to the method will be required.
- 384-well plate, polypropylene, microarray, Genetix (Promega Cat.# V5311)
- MagnaBot® 384 Magnetic Separation Device (Promega Cat.# V8241)
- Turbulator2™ Reservoir (ACME Automation Cat.# G5058)
Includes: polypropylene reservoir; silicone tubing, ½"; Gast vacuum pump, DOA-P104-AA (www.acme-automation.com)

IV. Before You Begin

A. Preparation of Solutions

Turbulator2™ Notes: The Turbulator2™ reservoir must be attached to a vacuum pump before the MagneSil™ GREEN PMPs are added to the reservoir. This is done by attaching the tubing from the Turbulator2™ reservoir a vacuum pump, turning the vacuum pump "on," and adjusting the pressure between 2 and 5 inches Hg. When the Turbulator2™ reservoir is placed onto the deck, press the reservoir firmly into place. Ensure that the tubing from the reservoir is not over the light curtain on the Biomek® FX workstation.

MagneSil™ GREEN PMPs Notes: Add 50ml of MagneSil™ GREEN to the Turbulator2™ before running the method. The entire volume of resin will not be used during the method. This volume is necessary to eliminate splattering of the resin while the Turbulator2™ reservoir is running. Unused PMPs may be poured back into the bottle after the method is complete.

Resuspension and dispensing of the MagneSil™ GREEN PMPs are explained in the comment lines of the 384-well sequencing clean-up electronic method for the Biomek® FX Workstation.

B. Sample Preparation Before Automated Processing

Ensure that the 10µl of unpurified sequencing reaction volume is at the bottom of the well of the 384-well sample PCR plate by centrifuging the sample plate briefly at high speed.

V. Beckman Biomek® FX Workstation Requirements

A. Instrument Requirements for the Beckman Biomek® FX Workstation

Any single-arm, 384-channel Biomek® FX workstation is able to run this method. The method can also be adapted for a dual-arm Biomek® FX workstation with at least one 384-channel pod.

Part Description	Quantity	Ordering Information
Minimum: Biomek® FX Software, version 2.1		Contact Beckman
384-channel POD	1	Contact Beckman
4 x 3 ALP	1	Contact Beckman
1 x 1 ALP	2	Contact Beckman
384-well Tip Wash ALP	1	Contact Beckman

B. Labware Requirements for the Beckman Biomek® FX

Part Description	Quantity	Ordering Information
AP 384 30µl Tip Box	2	Beckman Coulter Part# 719222
AP 384 30µl Tip Box Lid	2	Included with Tip Box
Turbulator2™ Reservoir	1	ACME Automation Part# G5058
Microseal® 384-well, polypropylene, microplate V.2.0	1	M.J. Research, Inc. Part# MSP-3842
384-well plate, polypropylene, microarray	1	Promega Cat.# V5311
384-well reaction plate, polypropylene	1	Applied Biosystems Part# 4309849
MagnaBot® 384 Magnetic Separation Device	1	Promega Cat.# V8241

C. Initial Deck Layout for Beckman Biomek® FX Workstation

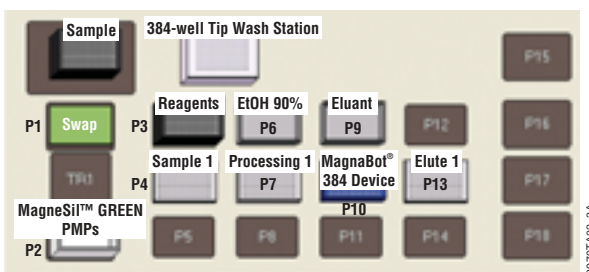


Figure 1. MagneSil™ Sequencing Reaction Clean-Up System deck layout on a Biomek® FX workstation. Your specific deck layout may be different depending on your Biomek® FX workstation configuration.

ALP Name	Part Siting on ALP
Tip Loader	AP_384 30µl Biomek® FX tips
P1	Swap Space. This is not a specific piece of labware. It is a place holder used to swap tips during the method.
P2	Turbulator 2™ Reservoir containing 50ml of MagneSil™ GREEN PMPs
P3	AP_384 30µl Biomek® FX tips
P4	384-well PCR plate containing 10µl of sequencing reactions/well
P5	Empty
P6	Beckman Coulter upside-down tip box lid containing 45ml of 90% ethanol
P7	W1 Labware Reserved Spot/384-well microarray plate
P8	Empty
P9	Beckman Coulter upside-down tip box lid containing 25ml of nuclease-free water
P10	MagnaBot® 384 Magnetic Separation Device
P13	Empty 384-well PCR plate
P11,12, 14–18	Empty. Positions only necessary for multiple plate methods.
TR1	Left Trash ALP. Only necessary for multiple plate methods.
Tip Wash ALP	384-well Tip Wash Station

D. Pre-Run Beckman Biomek® FX Workstation Specific Requirements

The Beckman 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, an end-user's deck may differ from the deck used for writing a Biomek® FX method. Therefore, mapping an imported method onto the end-user's deck configuration is generally necessary. Follow the instructions provided: [Biomek® Deck Mapping](http://www.promega.com/automethods/beckman/biomekfx) (www.promega.com/automethods/beckman/biomekfx)

Prior to the first run of the MagneSil™ 384-Well Sequencing Reaction Clean-Up method on the Beckman Biomek® FX workstation, it is necessary to ensure that the deck has been properly framed. Failure to do so may result in bent tips during the run.

Position the AP 384 30µl tip box on the Tip Loader so that it is pushed against the front left corner of the ALP. This will ensure proper seating of the tips into the mandrels of the 384 Multichannel Pod.

VI. Description of Automated Wizard® MagneSil™ 384-Well Sequencing Reaction Clean-Up Method

This overview describes general liquid handling steps required for 384-well sequencing reaction clean-up of a 10µl sample and can be adapted to a variety of automated liquid handling robots. For additional information about adapting this protocol to liquid handling robots other than those referenced above, please see Section VII.

A. Binding DNA to MagneSil™ GREEN PMPs

1. **MagneSil™ GREEN Transfer.** Thirty microliters (3X the volume of a 10µl sequencing reaction) of MagneSil™ GREEN PMPs is transferred from the Turbulator2™ reservoir to the processing plate. The MagneSil™ GREEN PMPs are transferred in a two-step process, 20µl followed by 10µl of PMPs.
2. **Sequencing Reaction Transfer.** Ten microliters (the sequencing reaction volume) is transferred from the sample PCR plate to the processing plate containing the MagneSil™ GREEN PMPs, and they are mixed by pipetting. Twelve microliters of the mixed PMPs and sequencing reactions is transferred back to the sample PCR plate and mixed in the PCR plate by pipetting. The PMPs and sequencing reaction mix are transferred back to the processing plate from the PCR plate. This step ensures that the entire sequencing reaction is mixed with MagneSil™ GREEN for optimal recovery of unpurified sequencing products.
3. **Binding Mixes.** The sequencing reaction is mixed by pipetting (28µl) to bind products to the MagneSil™ GREEN PMPs. The robot pauses for 2 minutes. A second mix is performed to increase binding efficiency.
4. **On-Magnet Mix and Supernatant Removal.** The processing plate is transferred to the MagnaBot® 384 Magnetic Separation Device. The robot pauses for 10 seconds to capture PMPs to the sides of each well. The supernatant is mixed by pipetting to ensure complete capture of PMPs to the sides of each well. The robot pauses for 5 seconds and then transfers all of the supernatant from the processing plate sitting on the MagnaBot® 384 device to the 384-well Tip Wash Station. It is critical to ensure that the supernatant has been completely removed.

B. Washing MagneSil™ GREEN PMPs

1. **Processing Plate Transfer.** The processing plate is transferred from the MagnaBot® 384 device to the W1 labware reserved spot.
2. **90% Ethanol Transfer.** Twenty microliters (2X the sequencing reaction volume) of 90% ethanol is transferred to the processing plate. When using the Biomek® FX workstation, a tip touch on the liquid in the 384-well Tip Wash Station is performed to remove ethanol droplets.
3. **Wash Mixes.** The PMPs in 90% ethanol are mixed by pipetting (15µl) to wash contaminants from the PMPs and sequencing reactions. The robot pauses for 1 minute 45 seconds and then performs a second mix to increase the washing efficiency.
4. **On-Magnet Mix and Supernatant Removal.** The processing plate is transferred to the MagnaBot® 384 device. The robot pauses for 10 seconds to capture PMPs to the sides of each well. The supernatant is mixed by pipetting to ensure complete capture of PMPs. The robot pauses for 5 seconds. Twenty microliters (2X the sequencing reaction volume) is transferred from the processing plate sitting on the MagnaBot® 384 device to the 384-well Tip Wash Station.
5. **Wash Step Repeat.** Steps 1–4 are repeated for a total of 3 washes of the MagneSil™ GREEN PMPs with 90% ethanol.

C. Drying the MagneSil™ GREEN PMPs

1. **Final Supernatant Removal.** The robot pauses for 30 seconds to allow time for any residual ethanol to move to the bottom of each well. Twelve microliters (1.2X the sequencing reaction volume) is transferred from the processing plate to the 384-well Tip Wash Station. This step ensures that all possible ethanol has been removed from each well of the processing plate.
2. **Dry MagneSil™ GREEN PMPs.** The robot pauses with the processing plate on the MagnaBot® 384 device for a total of 420 seconds. This allows any ethanol remaining on the PMPs, or in the plate, to evaporate.
3. **Wash Sample and Reagent Tips.** While the processing plate is paused, the robot rinses the sample and reagent tips in the 384-well Tip Wash Station to remove any ethanol or PMPs from the tips before the elution step.

D. Eluting the DNA from MagneSil™ GREEN PMPs

1. **Processing Plate Transfer.** The processing plate is transferred from the MagnaBot® 384 device back to the W1 labware reserved spot.
2. **Elution Water Transfer.** Twelve microliters (1.2X the sequencing reaction volume) of elution water is transferred to the processing plate.
3. **Elution Mixes.** The purified sequencing reaction products are eluted from the PMPs by mixing by pipetting. The robot pauses for 1 minute and then performs a second mix to increase the elution efficiency.
4. **On-Magnet Mix and Eluate Transfer.** The processing plate is transferred to the MagnaBot® 384 device. The robot pauses for 10 seconds to capture PMPs to the sides of each well. The supernatant is mixed with pipette tips to ensure complete capture of PMPs. The robot pauses for 5 seconds. Ten microliters (the sequencing reaction volume) is transferred from the processing plate to the empty 384-well PCR elution plate.

VII. General Guidelines for Adaptation to Alternative Robotic Platforms

Following the final ethanol removal, the processing plate is dried for 7 minutes. This is critical to remove all possible residual ethanol. The use of a heating ALP may be incorporated for the drying step. The number of mixes may need to be increased to ensure complete resuspension of the PMPs in each well of the processing plate.

The MagneSil™ GREEN PMPs used for this purification process settle rapidly. This method uses the Turbulator 2™ mixing reservoir to keep the MagneSil™ GREEN PMPs in suspension. If the MagneSil™ GREEN is dispensed into a 384-well plate prior to running the method, we recommend thoroughly mixing the MagneSil™ GREEN PMPs on the automated platform prior to dispensing to samples.

The single-plate method described here uses a single box of tips for dispensing reagents and a second for manipulating samples. This same setup can be used for multiple-plate methods as well, with one box of tips being used for all reagents dispensings and one box of tips for all sample manipulations.

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

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All prices and specifications are subject to change without prior notice.

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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