# Automated Wizard® MagneSil® Plasmid DNA Purification System Protocol

**Automated Protocol #EP001**

**DESCRIPTION OF THE LABORATORY ROBOTICS METHODS WITH PRODUCTS A1630, A1631 AND A1635. *PLEASE DISCARD PREVIOUS VERSIONS.***
**All technical literature is available on the Internet at www.promega.com**
**Please visit the web site to verify that you are using the most current version of this Automated Protocol.**



## I. Description

This document describes automation of the Wizard® MagneSil® Plasmid DNA Purification System[a,b]. Specific instructions are provided for the Beckman Coulter Biomek® 2000, Biomek® FX and Tecan Genesis® RSP150 automated liquid-handling workstations. Information on obtaining validated methods for these workstations is available at: **www.promega.com/automethods/** General automation guidelines are provided for adaptation to other liquid-handling platforms in Section VIII. For troubleshooting chemistry issues, please refer to the *Wizard® MagneSil® Plasmid DNA Purification System Technical Bulletin* #TB286.

**Note:** All Promega Technical Bulletins are available at: www.promega.com/tbs/

## II. Product Components

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid DNA Purification System | 4 × 96 preps | A1630 |

Each system contains sufficient reagents for 4 × 96 isolations.

Includes:

- 50ml Cell Resuspension Solution
- 60ml Cell Lysis Solution
- 60ml Neutralization Solution
- 19ml MagneSil® BLUE
- 30ml MagneSil® RED
- 50ml Elution Buffer
- 16 Collection Plates
- 1 Protocol

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid DNA Purification System | 8 × 96 preps | A1631 |

Each system contains sufficient reagents for 8 × 96 isolations.

Includes:

- 90ml Cell Resuspension Solution
- 125ml Cell Lysis Solution
- 120ml Neutralization Solution
- 38ml MagneSil® BLUE
- 60ml MagneSil® RED
- 100ml Elution Buffer
- 32 Collection Plates
- 1 Protocol

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid DNA Purification System | 100 × 96 preps | A1635 |

Each system contains sufficient reagents for 100 × 96 isolations.

Includes:

- 3 × 500ml Cell Resuspension Solution
- 3 × 500ml Cell Lysis Solution
- 3 × 500ml Neutralization Solution
- 5 × 100ml MagneSil® BLUE
- 7 × 100ml MagneSil® RED
- 3 × 500ml Elution Buffer
- 100 Collection Plates
- 1 Protocol

***Do not freeze*** the MagneSil® Paramagnetic Particles.

### Items Available Separately

| Product | Size | Cat.# |
|---|---|---|
| MagneSil® BLUE | 100ml | A2201 |
| MagneSil® RED | 100ml | A1641 |
| Cell Resuspension Solution | 500ml | A7114 |
| Cell Lysis Solution | 500ml | A7124 |
| Neutralization Solution | 500ml | A7132 |

**Storage Conditions:** Store all Wizard® MagneSil® Plasmid DNA Purification System components at 22–25°C.

## III. Before You Begin

### Materials to Be Supplied by the User

- culture medium containing appropriate antibiotic
- tabletop centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters (e.g., tabletop model or Beckman Coulter J2HC, Cat.# 362701)
- deep-well (2ml) 96-well plate (e.g., Beckman Coulter deep square-well plate, Cat.# 140504, or equivalent)
- 80% ethanol
- 1 plate sealer

### Preparation of Cell Pellets

Pellet the bacterial culture grown in a 2ml deep-well culture plate with square wells (e.g., Beckman Coulter deep-well titer plate, Cat.# 267007) by centrifugation for 15 minutes at 1,500 × *g* in a tabletop centrifuge. Cells should contain high-copy-number plasmids. For additional information on choosing a bacterial strain, see the *Wizard® MagneSil® Plasmid DNA Purification System Technical Bulletin* #TB286. As much as 6.0 $O.D._{600}$ of total cell mass may be processed per well. Pour off the supernatant and blot the plate upside down on a paper towel to remove excess liquid. Place this sample plate containing the cell pellets on the deck of the workstation for processing.

## IV. Automated Processing Requirements for the Biomek® 2000 Workstation

This section lists the instrument and labware requirements for the plasmid DNA purification method using the Wizard® MagneSil® Plasmid DNA Purification System on the Biomek® 2000. For specific instrument and labware requirements or multi-plate MagneSil® plasmid purification please inquire with Promega Technical Services or visit **www.promega.com/automethods/**

### A. Instrumentation Requirements for the Biomek® 2000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required for plasmid DNA purification using the Wizard® MagneSil® Plasmid DNA Purification System on a Biomek® 2000.

| Part Description | Quantity | Beckman Coulter Part Number |
|---|---|---|
| Biomek® 2000 Workstation, 50/60Hz, 100–120V | 1 | 609000 |
| Biomek® 2000 Controller NT | 1 | 609875 |
| IBM® Monitor | 1 | 974571 |
| BioWorks™ 3.2 for Beckman Coulter Computer | 1 | 609983 |
| Biomek® 2000 Left Side Module | 1 | 609048 |
| Gripper Tool System for Biomek® 2000 | 1 | 609001 |
| MP200 Pipetting Tool | 1 | 609025 |
| Tip Rack Holder | 3 | 609121 |
| Gray Labware Holder | 7 | 609120 |
| Reservoir Holder | 2 | 372795 |
| Single Half Module Reservoir | 1 | 372786 |
| Quarter Vertical Reservoir | 4 | 372788 |
| Beckman Coulter DPC MicroMix® 5 Shaker | 1 | 380560 |
| Beckman Coulter DPC MicroMix® 5 Shaker Integration Kit | 1 | 380561 |

## B. Labware Requirements for the Biomek® 2000

| Part Description | Ordering Information |
|---|---|
| MagnaBot® 96 Magnetic Separation Device | Promega Cat.# V8151 |
| Collection plates | Provided in Wizard® MagneSil® Plasmid DNA Purification System |
| 96-well 2ml square deep-well plate | Beckman Coulter Part# 140504 |

## C. Biomek® 2000 Initial Deck Configuration

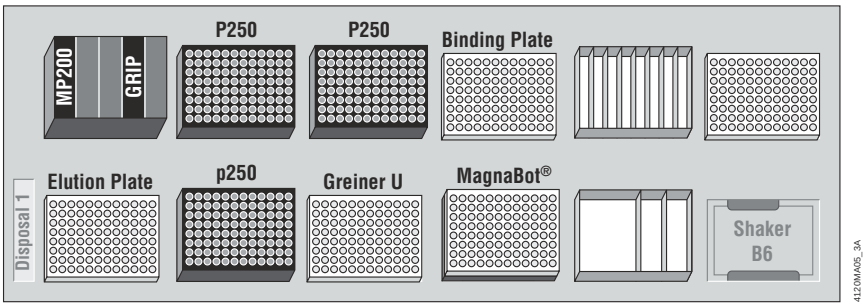
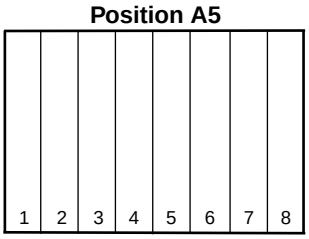



**Figure 1. Biomek® 2000 initial deck configuration.**

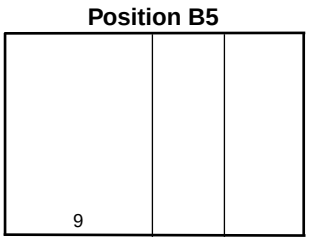
| | |
|---|---|
| Position A1 | Tool rack containing MP200 and Gripper tools |
| Position A2 | Tip rack holder, P250 tips |
| Position A3 | Tip rack holder, P250 tips |
| Position A4 | Labware holder, empty 96-well collection plate (“Binding Plate”) |
| Position A5 | Reservoir with reagents (see Figure 2 for configuration) |
| Position A6 | Shaker position, 96-well square deep-well culture plate containing bacterial cell pellets prepared in Section III. |
| Position B1 | Labware holder, empty 96-well collection plate |
| Position B2 | Tip rack holder, P250 tips |
| Position B3 | Labware holder, empty 96-well collection plate |
| Position B4 | MagnaBot® 96 Magnetic Separation Device with an empty 96-well collection plate sitting on top |
| Position B5 | Reservoir with reagents (see Figure 2 for configuration) |
| Position B6 | Empty shaker position |

### D. Biomek® 2000 Reagent Dispense Volumes

Prior to beginning run, dispense Wizard® MagneSil® Plasmid DNA Purification System reagents according to the following configuration:




1. 3ml MagneSil® RED
2. 3ml MagneSil® RED
3. 1.7ml MagneSil® BLUE
4. 1.7ml MagneSil® BLUE
5. 12ml Elution Buffer
6. 15ml Neutralization Solution
7. 15ml Cell Lysis Solution
8. 12ml Cell Resuspension Solution




9. 50ml 80% Ethanol (EtOH)

**Figure 2. Contents of the reagent reservoirs for the Automated Wizard® MagneSil® Plasmid DNA Purification System protocol.**

### E. Biomek® 2000 Specific Pre-Run Recommendations

Automated processing on the Biomek® 2000 Workstation requires integration of the DPC MicroMix® 5 Shaker. This shaker and its integration kit are available from Beckman Coulter. For more information on integration of the DPC MicroMix® 5 Shaker on the Biomek® 2000 Workstation, follow these instructions: **www.promega.com/automethods/beckman/biomek2000/default.asp**

Before running the method, it will need to be imported into the BioWorks™ Software. Please follow the instructions for importing Biomek® 2000 Methods: **www.promega.com/automethods/beckman/biomek2000/default.asp**

## V. Automated Processing Requirements for the Biomek® FX Workstation

### A. Instrumentation Requirements for the Biomek® FX

| Part Description | Quantity | Beckman Coulter Part Number |
|---|---|---|
| Minimum: Biomek® FX Software version 2.1 | 1 | Contact Beckman |
| 96-channel POD | 1 | Contact Beckman |
| Minimum number of Labware Positions by 1 POD | 13 | Contact Beckman |
| Tip Loader | 1 | Contact Beckman |
| Linear Shaker ALP | 1 | Contact Beckman |
| MagnaBot® 96 Magnetic Separation Device | 1 | Promega Cat.# V8151 |

## B. Labware Requirements for the Biomek® FX

| Part Description | Beckman Coulter Part Number |
|---|---|
| Collection plates | Provided in Wizard® MagneSil® Plasmid DNA Purification Kit |
| 96-well 2ml square deep-well plate | 267007 |

## C. Biomek® FX Initial Deck Configuration

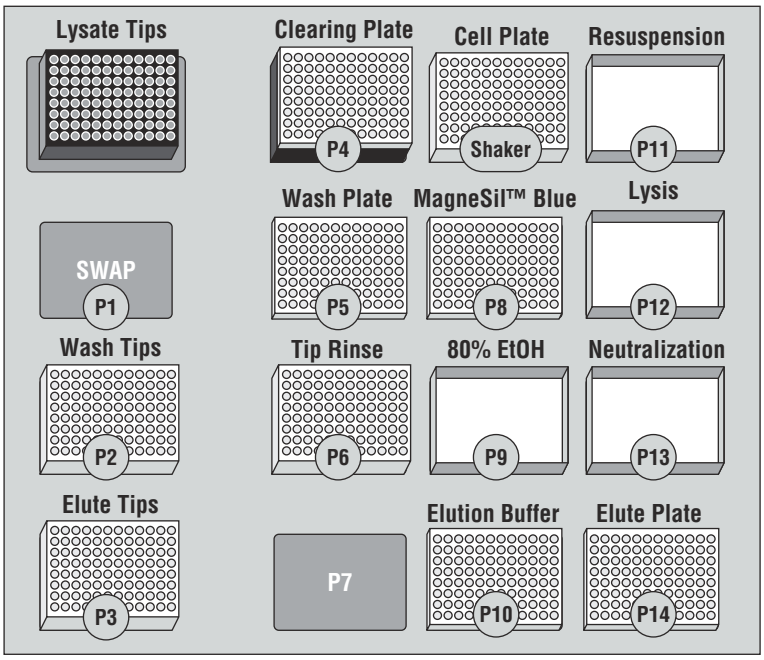



**Figure 3. Biomek® FX initial deck configuration.**

| ALP Name | Equipment |
|---|---|
| Tip Loader | 200µl non-ART Biomek® FX tips |
| P1 | Swap spot |
| P2 | 200µl non-ART Biomek® FX tips |
| P3 | 200µl non-ART Biomek® FX tips |
| P4 | MagnaBot® Separation Device with a Greiner 96-well U-bottom plate on top |
| P5 | Greiner 96-well U-bottom plate containing 50µl MagneSil® RED per well |
| P6 | 96-well square deep-well plate filled completely with deionized water |
| P7 | Empty |
| Shaker | 96-well square deep-well plate containing bacterial cell pellet sample |
| P8 | Greiner 96-well U-bottom plate containing 40µl MagneSil® BLUE per well |
| P9 | Upside-down tip box lid containing 80% ethanol |
| P10 | Greiner 96-well U-bottom plate containing 125µl Elution Buffer per well |
| P11 | Upside-down tip box lid containing 40ml Cell Resuspension Solution |
| P12 | Upside-down tip box lid containing 40ml Cell Lysis Solution |
| P13 | Upside-down tip box lid containing 40ml Neutralization Solution |
| P14 | Greiner 96-well U-bottom plate used for elution |

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

The Biomek® FX automated platform allows users the flexibility to configure the robot's deck according to need. Because of this flexibility, 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. Follow the instructions provided: *Biomek® FX Deck Mapping*
**www.promega.com/automethods/beckman/biomekfx/default.asp**

## VI. Automated Processing Requirements for the Tecan Genesis® RSP150 Workstation

### A. Instrumentation Requirements for the Genesis® RSP150

| Part Description | Quantity | Ordering Information |
|---|---|---|
| Gemini version 3.5 software | 1 | Contact Tecan |
| 1ml syringes | 8 | Contact Tecan |
| Standard fixed tips | 8 | 612530 |
| Carrier for Three Reagent Troughs (100ml) | 3 | 61-449 |
| Reagent Troughs 100ml (100 pcs) | 1 | 70-744 |
| Robotic manipulator arm (RoMa) | 1 | Contact Tecan |
| Te-Shake orbital shaker (1 or 2 position) | 1 | Contact Tecan |
| Carrier for Microplates (3 position) | 2 | 61-418 |

### B. Labware Requirements for the Genesis® RSP150

| Part Description | Quantity | Ordering Information |
|---|---|---|
| MagnaBot® 96 Magnetic Separation Device | 1 | Promega Cat.# V8151 |
| MagnaBot® Adapter T1 | 1 | Promega Cat.# V8481 |
| 2ml deep-well plate (or comparable) | 1 | Marsh Cat.# AB-0932 |
| Polystyrene U-bottom multiwell plate | 4 | Provided in Wizard® MagneSil® Plasmid DNA Purification Kit |

## C. Genesis® RSP150 Initial Workspace Configuration

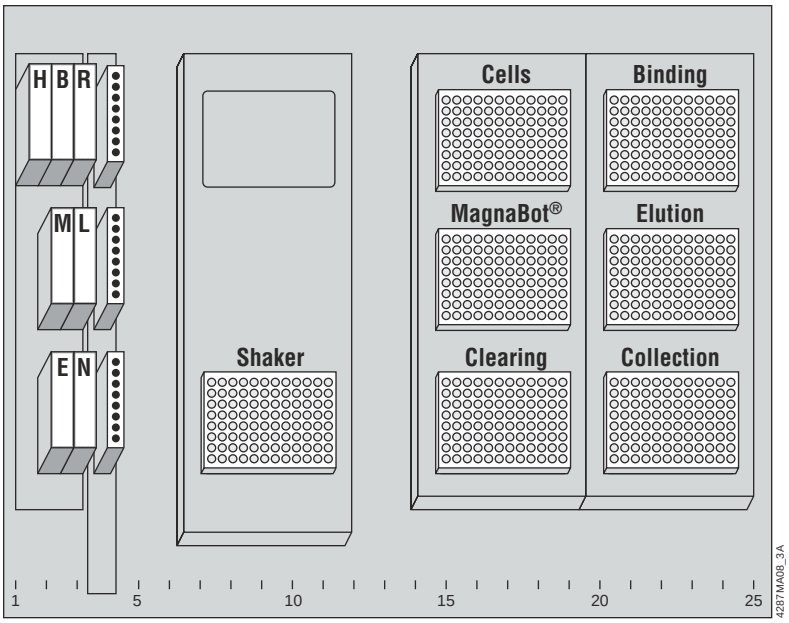



**Figure 4. Tecan Genesis® RSP150 initial workspace configuration.**

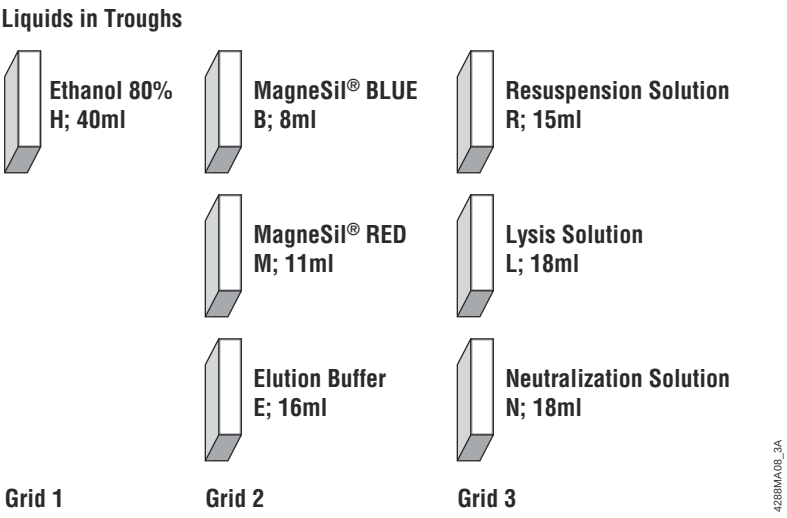



**Figure 5. Placement of liquids and reagents in containers.**

**Setting up the Workspace**

Carriers, racks and containers are placed on the Genesis® RSP150 workspace as shown in Figures 4 and 5:

| Grid Number | Instrumentation | Labware | Labware Position |
|---|---|---|---|
| 1 | 100ml trough carrier | 100ml trough | Rear |
| 2 | 100ml trough carrier | 100ml trough | Rear |
| | | 100ml trough | Middle |
| | | 100ml trough | Front |
| 3 | 100ml trough carrier | 100ml trough | Rear |
| | | 100ml trough | Middle |
| | | 100ml trough | Front |
| 4 | Wash Station | | |
| 6 | Te-Shake | 96-well microplate | Front |
| 13 | Microplate carrier | Culture plate | Rear |
| | | MagnaBot® on MagnaBot® Adaptor T1 | Middle |
| | | Microplate for Clearing | Front |
| 19 | Microplate carrier | Microplate for Binding | Rear |
| | | Microplate for Elution | Middle |
| | | Microplate for Collection | Front |

### D. Genesis® RSP150 Specific Pre-Run Recommendations

Due to differences in instrument reference positions, carriers, plate types and Te-Shake configurations, it is recommended to check and adjust the following settings prior to running the method:

a. X,Y,Z coordinates for each plate and carrier type used in the method
b. RoMa vectors
c. Te-Shake: Orbit = 3mm; Counterweight = none

## VII. Description of the Automated Wizard® MagneSil® Plasmid DNA Purification System

This overview describes the general liquid-handling steps required for the Automated Wizard® MagneSil® Plasmid DNA Purification 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 VIII, General Guidelines for Adaptation to Alternative Robotic Platforms.

1. **Cell Resuspension.** 90µl of Cell Resuspension Solution is added to each well of the deep-well culture plate. The cells are resuspended by thorough tip mixing, then shaken for 2.5 minutes to ensure the cell pellets are well resuspended.
2. **Cell Lysis.** 120µl of Cell Lysis Solution is added to each well of the deep-well culture plate and shaken for 3 minutes to mix.
3. **Cell Lysate Neutralization.** 120µl of Neutralization Solution is added to each well of the deep-well culture plate and shaken for 2 minutes to mix. A flat, floating precipitate should form in each well.
4. **Preparation for Cell Lysate Clearing.** The lysate clearing process begins with a transfer of 25µl of MagneSil® BLUE to each well of the cell culture

plate and pipetting up and down to mix the MagneSil® BLUE and cell lysate. The deep-well culture plate then shakes for 1 minute to mix.

5. **Cell Lysate Clearing.** 300µl of cell lysate is transferred from the deep-well culture plate to a working plate (Greiner U-bottom 96-well plate). The cell lysate is then cleared by placing the 96-well plate onto the MagnaBot® for 90 seconds. This allows the bound lysate/cell debris to remain localized on the magnet, while 240µl of supernatant containing plasmid DNA is removed and transferred to a U-bottom 96-well plate containing 50µl of MagneSil® RED.
6. **DNA Binding.** The MagneSil® RED and sample are mixed well by pipet-tip mixing. The 96-well plate is then placed on the MagnaBot® to capture the particles containing plasmid DNA. The supernatant is removed to waste.
7. **Washing.** The MagneSil® RED particles are washed 3 times with 100µl of 80% EtOH. During each wash, the particles are mixed by shaking for 1 minute. The particles are captured on the MagnaBot®, and the supernatant is removed to waste.
8. **Drying and Elution.** Following the last EtOH wash, the particles are air-dried for 10 minutes. Heat-drying may be performed, which will shorten this incubation time. Following the drying step, the plasmid DNA is eluted from the particles by adding 100µl of Elution Buffer and mixing well by pipetting. The particles are then captured on the MagnaBot®, and the supernatant is saved in a clean 96-well multiwell plate.
9. **Method Completion.** Purified plasmid DNA is contained in the 96-well multiwell elution plate.

## VIII. General Guidelines for Adaptation to Alternative Robotic Platforms

Following the final ethanol removal, it is critical to pause and allow the plate to dry to remove all possible residual ethanol. Carryover of ethanol in eluted purified products may inhibit downstream applications. If carryover of ethanol in eluted purified DNA is a problem, integration of a heating step during drying will improve evaporation of ethanol.

The MagneSil® BLUE and RED paramagnetic particles used for this purification settle rapidly. Ensure that the MagneSil® Paramagnetic Particles are completely resuspended in the bottle before dispensing into processing plates. When pipetting MagneSil® Paramagnetic Particles, reduce aspiration speeds from maximum settings to prevent aspiration of the particles up into liquid handling instrumentation.

Integrated shakers may differ between automated liquid-handling systems. MagneSil® Paramagnetic Particles should be mixed so that the particles are thoroughly resuspended in solution during the purification process for optimal performance. Take care that shaker settings are not so vigorous to cause splashing of the MagneSil® Paramagnetic Particles between wells, therefore causing cross-contamination of samples.

[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.

[b]U.S. Pat. No. 6,284,470 has been issued to Promega Corporation for kits for cell concentration and lysate clearance using paramagnetic particles. Other patents are pending.





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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