# Automated MagneHis™ Protein Purification System

**Automated Protocol #EP011**

**DESCRIPTION OF THE BECKMAN COULTER BIOMEK® 2000 AND BIOMEX® FX, AND TECAN GENESIS® AND FREEDOM EVO™ METHODS FOR PRODUCT V8550.**
**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 document describes automation of the MagneHis™ Protein Purification System[a,b,c]. Specific instructions are provided for the Beckman Coulter Biomek® FX and Biomek® 2000, and Tecan Genesis® and Freedom EVO™ automated liquid-handling workstations. Validated methods for these liquid-handling workstations are available at: **www.promega.com/automethods/**. General automation guidelines are provided for adaptation to other liquid handling platforms. For troubleshooting chemistry issues please refer to the *MagneHis™ Protein Purification System Technical Manual* #TM060.

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

## II. Product Components

| Product | Size | Cat.# |
|---|---|---|
| MagneHis™ Protein Purification System | 10ml | V8550 |

Includes:

- 250ml MagneHis™ Binding/Wash Buffer
- 50ml MagneHis™ Elution Buffer
- 60ml FastBreak™ Cell Lysis Reagent, 10X
- 10ml MagneHis™ Ni-Particles
- 2 vials DNase I (lyophilized)
- 1 Protocol

***Do not freeze*** the MagneHis™ Ni-Particles.

**Storage Conditions:** Store all MagneHis™ Protein Purification System components at 4°C. **Do not freeze the MagneHis™ Ni-Particles.** The DNase I (lyophilized) may be stored at room temperature. Upon resuspension in water, store the DNase I in aliquots at –20°C. For convenience, the resupended DNase I may be stored at 4°C for up to one week. **FastBreak™ Cell Lysis Reagent** may form a precipitate at low temperature. If this occurs, warm the reagent to room temperature before use.

## III. Before You Begin

### Materials to Be Supplied by the User

- Tabletop centrifuge capable of 1,500 x *g*.
- Deep Well MagnaBot® 96 Magnetic Separation Device (Promega Cat.# V3031)
- 1/4 inch Foam Spacer (Cat.# Z3301)
- 2.2ml deep-well, square-well culture plates (Promega Cat.# V6781)
- 1.2ml deep-well, square-well culture plates (Promega Cat.# V6771)
- U-bottom multiwell plates (Promega Cat.# A9161)

### For Biomek® FX:

- Pyramid-bottom reservoir plates (Promega Cat.# V6801)
- Beckman Coulter Orbital Shaker ALP

### For Biomek® 2000:

- Twinivoir low-volume, quarter-vertical reservoir (Acme-Automation Cat.# C5001)
- Variomag Teleshake (3mm orbit) (Promega Cat.# V6751)
- Shaker Integration Plate for Biomek 2000 (Promega Cat.# V3691)

### For Tecan Workstations:

- Tecan Te-Shake Orbital shaker (3mm orbit)

**Sample Preparation Before Automated Processing:** If the bacterial cell pellets are frozen, thaw at room temperature for 10–15 minutes before use.

For cell pellets, dilute the supplied FastBreak™ Cell Lysis Reagent to 1X concentration with water before use. For cell cultures, the supplied 10X FastBreak™ Reagent is added directly to the cell cultures at a 1:10 ratio (i.e., 100µl 10X Fastbreak™ Reagent to 900µl culture volume).

## IV. Automated Processing Requirements for the Beckman Biomek® FX

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

| Part Description | Quantity | Beckman Coulter Part Number |
|---|---|---|
| Minimum: Biomek® FX software version 2.5 | | Contact Beckman Coulter |
| 96-channel POD | 1 | Contact Beckman Coulter |
| Labware positions for 1 POD | 11 | Contact Beckman Coulter |
| Tip loader ALP | 1 | 719356 |
| Orbital shaker ALP (optional) | 1 | Contact Beckman |

### B. Labware Requirements for the Biomek® FX

| Part Description | Quantity | Part Number |
|---|---|---|
| Deep Well MagnaBot® 96 Magnetic Separation Device | 1 | Promega Cat.# V3031 |
| 2.2ml deep-well plate or comparable | 2 | Promega Cat.# V6781 |
| 1/4 inch Foam Spacer | 1 | Promega Cat.# Z3301 |
| 1.2ml deep-well plate (or comparable) | 1 | Promega Cat.# V6771 |
| Pyramid-bottom 96-well reservoirs (or comparable) | 3 | Promega Cat.# V6801 |
| Polystyrene U-bottom multiwell plates (or comparable) | 2 | Promega Cat.# A9161 |
| 200 tips-barrier (rack) | 3 | Beckman Cat.# 717253 |

## C. Initial Deck Layout for the Biomek® FX

This is an example of the MagneHis™ Protein Purification System deck layout on a Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

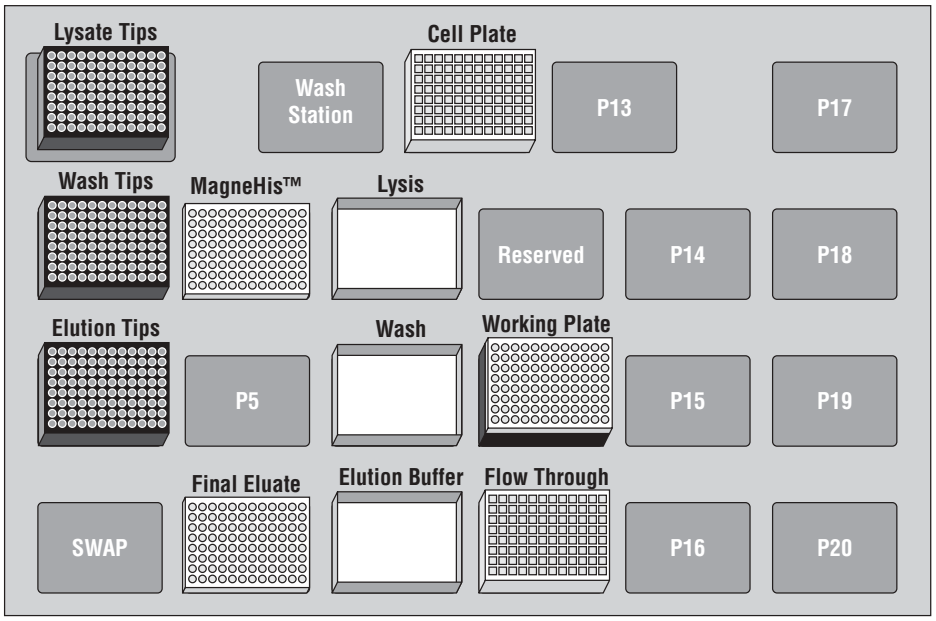


**Figure 1. Inital deck configuration of the Beckman Biomek® FX.**

| ALP Name | Equipment |
|---|---|
| Tip Loader | 200µl ART® Biomek® FX Tips |
| P1 | 200µl ART® Biomek® FX Tips |
| P2 | 200µl ART® Biomek® FX Tips |
| P3 | Empty: Tip box swap spot |
| WS1 | Tip Wash Station (96 channel; not necessary for method) |
| P4 | 96-well, round-bottom plate containing 45µl of MagneHis™ Resin/well (labeled “MagneHis”) |
| P5 | Empty |
| P6 | Empty 96-well round-bottom plate (labeled “Final Eluate”) |
| P7 | Pyramid-bottom reservoir plate containing 25ml FastBreak™ Cell Lysis Reagent (10X concentration for cultures; 1X concentration for cell pellets) + 25µl DNase I, if desired (labeled “Lysis”) |
| P8 | Pyramid-bottom reservoir plate containing 50ml MagneHis™ Binding/Wash Buffer (labeled “Wash”) |
| P9 | Pyramid-bottom reservoir plate containing 25ml MagneHis™ Elution Buffer (labeled “Elution”) |
| P10 | Empty. Reserved for plate movement |
| P11 | Empty 1.2ml 96-well, round-bottom plate (labeled “Working Plate”) on a Deep Well MagnaBot® 96 Magnetic Separation Device with 1/4 inch Spacer |
| P12 | Empty 2.2ml deep-well square-well plate to collect flowthrough (labeled Flow-thru”) |
| Orbital 1 | 2.2ml deep-well square-well culture plate containing 900µl cell culture in suspension or cell pellets (labeled “Cell Plate”) |

### 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 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 do this, follow the instructions provided in the document **Deck Mapping on the Biomek® FX** (www.promega.com/automethods/beckman/biomekfx/default.asp).

## V. Automated Processing Requirements for the Beckman Biomek® 2000

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

| Part Description | Quantity | Beckman Coulter Part Number |
|---|---|---|
| Biomek® 2000 Workstation, 50/60 Hz, 100-120V | 1 | 609000 |
| Biomek® 2000 controller NT | 1 | 609875 |
| BioWorks 3.2 for Beckman Coulter computer | 1 | 609983 |
| MP200 pipetting tool | 1 | 609025 |
| Gripper tool system for Biomek® 2000 | 1 | 609001 |
| Tip rack holder | 2 | 609121 |
| Gray labware holder | 6 | 609120 |
| Reservoir holder | 1 | 372795 |
| Quarter vertical reservoir | 1 | 372788 |
| Quarter single reservoir | 2 | 372790 |

### B. Labware Requirements for the Biomek® 2000

| Part Description | Quantity | Ordering Information |
|---|---|---|
| Deep Well MagnaBot® 96 Magnetic Separation Device | 1 | Promega Cat.# V3031 |
| 1/4 inch Foam Spacer | 1 | Promega Cat.# Z3301 |
| 2.2ml deep-well plate or comparable | 2 | Promega Cat.# V6781 |
| 1.2ml deep-well plate | 1 | Promega Cat.# V6771 |
| Polystyrene U-bottom multiwell plates or comparable | 1 | Promega Cat.# A9161 |
| Biomek® 2000 standard 250 tips (rack) | 2 | Beckman Cat.# 717253 |
| Twinivoir low volume quarter vertical reservoir | 1 | Acme-Automation Cat.# C5001 |

## C. Initial Deck Layout for the Biomek® 2000

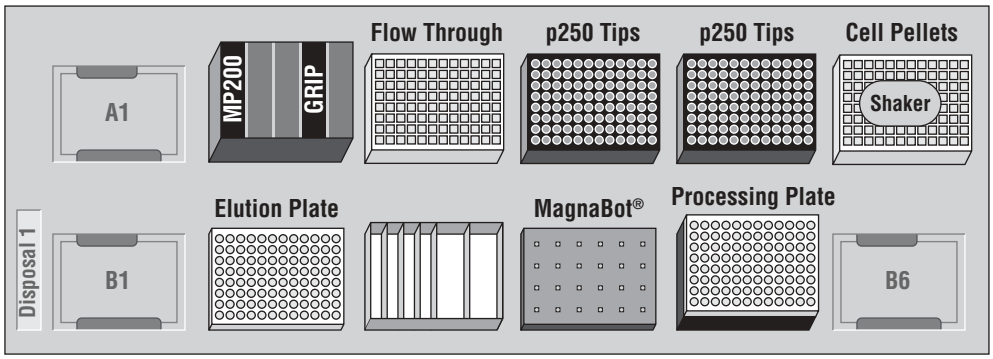


**Figure 2. Beckman Biomek® 2000 initial deck configuration.**

| Position | Equipment |
|---|---|
| A1 | Empty (not used in method) |
| A2 | Tool rack containing MP200 and Gripper tools |
| A3 | Labware holder, 2.2ml deep-well, square-well plate for flowthrough (labeled “Flow Thru”) |
| A4 | Tip rack holder, P250 tips |
| A5 | Tip rack holder, P250 tips |
| A6 | Teleshake. 2.2ml deep-well, square-well plate containing samples (labeled “Culture Plate” or “Cell Pellets”) |
| B1 | Empty (not used in method) |
| B2 | Labware holder, empty 96-well U-bottom plate (labeled “Elution Plate”) |
| B3 | Labware holder, reservoir holder, 1 quarter vertical reservoir, 1 Twinivoir reservoir, and 2 quarter single reservoirs |
| B4 | Labware holder, Deep Well MagnaBot® 96 Magnetic Separation device with 1/4 inch Foam Spacer |
| B5 | Labware holder, empty 1.2ml deep-well U-bottom plate (labeled “Processing Plate”) |
| B6 | Labwate holder |

## D. Reagent Dispense Volumes for the Biomek® 2000

Prior to beginning run, the following MagneHis™ Protein Purification System reagents need to be dispensed appropriately on the deck of the Biomek® 2000:

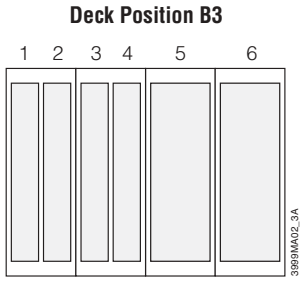


**Figure 3. Reagent dispense volumes at Deck Position B3.**

1. 11ml Elution Buffer
2. 11ml 10X FastBreak™ Cell Lysis Reagent (for cell cultures)
   **or** 22ml 1X FastBreak™ Cell Lysis Reagent (for cell pellets)
   25µl DNase I may be added to the FastBreak™ Reagent if desired.
3. 4ml MagneHis™ Ni-Particles
4. Empty
5. 45ml Binding/Wash Buffer
6. Empty (for waste collection)

## VI. Automated Processing Requirements for the Tecan Genesis® and Freedom EVO™ Workstations

### A. Instrument Requirements for the Tecan Workstations

| Part Description | Quantity | Tecan Part Number |
|---|---|---|
| Tecan Genesis RSP100 RSP150 or RSP200 or Freedom EVO™ 100, 150 or 200 | 1 | Contact Tecan |
| Gemini 4.2 software | 1 | Contact Tecan |
| 1ml syringes | 8 | Contact Tecan |
| Standard fixed tips | 8 | 612501 |
| 100ml reservoir carriers | 2 | 613020 |
| 100ml reservoirs | 4 | 613021 |
| Robotic manipulator arm (RoMa) | 1 | Contact Tecan |
| Te-Shake orbital shaker (1 or 2 position) | 1 | Contact Tecan |
| 3-position microplate carrier | 2 | Contact Tecan |

### B. Labware Requirements for the Tecan Workstations

| Part Description | Quantity | Ordering Information |
|---|---|---|
| Deep-well MagnaBot® 96 Magnetic Separation Device | 1 | Promega Cat.# V3031 |
| 1/4 inch Foam Spacer | 1 | Promega Cat.# Z3301 |
| 1.2ml deep-well plate | 1 | Promega Cat.# V6771 |
| MagnaBot® Adaptor T1 | 1 | Promega Cat.# V8481 |
| 2.2ml deep-well plate (or comparable) | 2 | Promega Cat.# V6781 |
| Polystyrene U-bottom multiwell plate (or comparable) | 1 | Promega Cat.# A9161 |

## C. Initial Deck Layout for the Tecan Workstations

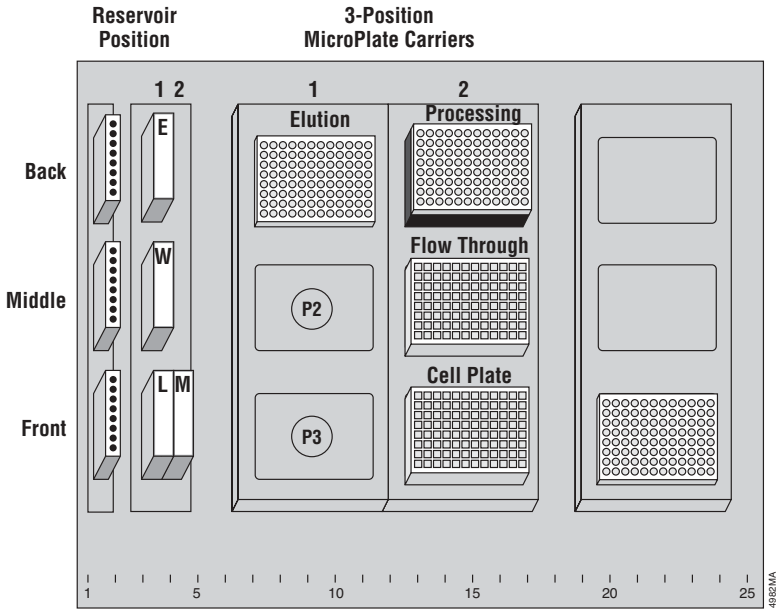


**Figure 5. Tecan Genesis® and Freedom EVO™ initial deck configuration.**

| Reservoir Position | Column 1 | Column 2 |
|---|---|---|
| Back | 15ml MagneHis™ Elution (labeled E) | Empty |
| Middle | 35ml MagneHis™ Binding/Wash (labeled W) | Empty |
| Front | 25ml 1X FastBreak™ Lysis Reagent (for cell pellets) **or** 15ml 10X FastBreak™ Lysis Reagent (for cell cultures) (labeled L) + 25µl DNase I, if desired | 10ml MagneHis™ Resin (labeled M) |

| Te-Shake Position | Rack on Carrier |
|---|---|
| Back | Empty |
| Front | Empty (deck image shows a plate for pipetting to later) |

| 3-Position Microplate Carrier | Rack on Carrier |
|---|---|
| Position 1 | Empty U-bottom multiwell plate (labeled “Elution”) |
| Position 2 | Empty |
| Position 3 | Empty |

| 3-Position Microplate Carrier | Rack on Carrier |
|---|---|
| Position 1 | Empty 1.2ml deep-well plate on MagnaBot® T1 Adaptor, Deep Well MagnaBot® 96 Magnetic Separation Device with 1/4 inch Foam Spacer |
| Position 2 | 2.2ml deep-well, square-well plate(labeled “Flow-through”) |
| Position 3 | 2.2ml deep-well plate containing either cell pellets or cell culture. |

### D. Tecan Workstation 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 Automated MagneHis™ Protein Purification System

This overview describes general liquid handling steps required for the automated MagneHis™ Protein Purification 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 VIII, General Guidelines for Adaptation to Alternative Robotic Platforms.

1. **MagneHis™ Particle Dispense.** MagneHis™ Ni-Particles are mixed in the reservoir, then 30µl aliquots are dispensed into a 1.2ml deep-well plate.
2. **Cell Pellet Resuspension/Lysis.** Cell pellets contained in the deep-well 96-square-well culture plate are resuspended with FastBreak™ Cell Lysis Reagent by a combination of mixing and shaking for 5 minutes. If processing cell pellets, 200µl 1X FastBreak™ Reagent is used. If processing cell cultures, 100µl 10X FastBreak™ Reagent is used.
   **Note:** to overcome sticky or 'slimy' cell lysates add 1µl/well of resuspended DNase I.
3. **Bind Protein to MagneHis™ Ni-Particles.** The lysate is added to the MagneHis™ Ni-Particles to initiate binding of His-tagged proteins. The lysate is mixed well with the particles by shaking for 1 minute. After shaking, the plate is placed onto the Deep Well MagnaBot® Device to capture the particles. The supernatant, containing unbound materials, can be saved for analysis or discarded to waste.
4. **Washes.** Three washes are performed with the MagneHis™ Binding/Wash Buffer to remove residual impurities from the sample. During each wash, 100–150µl of MagneHis™ Binding/Wash Buffer is added to the MagneHis™ Ni-Particles. The particles are mixed well by shaking for 1 minute. After shaking, the particles are captured on the Deep Well MagnaBot® Device, and the supernatant is discarded to waste. This process is repeated 2 more times.
5. **Elution.** The samples are eluted from the particles by adding 100µl of MagneHis™ Elution Buffer and mixing well for 1 minute on the shaker. After shaking, the particles are captured on the Deep Well MagnaBot® Device, and the supernatant is saved in a collection plate for analysis.

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

The MagneHis™ Ni-Particles settle over time. It is recommended to thoroughly mix the MagneHis™ Ni-Particles on the automated platform prior to dispensing into samples. Resuspension of the MagneHis™ Ni-Particles can be accomplished by thorough mixing or shaking.

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[a]Patent Pending.

[b]Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

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**Promega Corporation**
2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Telephone 608-274-4330
Fax 608-277-2516
Internet www.promega.com