

# Automatic Protein Purification



## Automated Polyhistidine-Tagged Protein Purification Using the MagneHis™ Protein Purification System

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### Abstract

Automated high-throughput protein purification using Promega's MagneHis™ Protein Purification System provides a rapid, reliable and walkaway system for the purification of bacterially expressed, polyhistidine-tagged proteins. Automated methods have been developed to purify proteins from 1ml of bacterial cell culture on several automated workstations. The MagneHis™ protein chemistry is easily scalable to accommodate a variety of sample volumes.

We have developed a magnetic, mobile-solid phase system to purify polyhistidine-tagged proteins from bacterial cultures in an automated, walkaway manner.

### Introduction

Rapid exploration of novel proteins requires high-quality, easy-to-use high-throughput protein purification. Expression and purification of recombinant proteins using a polyhistidine tag is a commonly used strategy for obtaining specific highly pure proteins in large quantities. We have developed a magnetic, mobile-solid phase system to purify polyhistidine-tagged proteins from bacterial cultures in an automated walkaway manner. By automating the purification process in settings where the workflow and sample throughput warrant, scientists can shift their efforts from sample processing to data analysis.

The MagneHis™ Protein Purification System<sup>(a,b)</sup> contains a unique Cell Lysis Reagent that is complementary to automation. This component allows efficient resuspension and lysis of bacterial cell pellets, without sonication and centrifugation (1), unlike other polyhistidine-tagged protein purification systems. We present data generated from several automated workstations in standard 96-well plate formats. Depending on the automated platform being used, this chemistry is easily scalable to process larger sample volumes with corresponding increases in yield.

### Automated Workstations

Automated methods were developed on four different instruments including , from low- to high-throughput capabilities: Thermo Labsystems Kingfisher®, Beckman Biomek® 2000 Laboratory Automation Workstation (Figure 1), Tecan Genesis® RSP (Figure 2), and Beckman Biomek® FX Laboratory Workstation (Figure 3). The Biomek® 2000, Biomek® FX and the Tecan Genesis® RSP

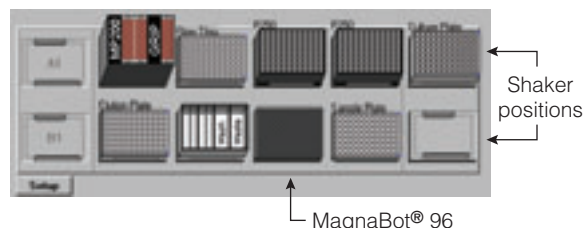


Figure 1. Initial deck configuration of the Beckman Biomek® 2000.

Hardware and labware requirements include the BioWorks™ Version 3.2 software, MP200 and Gripper tools, 1 Quarter Divided Vertical Reservoir, 2 Quarter Reservoirs, 1 Twinivior Low Volume Reservoir (ACME-Automation), Beckman DPC MicroMix® 5 Shaker and Integration Kit, 2 boxes of P250 tips, one 2ml deep-well plate, 3 Greiner U-bottom polystyrene 96-well plates, 1 MagnaBot® 96 Magnetic Separation Device.

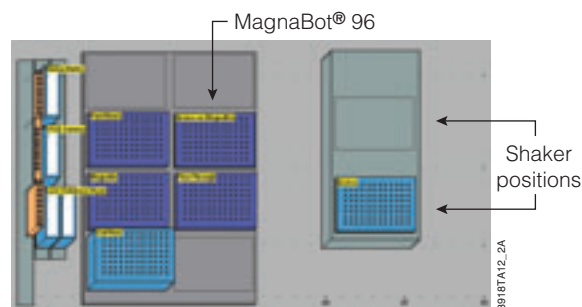


Figure 2. Initial deck configuration of the Tecan Genesis® RSP 150.

Hardware and labware requirements include the Gemini Version 4.0 software, 1ml syringes, 8 standard fixed tips, two 100ml reservoir carriers, four 100ml reservoirs, Te-Shake orbital shaker (1 or 2 position shaker), Robotic Manipulator Arm, one 8-position microplate carrier, one 2ml deep-well plate, 4 Greiner U-bottom polystyrene 96-well plates, a MagnaBot® 96 Magnetic Separation Device, 1 MagnaBot® Adaptor T1 (Cat.# V8481).

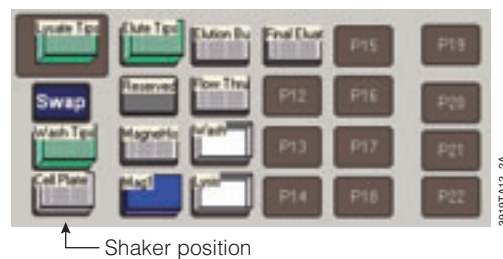


Figure 3. Initial deck configuration of the Beckman Biomek® FX.

Hardware and labware requirements include Version 2.1 software, 96-channel POD, Orbital Shaker ALP, 11 accessible positions, 3 boxes of P250 tips, one 2ml deep-well plate, 4 Greiner U-bottom polystyrene 96-well plates, 2 Greiner pyramid bottom reservoirs, 1 MagnaBot® 96 Magnetic Separation Device.

instruments are liquid handlers, capable of processing 96-sample formats, moving plates and manipulating liquid reagents throughout the procedure. The Thermo Labsystems Kingfisher® is unique in that it operates by moving magnetic particles, not liquid reagents, through a series of wells containing manually predispensed lysate, wash buffer and elution reagents. Due to this difference in format, the Kingfisher® is capable of processing up to 24 samples in a single automated run.

### Automated Procedure

Polyhistidine-tagged proteins 18 to 123kDa in size were expressed in either JM109 or BL21(DE3)pLysS *E. coli* bacterial strains and purified using the MagneHis™ Protein Purification System. These proteins included RNase HI, humanized *Renilla* luciferase, RNasin® Ribonuclease Inhibitor<sup>(d)</sup>, thermostable firefly luciferase, methionyl tRNA synthetase and β-galactosidase. Bacterial cultures were grown in 96-well deep-well square culture plates at 37°C (or at 25°C for cells expressing RNasin® Ribonuclease Inhibitor) in 1ml of LB medium per well to < 2.0 OD<sub>600</sub>. Cells were pelleted by centrifugation. Each automated workstation was optimized to perform the following steps of the purification process. Note that unique mechanical operations make the Kingfisher® procedure different than those of the Biomek® 2000, Biomek® FX and Tecan Genesis® platforms.

**Cell Pellet Resuspension/Lysis.** For the Biomek® 2000, Biomek® FX and Tecan Genesis® RSP instruments, the 96-well, deep-well square culture plate containing the pelleted cells received 200µl of MagneHis™ Cell Lysis Reagent per well. The cell pellets were resuspended by a combination of tip mixing and orbital shaking for 5 minutes. Efficient resuspension of the cells required both tip mixing and shaking.

For the Kingfisher® method, the pelleted cells were first resuspended manually with 200µl MagneHis™ Cell Lysis Reagent and then transferred into Row B of 2 Kingfisher® purification plates.

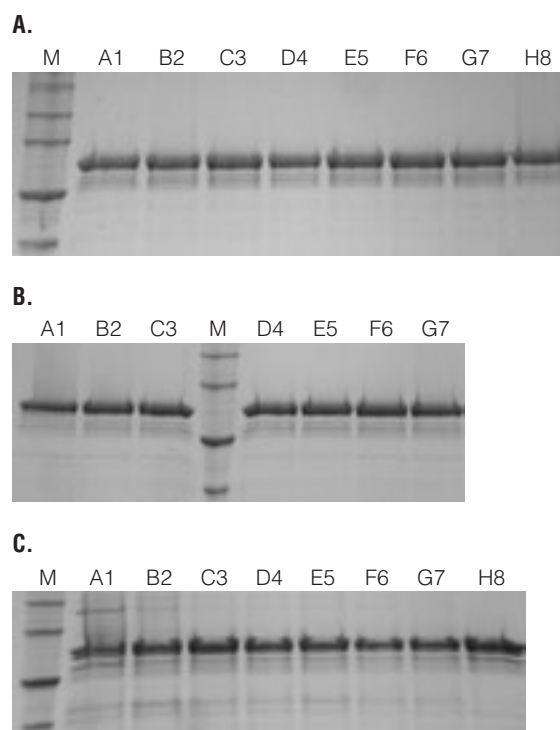
**Protein Binding to MagneHis™ Ni-Particles.** For each 1ml sample of bacterial culture, 30µl of MagneHis™ Ni-Particles was added to an empty well of a Greiner U-bottom multiwell plate. The resuspended cell lysates were then added to the MagneHis™ Ni-Particles. The plates were thoroughly mixed on an orbital shaker, and the MagneHis™ Ni-Particles, which selectively bind the polyhistidine-tagged proteins, were captured on the MagnaBot® 96 Magnetic Separation Device (Cat.# V8151). All unbound proteins were collected as flowthrough for analysis (optional). Note that with this volume and plate type it was necessary to process half of the lysate volume at a time due to the space limitations of the wells.

**Washes.** Three 100µl washes with the MagneHis™ Binding/Wash Buffer removed contaminants and residual unbound materials. During each wash, the samples were placed on the orbital shaker for efficient resuspension of the MagneHis™ Ni-Particles.

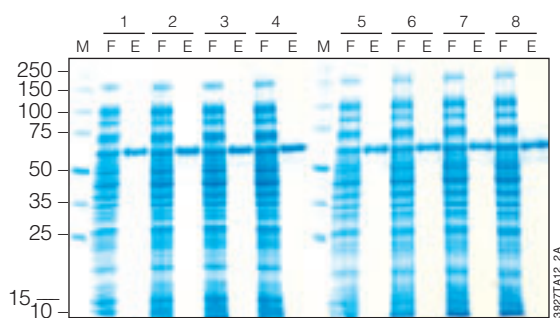
**Elution.** For each sample 100µl of MagneHis™ Elution Buffer was added, and samples were resuspended on the orbital shaker. After shaking, the samples were separated magnetically and the supernatants saved in a fresh 96-well plate for analysis.

### Results

Following each automated run, 20µl of the purified, eluted proteins from various wells across the plate were analyzed by SDS-PAGE. Figures 4 and 5 show purification of polyhistidine-tagged thermostable firefly luciferase using the Biomek® FX (Figure 4, Panel A), Biomek® 2000 (Figure 4, Panel B), Tecan Genesis® RSP (Figure 4, Panel C), or Kingfisher® (Figure 5). Each instrument generated purified protein with a consistent yield across the 96-well plate. Additional proteins, ranging in size from 18 to 123kDa, were also purified using the MagneHis™ Protein Purification System with similar results (data not shown).



**Figure 4. Automated protein purification of polyhistidine-tagged thermostable firefly luciferase using the MagneHis™ Protein Purification System.** Luciferase was purified from bacterial cultures in a 96-well format on the Beckman Biomek® FX (Panel A), Biomek® 2000 (Panel B), and Tecan Genesis® RSP (Panel C). The designations A1–H8 refer to the location of the well within the plate. Lane M corresponds to the Broad Range Protein Molecular Weight Markers (Cat.# V8491).



**Figure 5. Automated protein purification of polyhistidine-tagged thermostable firefly luciferase on the Kingfisher® using the MagneHis™ Protein Purification System.** Eight samples of bacteria expressing polyhistidine-tagged thermostable firefly luciferase were cultured. For each sample, purified protein was eluted from the MagneHis™ Ni-Particles (lanes E), and the flowthrough fractions of proteins not captured by the MagneHis™ Ni-Particles were collected (lanes F). Lane M contains the Broad Range Protein Molecular Weight Markers (Cat.# V8491).

## Instrument Configuration and Throughput Considerations

The Biomek® FX workstation processed all 96 samples simultaneously due to the 96-channel POD configuration. This increased sample throughput considerably. Our Tecan Genesis® RSP 150 model was equipped with 8 standard fixed tips and, like the Biomek® 2000, can pipet 8 samples at a time, thereby increasing the flexibility in the number of samples processed during a single run but decreasing overall sample throughput (Table 1). A Tecan Genesis® RSP with a TeMo 96-channel pipetting tool would result in throughput similar to that of the Biomek® FX workstation.

Robotic throughput varies depending on the instrument and its configuration. While it is possible to process samples faster manually than with the Biomek® 2000 or Tecan Genesis® RSP workstations (Table 1), manual processing of 96 samples at a time is labor intensive, tedious and prone to error.

**Table 1. MagneHis™ Protein Purification System Throughput.**

Method	Time per 96-Well Plate	Time per Sample	Throughput in 8-Hour Shift
Biomek® FX	25 minutes	15 seconds	20 plates
Biomek® 2000	90 minutes	60 seconds	5 plates
Genesis® RSP	90 minutes	60 seconds	5 plates
Kingfisher®	NA <sup>1</sup>	50 seconds	6 plates <sup>2</sup>
Manual	45 minutes <sup>3</sup>	30 seconds	8 plates

<sup>1</sup> Two Kingfisher® plates, each containing 12 samples, processed simultaneously in 20 minutes during a single automated run.

<sup>2</sup> Forty-eight plates, each containing 12 samples (576 samples total) in an 8-hour shift. Equivalent to six 96-well plates.

<sup>3</sup> Approximate.

The Thermo Labsystems Kingfisher® is a very practical instrument for processing small numbers of samples in an automated format relatively quickly without user intervention (Table 1). Two plates of 12 samples each can be processed simultaneously with this instrument. The following reagents must be dispensed manually into each plate prior to the run:

Row A: MagneHis™ Ni-Particles

Row B: Cell pellets resuspended in MagneHis™ Cell Lysis Reagent

Row C: MagneHis™ Binding/Wash Buffer

Row D: MagneHis™ Binding/Wash Buffer

Row E: MagneHis™ Binding/Wash Buffer

Row F: MagneHis™ Elution Buffer

## Conclusions

We have automated the MagneHis™ Protein Purification System for the rapid purification of polyhistidine-tagged proteins on several automated workstations. The innovative MagneHis™ Cell Lysis Reagent eliminates the need for manual intervention (no sonication or centrifugation steps during the purification process) and allows for a truly walkaway protein purification system. Methods have been developed for the Biomek® 2000, Biomek® FX, Tecan Genesis® RSP and Kingfisher® automated platforms. These methods allow a quick start-up in your laboratory and offer several key advantages for end-users. These advantages include a simple process that is easily automated on a variety of platforms for high-throughput applications, chemistry that is easily scaled up for larger sample volumes and high-quality purified proteins with few background bands.

## Reference

1. Godat, B. *et al.* (2003) *Promega Notes* **83**, 2–5

## Protocols

- ◆ *MagneHis™ Protein Purification System Technical Manual #TM060*, Promega Corporation ([www.promega.com/tbs/tm060/tm060.html](http://www.promega.com/tbs/tm060/tm060.html))



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## Ordering Information

Product	Size	Cat.#
MagneHis™ Protein Purification System <sup>(a,b)</sup>	2ml	V8500
	10ml	V8550
MagneHis™ Ni-Particles <sup>(a,b)</sup>	2ml	V8560
	10ml	V8565
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
MagnaBot® Adapter T1	1 each	V8481

<sup>(a)</sup> Patent Pending.

<sup>(b)</sup> 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.

<sup>(c)</sup> U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

<sup>(d)</sup> U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

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