

Automated HisLink™ 96 Protein Purification System

Automated Protocol #EP028

DESCRIPTION OF THE BIOMEK® 2000 METHOD FOR PRODUCTS
V3680 AND V3681

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I. Description

This document describes automation of the HisLink™ 96 Protein Purification System^(a,b,c). Specific instructions are provided for the Beckman Biomek® 2000 automated liquid handling workstation. The method for this liquid handling workstation is 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 *HisLink™ 96 Protein Purification System Technical Bulletin #TB342*.

II. Product Components

Product	Size	Cat. #
HisLink™ 96 Protein Purification System	1 × 96 preps	V3680

Each system contains sufficient reagents for 96 manual purifications from 1ml of bacterial culture. Includes:

- 15ml FastBreak™ Cell Lysis Reagent, 10X^(a,b,c)
- 1 vial DNase I
- 25ml HisLink™ Resin
- 110ml Binding/Wash Buffer
- 25ml Elution Buffer
- 1 Filtration Plate
- 1 Collection Plate
- 1 Protocol

Product	Size	Cat. #
HisLink™ 96 Protein Purification System	5 × 96 isolations	V3681

Each system contains sufficient reagents for 480 manual purifications from 1ml of bacterial culture. Includes:

- 60ml FastBreak™ Cell Lysis Reagent, 10X
- 1 vial DNase I
- 2 × 50ml HisLink™ Resin
- 2 × 280ml Binding/Wash Buffer
- 125ml Elution Buffer
- 5 Filtration Plates
- 5 Collection Plates
- 1 Protocol

Storage Conditions: Store all HisLink™ 96 reagents at 4°C. The plates may be stored at 4°C or at room temperature. Following reconstitution with water, DNase I should be stored in aliquots at –20°C. **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

- Nuclease-Free Water (Cat.# P1195)
- 96-well deep-well plates (e.g., ABgene 2.2ml storage plate, Marsh Bio Products Cat.# AB-0932)
- Wide Bore 250µl Tips (Axygen Cat.# BT-255-WB-R or Molecular BioProducts Cat.# 917-262G)

III.A. Preparation of Cell Culture

Bacterial cultures can be grown in tubes, flasks or 96-well, deep-well plates (Marsh Bio Products Cat.# AB-0932). Grow the culture containing the appropriate polyhistidine-tagged fusion protein to an O.D.₆₀₀ of 0.4–0.6, then induce protein expression. For IPTG induction, add IPTG to a final concentration of 1mM and incubate at 37°C for 3 hours or 25°C overnight. Induction time and IPTG concentration may require optimization. Cultures with concentrations of up to 8.0 O.D.₆₀₀ units/ml have been successfully used with this system. Cells do not need any centrifugation or freezing for this purification system

III.B. Considerations When Adding Lysozyme

The HisLink™ 96 System is designed to lyse cells without the addition of lysozyme. Lysozyme, if added, will co-purify with your polyhistidine-tagged protein unless 500mM NaCl is added to the wash buffer.

III.C. Preparation of FastBreak™ Reagent/DNase I Solution

1. Add the indicated amount of Nuclease-Free Water to the vial of DNase I. (Use 80µl of Nuclease-Free Water for the 1 plate system [Cat.# V3680]. Use 275µl of Nuclease-Free Water for the 5 plate system [Cat.# V3681]).
2. Mix completely to dissolve the powder.
3. Remove the DNase solution from the vial and add the indicated amount of Nuclease-Free Water. Mix well. (Use 1ml of Nuclease-Free Water for the 1 plate system [Cat.# V3680]. Use 4.75ml of Nuclease-Free Water for the 5 plate system [Cat.# V3681]).

Note: Once resuspended, the DNase I solution can be dispensed into working aliquots (e.g., 1ml), stored at –20°C for 6 months, and is stable for 8 freeze-thaw cycles.

4. To prepare the FastBreak™ Reagent/DNase I Solution, add 1ml of DNase I solution to 11ml of FastBreak™ Cell Lysis Reagent, 10X, and mix well.

Note: Once prepared, the FastBreak™ Reagent/DNase I solution can be stored at –20°C for 6 months and is stable for up to 5 freeze-thaw cycles.

IV. Automated Processing Requirements for the Biomek® 2000 Workstation

IV.A. Instrumentation Requirements for the Biomek® 2000

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the HisLink™ 96 Protein Purification System on a Beckman Biomek® 2000 instrument.

Description	Beckman Coulter Part Number
Biomek® 2000 Workstation, 50/60Hz, 100–120V	609000
Biomek® 2000 Controller NT	609875
IBM Monitor	974571
BioWorks™ 3.2 for Beckman Coulter Computer	609983
Gripper Tool System for Biomek® 2000	609001
Worksurface Spill Tray	609077
MP200 Pipetting Tool	609025
Tip Rack Holder (2)	609121
Gray Labware Holder (4)	609120
Collar Holders	609736
Vacuum Valve Unit	609005
Vacuum Filtration Manifold Base	609670
36mm Vacuum Collar	609597
Vacuum Regulator	609674
Tubing Kit, Filtration System	609676
Tubing Kit, Wash Unit	609687
Plastic Bottle, 4L	975796
Cap	975797
Reservoir Holder	372795
Quarter Single Reservoirs	372790
Quarter Vertical Reservoirs	372788
Full Reservoirs	372784

IV.B. Labware Requirements for the Biomek® 2000

Description	Ordering Information
96-well Elution Plate	provided in HisLink™ 96 Protein Purification System
HisLink™ 96 Filtration Plate	provided in HisLink™ 96 Protein Purification System
96-well 2.2ml deep-well culture plate	provided by user
250µl Wide Bore Tips	Axygen Cat. # BT-255-WB-R Molecular BioProducts Cat. # 917-262G

IV.C. Biomek® 2000 Initial Deck Configuration



Figure 1. Beckman Coulter Biomek® 2000 initial deck configuration.

- Position A1: Empty
 - Position A2: Tool rack containing MP200, gripper tools
 - Position A3: Empty
 - Position A4: Labware collar holder (**Place folded paper towel on collar holder for blotting filtration plate**)
 - Position A5: vacuum filtration manifold base, 36mm collar, HisLink™ Filtration Plate***
 - Position A6: Labware holder, HisLink Collection Plate
 - Position B1: tip rack holder, Wide Bore P250 tips
 - Position B2: tip rack holder, Wide Bore P250 tips
 - Position B3: labware holder, reservoir holder, one quarter vertical reservoir, one quarter single reservoir and two empty positions
 - Position B4: Labware holder, full reservoir
 - Position B5: Labware holder, 2.2ml deep well plate containing 1ml bacterial cultures per well
 - Position B6: Empty
- *** Be sure to set Vacuum Pressure at 10 inches of Hg for this method.

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

Prior to beginning the run, dispense the following reagents as shown at position B3 on the deck of the Biomek® 2000.

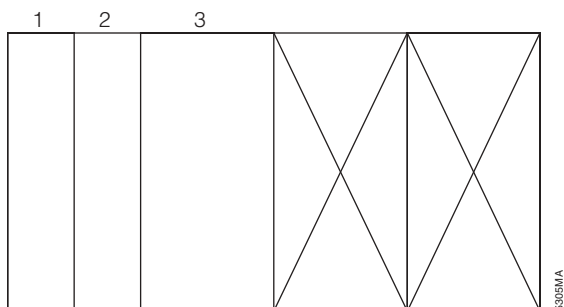


Figure 2. Deck position B3.

1. Fifteen milliliters of HisLink™ Resin. Be sure to thoroughly resuspend the resin before dispensing it to the reagent reservoir. If you are processing less than a full plate of samples, you can add less resin to this reservoir. To calculate how much HisLink™ Resin to add, multiply the number of samples by 0.15ml and then add 2ml for the dead volume in the trough (e.g., for 30 samples use $[30 \times 0.15\text{ml}] + 2\text{ml} = 6.5\text{ml}$).
2. Twelve milliliters of FastBreak™ Reagent/DNase I Solution. If you are processing less than a full plate of samples, you can add less FastBreak™ Reagent/DNase I Solution to this reservoir. To calculate how much FastBreak™ Reagent/DNase I Solution to add, multiply the number of samples by 0.1ml and then add 2ml for the dead volume in the trough (e.g., for 30 samples use $[30 \times 0.1\text{ml}] + 2\text{ml} = 5\text{ml}$).
3. Twenty-five milliliters of HisLink™ Elution Buffer. If you are processing less than a full plate of samples, you can add less HisLink™ Elution Buffer to this reservoir. To calculate how much Elution Buffer to add, multiply the number of samples by 0.2ml and then add 2ml for the dead volume in the trough (e.g., for 30 samples use $[30 \times 0.2\text{ml}] + 2\text{ml} = 8\text{ml}$).

To the full reservoir at position B4 on the deck of the Biomek® 2000 dispense 110ml of HisLink™ 96 Binding/Wash Buffer. If you are processing less than a full plate of samples, you can add less HisLink™ 96 Binding/Wash Buffer to this reservoir. To calculate how much Binding/Wash Buffer to add, multiply the number of samples by 1ml and then add 2ml for the dead volume in the trough (e.g., for 30 samples use $[30 \times 1\text{ml}] + 2\text{ml} = 32\text{ml}$)

Note: Any extra DNase I Solution can be dispensed into aliquots (e.g., 1ml) and stored at -20°C for up to 6 months and is stable for up to 8 freeze-thaw cycles

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

Before running, the method must be imported into the BioWorks™ Software. To import the method, please follow the instructions provided in the document *Importing Biomek® 2000 Programs* (www.promega.com/automethods/beckman/biomek2000/default.asp). The Biomek® 2000 method is available at: www.promega.com/automethods/

Defining Sample Number for Processing

This method provides a simple way to modify the number of samples processed during the run. The number of samples processed can be changed from the default 96 samples/run to any number of samples divisible by 8. Therefore, runs of 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 or 96 samples can be performed. To change the number of samples processed in each run, the HisLink™ plate pattern needs to be redefined and the pause during the Lysis/Binding mix needs to be adjusted.

Defining HisLink™ Pattern

In the method edit screen of the BioWorks™ software for the “HisLinkFinal” method, click on EDIT in the menu bar. In the EDIT menu bar select “Patterns.” This will bring up the “EDIT Global Window.” In this window, highlight the “HisLink” pattern. This selection will activate a number of buttons in the window. Click on the EDIT button. This will bring up another window that is titled “HisLink.” On the right side of the window is a small box labeled “Edit Pattern.” In the “Edit Pattern” box, select the “Allow Changes” option. Select the CLEAR button at the bottom of the window. This clears the selection of wells being processed. Hold the cursor over the representation of the plate and select the wells to be processed. Selected wells will turn blue. Once the wells are selected, click OK. You will return to “EDIT Global Window.” If running less than 96 samples, use a plate sealer to cover the unused wells on the HisLink™ 96 Filtration Plate. Unused wells can be used at a later time.

Defining the Lysis/Binding Mix Pause Time

During the method the total number of mixes for lysis and binding are set at 10 cycles of 3 mixes per column of samples. It is necessary for this mixing to take place over at least a 30-minute period to ensure thorough sample lysis and efficient binding. To do this a pause is inserted during the Lysis/Binding mixes to ensure that the mixes take at least this long. If running less than a full plate of samples, change the “Pause Labware B5” step time to reflect the number of samples being processed according to the table below.

Number of Sample Columns	Pause Time (minutes)
1–2	02:30:00
3–4	01:15:00
5–6	00:00:30
7–12	00:00:01

V. Description of the Automated HisLink™ 96 Protein Purification Procedure

This overview describes the general liquid handling steps required for automated HisLink™ 96 Protein Purification. The procedure can be adapted for performance on a variety of automated liquid handling robots. Additional information for adaptation to liquid handling robots other than the Biomek® 2000 is provided in Section VI.

1. **Addition of Lysis Buffer to 1ml Cell Cultures.**
100µl of FastBreak™ Reagent/DNase I Solution is transferred to each sample in a 96-well 2.2ml deep-well plate.
2. **Addition of HisLink™ Resin to Samples.**
75µl of HisLink™ Resin is aspirated from a settled bed of resin, dispensed to each sample, and mixed with pipet mixing.
3. **Mixing of Samples for Lysis and Binding.**
Samples are tip mixed for 10 cycles of 3 mixes per column to aid cell lysis and binding of polyhistidine-tagged proteins to the HisLink™ Resin. Samples are aspirated from the bottom of the well and dispensed near the top of the liquid to circulate the resin. Depending on the number of samples being processed, a pause is inserted during mixing to bring the total time of this step to at least 30 minutes.
4. **Transfer of Samples to HisLink™ Filtration Plate.**
The samples are transferred to the HisLink™ 96 Filtration Plate that is sitting on top of the vacuum manifold apparatus. Samples should flow through the Filtration Plate. After all samples are transferred the vacuum is applied for 20 seconds to aspirate any remaining liquid to waste.
5. **Washes 1 through 4.**
Two hundred and fifty microliters of Binding/Wash Buffer is dispensed to each well of the HisLink™ 96 Filtration Plate. After the Binding/Wash Buffer has been added to all wells the vacuum is applied for 20 seconds, and the wash solution is pulled through the HisLink™ 96 Filtration Plate.
6. **Preparation for elution.**
A gripper tool disassembles the vacuum manifold stack. First the HisLink™ Filtration Plate is removed from the vacuum collar and blotted on the paper towel at the Collar Holder position to remove any bubbles on the bottom of the filter plate. The HisLink™ Filtration Plate is then placed back on the vacuum collar. Next, the vacuum collar is moved from the vacuum manifold to a holding position. The gripper then moves a 96-well Collection Plate into the vacuum manifold. The vacuum manifold stack is then reassembled by moving the HisLink™ 96 Filtration Plate and vacuum collar back onto the vacuum manifold over the top of the Collection Plate (Figure 3).



Figure 3. Example of vacuum manifold disassembly, placement of the Collection Plate and reassembly of vacuum manifold for elution of purified polyhistidine-tagged protein on the Beckman Biomek® 2000. Panel A. Disassembly of vacuum manifold. Panel B. Placement of Collection Plate. Panel C. Reassembly of vacuum manifold.

7. Elution of purified polyhistidine-tagged protein.

Two hundred microliters of HisLink™ 96 Elution Buffer is transferred from the reservoir to each well of the HisLink™ 96 Filtration Plate. After a three-minute pause, the vacuum is applied for 1 minute. Elution Buffer is pulled through the HisLink™ 96 Filtration Plate, eluting the purified proteins into the 96-well Elution Plate.

8. Method ends.

The vacuum collar and HisLink™ 96 Filtration Plate are moved to the collar holder position and the Elution Plate is moved to position A6. Dispose of the HisLink™ 96 Filtration Plate after use.

VI. General Guidelines for Adaptation to Alternative Robotic Platforms

This method uses vacuum filtration for washing and elution of samples. Make sure that the vacuum pump you are using is set to pull a vacuum of about 10 inches Hg to ensure that sufficient pressure is applied. Vacuum pressures lower or higher than this will cause problems with the purification.

The Binding/Wash solution provided with the kit is recommended for optimal removal of background proteins. If further background reduction is necessary we recommend adding NaCl to the Binding/Wash Solution up to a concentration of 500mM.

Pause steps built into the purification procedure improve cell lysis and protein binding. Removal of these pauses may decrease the purity and recovery of protein products.

Blotting of the Filtration Plate on the paper towel before the elution step is necessary to remove bubbles from the bottom of the filter plate. Failure to do this may lead to well-to-well cross-contamination problems.

The recommended elution volume for the HisLink™ 96 Protein Purification System is 200µl. Decreases in elution volume may increase the concentration of eluted product but may also result in a decrease in the efficiency of recovery.

^(a)Patent Pending.

^(b)This product is licensed for use under U.S. Pat. No. 6,174,704.

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