

Product Information

**Automated Protocol for
High-throughput Recombinant Protein Purification
Using the Biomek® FX (Beckman Coulter)**HIS-Select® HF Nickel Affinity Gel Catalog Number **H0537**

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Automation Guide

I. Description

An automated protocol has been developed using the HIS-Select[®] High Flow (HF) Nickel Affinity Gel to provide a simple and rapid system for high-throughput, medium-scale purification of histidine-tagged recombinant proteins. The HIS-Select HF Affinity Gel utilizes a proprietary quadridentate chelator that is designed to capture proteins with histidine-tags while exhibiting low non-specific binding of other proteins. The filtration-based purification method eliminates the need for multiple centrifugation steps common in traditional purification protocols, and is ideal for use with robotic platforms. HIS-Select HF Affinity Gel is added directly to cell lysates. After a short incubation, the mixture is transferred to a 96-well filter plate where it is subjected to vacuum. Following a single wash step, purified protein is eluted from the resin. 96 samples are processed in just 45 minutes.

The automated method utilizes the Biomek[®] FX workstation from Beckman Coulter. The protocol has been developed to purify up to 350 µg of protein per well from 5 ml of culture, depending upon the expression rate of the protein. For applications requiring a greater yield of protein, this method can be scaled up by using a larger culture volume.

II. Product Components

Product Description	Catalog Number	Package Size
HIS-Select HF Nickel Affinity Gel	H0537	25 ml (1 Plate)
HIS-Select HF Nickel Affinity Gel	H0537	100 ml (4 Plates)
HIS-Select HF Nickel Affinity Gel	H0537	500 ml (20 Plates)

III. Storage

The HIS-Select HF Affinity Gel is stored at 2–8 °C. Mix the suspension in the bottle by gentle inversion until homogeneous prior to use.

IV. Materials to Be Supplied by the User

1. 2 ml Square Filter Plate (Seahorse Bioscience, formerly Innovative Microplates, F20000)
2. Cell Lysate that contains a histidine-tagged protein
3. HIS-Select Wash & Elution Buffer Kit (Sigma-Aldrich, HS0100) consisting of HIS-Select Equilibration/Wash buffer (H5288) and HIS-Select Elution buffer (H5413)
4. 1 × 96-well, 1.1 ml, square well, v-bottom plate (Seahorse Bioscience, formerly Innovative Microplates, S30026)
5. 2 × 96-well deep well plate with pyramidal bottom (Seahorse Bioscience, formerly Innovative Microplates, S30009)
6. 2 × 12-column reagent reservoir with high profile (Seahorse Bioscience, formerly Innovative Microplates, S30019)

V. Cell Growth Recommendations

1. Grow cells expressing a histidine-tagged recombinant protein per standard procedures.
2. HIS-Select HF Affinity Gel has been used successfully with cells grown in either Luria Broth or Terrific Broth. Media with a high concentration of iron should be avoided, because the iron will displace the nickel on the HIS-Select HF Affinity Gel.
3. Prepare lysates using the CellLytic™ B Plus Kit (CB0050) per instructions.

VI. Instrument Requirements for the Biomek FX

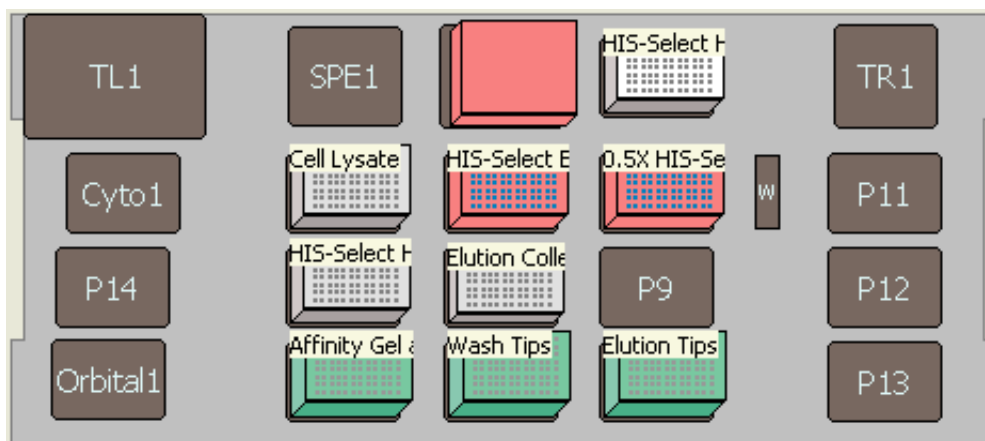
Part Description	Qty	Ordering Information
Beckman Vacuum Manifold	1	Contact Beckman
SPE1 ALP	1	Contact Beckman
36 mm Vacuum Manifold Collar	1	Contact Beckman
Collar and Manifold Holder ALP	2	Contact Beckman
Multichannel Pod (96 Mandrel 200 µl Head) with integrated Gripper	1	Contact Beckman
Tip Loading Station	1	Contact Beckman
Standard Passive ALPs	9	Contact Beckman
AP96 P250 Disposable Tip Box	3	717251 (Beckman)

VII. Automated HIS-Select HF Affinity Gel

A. Reagent Preparation

1. *HIS-Select HF Nickel Affinity Gel*
 - a. Mix the gel in the bottle by gentle inversion until homogeneous. To process 96 samples, 25 ml of affinity gel slurry is required. Centrifuge gel at $100 \times g$ for 10 minutes and remove storage buffer. Add 12.5 ml of 0.5× HIS-Select Equilibration/Wash buffer to the gel to create a 50% resin slurry. Mix until gel is homogenous. Transfer 0.5 ml of the slurry into each well of a 96 well deep well plate.
2. *HIS-Select Equilibration/Wash buffer*
 - a. Dilute the HIS-Select Equilibration/Wash buffer (H5288) 2-fold with water to 0.5× (5 mM Imidazole) if purifying a MAT tagged protein. If using another histidine tag, use 1× concentration of Equilibration/Wash buffer. Dispense at least 20 ml into each column of the 12-column reservoir.
3. *HIS-Select Elution buffer*
 - a. Dispense at least 20 ml of Elution Buffer (H5413) into each column of the 12-column reservoir.

B. Layout for *HIS-Select_HF_Filter_Plate* Method



Deck Position	Equipment
Holder1	36 mm collar for filtration
P1	96-well deep well plate with cell lysate
P2	96-well deep well plate with HIS-Select HF Affinity Gel
P3	AP96 P250 tip box for cell lysate and affinity gel
P4	12-column reservoir for HIS-Select Elution Buffer
P5	96-well, 1.1 ml square well plate for elution
P6	AP96 P250 tip box for cell wash buffer
P7	96-well 2 ml filter plate
P8	12-column reservoir for HIS-Select Equilibration/Wash Buffer
P10	AP96 P250 tip box for elution buffer

C. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated HIS-Select HF Affinity Gel procedure and can be customized to a variety of applications. For customized applications, see Section IX.

i. Getting Started

1. Set up deck: place the tip boxes, plates, and reservoirs at the appropriate positions on the deck as described above.
2. Add reagents to the appropriate reservoirs as described above.
3. Place the 36 mm Manifold Collar with the 96-well filter plate at the Holder1 position.
4. For optimal performance, set the vacuum pressure between 15-20 psi.
5. Run the method using Biomek Software Version 3.1.
6. After running the automated method, the eluate can be analyzed.

ii. HIS-Select HF Filter Plate: Method Overview

Below is a brief summary of the *HIS-Select_HF_Filter_Plate* automated method. For complete program details download the automation program at www.sigmaaldrich.com/automation

1. Cells lysate (1.5 ml) is dispensed into each well of the HIS-Select HF Affinity Gel Deep Well Plate.
2. The affinity gel and lysate are mixed every 5 minutes during a 30-minute incubation.
3. The vacuum manifold collar is placed on the manifold.
4. The gel and lysate are then dispensed into a deep well filter plate.
5. Vacuum is applied to the plate for 25 seconds.
6. HIS-Select Equilibration/Wash Buffer (2 ml) is applied to the gel in the filter plate.
7. Vacuum is applied to the plate for 25 seconds.
8. The collar is removed from the manifold and the collection plate is placed in the manifold. The collar and filter plate are then placed on top.
9. HIS-Select Elution Buffer (1 ml) is applied to the gel in the filter plate.
10. Vacuum is applied to the plate for 25 seconds.
11. The filter plate and collar are removed from the manifold.

IX. Method Customization

A. Using a different type of filter plate

The automated method has been optimized with a 2 ml deep-well filter plate (F20000) from Seahorse Bioscience, formerly Innovative Microplates. This plate may be substituted with the filter plate of your choice. A filter plate with a volume capacity of >1.5 ml is recommended. In addition, filter plates with a PE or glass fiber frit and a pore size of 25 µm works best. The filter plate must also be compatible with the Biomek vacuum manifold. Use of a different filter plate will require some modifications to the automated method including creating a new labware definition.

B. Increasing yield of purified proteins

The automated method was developed to purify up to 350 µg of protein from 5 ml of culture. To increase the yield of target proteins, it is possible to increase the culture volume. This may require use of a 24-well plate to accommodate the increased lysate volume. A single 96-well filter plate can be used to process samples from up to four 24-well plates.

The volume of packed HIS-Select Affinity Gel used in the purification protocol is 0.25 ml per well, and this volume may also be increased to enhance purified protein yield. The HIS-Select Affinity Gel should still be prepared as a 50% slurry. It is recommended that the volume of packed HIS-Select Affinity Gel not exceed 0.5 ml.

X. Performance Characteristics

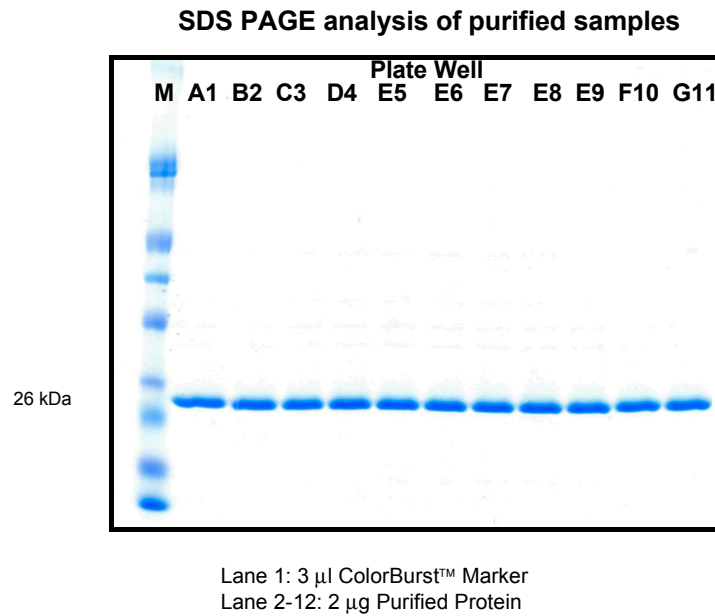


Figure 1. The automated Biomek FX method was used to purify a target histidine-tagged protein from 5 ml of an *E. coli* culture. Samples (10 μ l) were reduced and denatured for analysis by SDS-PAGE. The gel was then stained with EZBlue™ Gel Staining Reagent (Catalog Number G1041). Only the target protein of interest is detected on the gel.

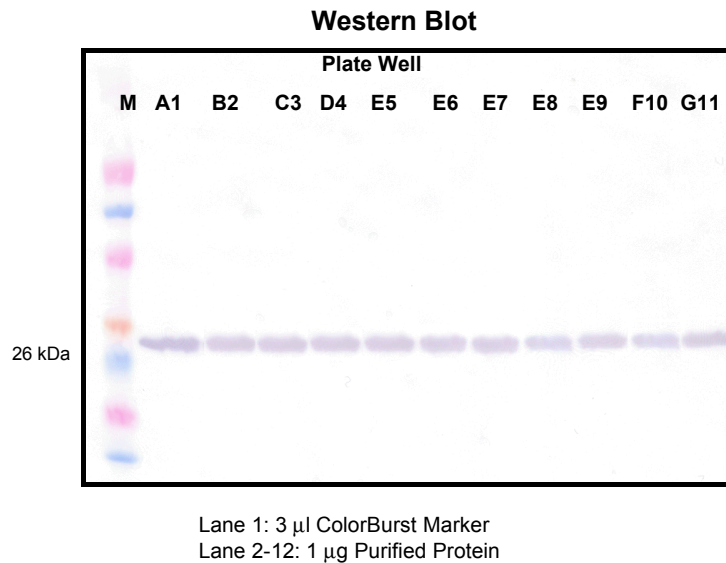
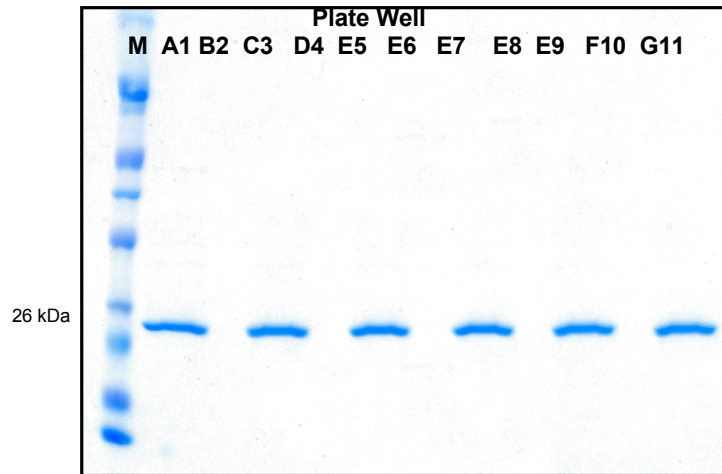


Figure 2. The automated Biomek FX method was used to purify a target histidine-tagged protein from 5 ml culture of *E. coli*. Purified protein (1 μ g) was transferred to the blot. Only the target protein of interest is detected.

Cross-Contamination Analysis



Lane 1: 3 μ l ColorBurst Marker
Lane 2-12: 2 μ g Purified Protein

Figure 4. *E. coli* cells expressing a protein of interest were dispensed into alternating columns of the filter plate. In lanes 2, 4, 6, 8, 10, and 12, the purified protein (20 μ l) was loaded corresponding to wells A1, C3, E5, E7, E9, and G11 on the filter plate. Samples in lanes 3, 5, 7, 9, and 11, corresponding to wells B2, D4, E6, E8, and F10 on the filter plate were run as negative controls (i.e., no protein). No cross-contamination was observed.

Total Protein Quantification by the Bradford Method

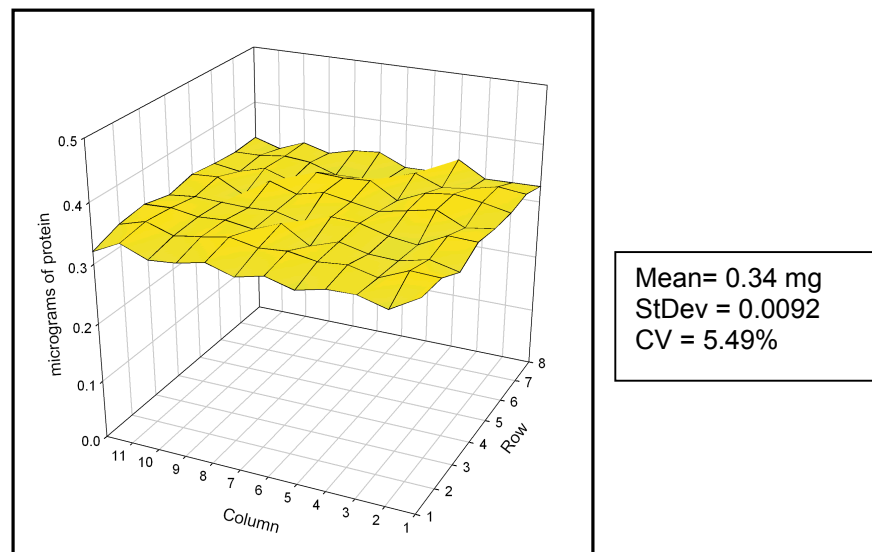


Figure 5. A GST-MAT target protein was purified using the automated method, and subsequently quantified by Bradford method. Total protein concentrations were determined from a standard curve that was generated using BSA as a control. Mean values and coefficients of variation were calculated for the whole plate.

XI. Troubleshooting

Problem	Cause	Solution
The HIS-Select Affinity Gel did not purify the protein of interest.	The protein of interest is insoluble and was not purified.	Determine whether or not protein of interest can be detected by Western blot assay. For crystallography studies a protein must be soluble.
	The protein of interest is soluble, but little to no protein of interest was purified.	Determine whether or not protein of interest can be detected by Western blot assay. If the protein of interest is still not detected, the expression system may have to be optimized for higher expression.
	The protein of interest is soluble and highly expressed, but the gel did not purify the protein.	Confirm that the protein of interest has a histidine-containing tag by either Western blot or by sequencing.
	Iron in medium is displacing nickel on the gel.	Use a medium with a lower iron concentration.
	Others	Refer to the Technical Bulletin of the HIS-Select HF Affinity Gel.
Negative control shows a protein of interest in the SDS-PAGE gel.	Reagents are contaminated.	Use new labware and a new batch of reagents. Test the reagents in an SDS-PAGE gel.

XII. Contact Information

Technical Service
 (800) 325-5832
 email: techserv@sial.com

Customer Service
 (800) 325-3010
 (800) 588-9160
www.sigma-aldrich.com/order

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