

ProductInformation

Automated Protocol for HIS-Select™ iLAP™ HC Nickel Coated Plate, 96-Well Clear Using the Sciclone ALH 3000 Workstation (Caliper Life Sciences)

HIS-Select iLAP HC Nickel Coated Plate Product Code **H 9412**

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Automation Guide

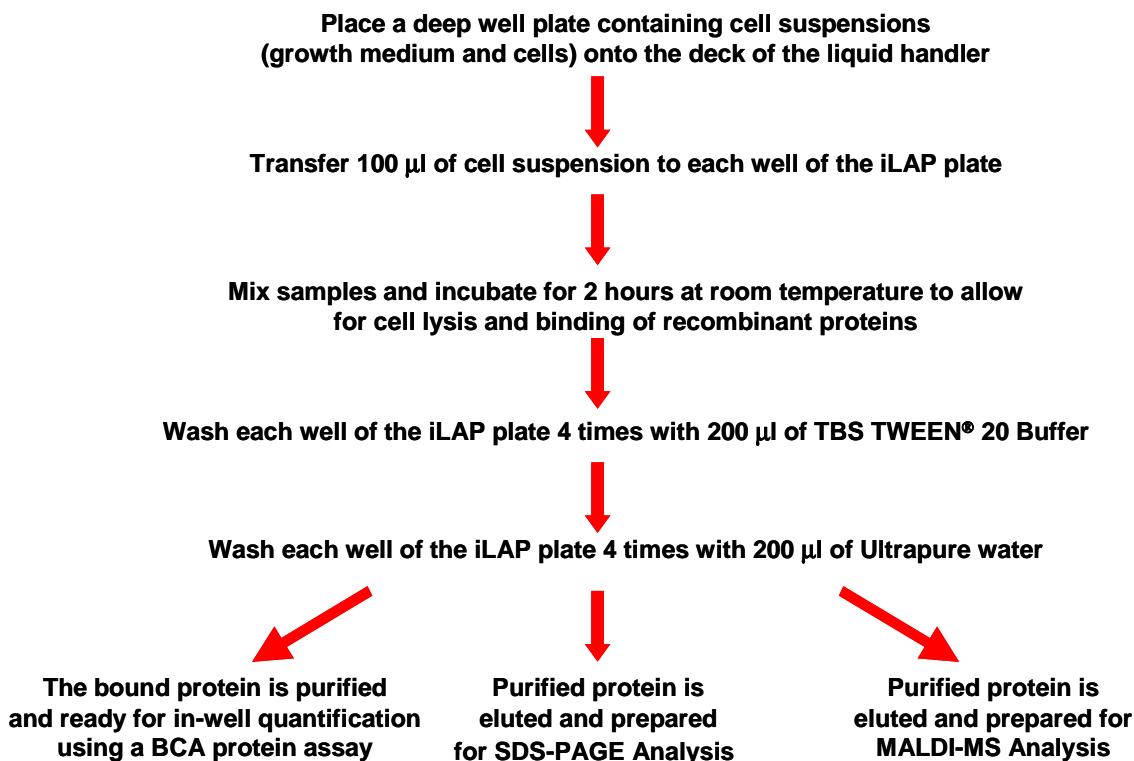
I. Description

The HIS-Select™ Integrated Lysis and Affinity Purification (iLAP™) High Capacity Nickel Coated Plate has been developed to provide rapid bacterial cell lysis and purification of histidine-tagged proteins in a walk-away, automated format. The iLAP 96-well plates are coated with both cell lysis reagents and the HIS-Select nickel-chelate matrix. This patented technology allows for cells to be lysed and the histidine-tagged protein to be captured in one step in a single well. Cell lysis and protein extraction are highly efficient and eliminate the need to harvest cells from culture.

The iLAP plates are coated with a proprietary high-density nickel-chelate matrix for high capacity affinity binding of recombinant histidine-containing fusion proteins. The HIS-Select coating provides a highly specific interaction allowing for protein purification with purities greater than 90% and a binding capacity of $\geq 4 \mu\text{g}$ per well. In addition, the coating contains an optimized mixture of reagents necessary for the fast and efficient lysis of bacterial cells directly from medium. The lysis components include a non-ionic detergent, lysozyme, endonuclease, and protease inhibitors.

The automated protocol for the iLAP plate using the Sciclone workstation provides a walk away method for the extraction and affinity purification of histidine-tagged proteins making it ideal for rapid colony screening and protein:protein interaction assays. In a few simple steps fusion proteins can be purified and are ready for downstream applications.

Workflow



II. Product Components

Product Description	Product Code	Package Size
HIS-Select iLAP HC Nickel Coated Plates	H 9412	1 ea
HIS-Select iLAP HC Nickel Coated Plates	H 9412	5 x 1 ea

III. Storage

The HIS-Select iLAP HC Nickel Coated Plates are stored at 2–8 °C. Prior to use, warm the plate to room temperature for 20 minutes.

IV. Materials to Be Supplied by the User

1. Bacterial cells in medium expressing a soluble histidine-tagged protein
2. Wash Buffer (TBST) – Tris Buffered Saline, pH 8.0, containing 0.05% TWEEN® 20 (Sigma-Aldrich, T 9039)
3. Imidazole, Molecular Biology Grade (Sigma-Aldrich, I 5513)
4. Sodium chloride (Sigma-Aldrich, S 3014)
5. Sodium phosphate (Sigma-Aldrich, S 0751)
6. Water, Molecular Biology Grade (Sigma-Aldrich, W 4502)
7. Bicinchoninic Acid (BCA) Kit for total protein quantification (Sigma-Aldrich, BCA-1)
8. Clear 96-well polystyrene plate (Sigma-Aldrich, M 0156) for BCA standard curve.
9. Plate lid for clear 96 well plate (Sigma-Aldrich, Z37,190-4)
10. Sample Buffer, Laemmli 2x Concentrate (Sigma-Aldrich, S 3401)
11. pH Meter with calibrating standards
12. Gel electrophoresis box with compatible consumables
13. 96-well reservoir with low profile and pyramidal bottom (Innovative Microplates, S30018)
14. 12-column reagent reservoir with high profile (Innovative Microplates, S30019)

V. Cell Growth Requirements

1. Grow cells expressing a histidine-tagged recombinant protein per standard procedures. iLAP plates have 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 surface of the iLAP plate.
2. Binding of the target fusion protein is concentration dependent. The best results will be achieved when the concentration of the target fusion protein is at least 0.1 mg/ml of cell suspension.

VI. Instrument Requirements for the Sciclone ALH 3000 Workstation

Part Description	Qty	Ordering Information
Deck Mounted Shaker	1	Contact Caliper
96-channel High Volume Head	1	Contact Caliper
Z8™ Pipettor	1	Contact Caliper
Gripper	1	Contact Caliper
I/O Box	1	Contact Caliper
Deck Locator	8	Contact Caliper
Tip Box Locator	7	# 76523 (Caliper)
200 µl Disposable Tip Box	7	# 56362 (Caliper)
Bulk Dispense Module with Syringe Pump	1-2	Contact Caliper

VII. Automated iLAP Protocol for SDS-PAGE Analysis

This section describes the setup and protocol for the *iLAP_SDS-PAGE* method. This automated method includes steps for the integrated lysis and purification, as well as elution of histidine-tagged proteins for downstream analysis by SDS-PAGE.

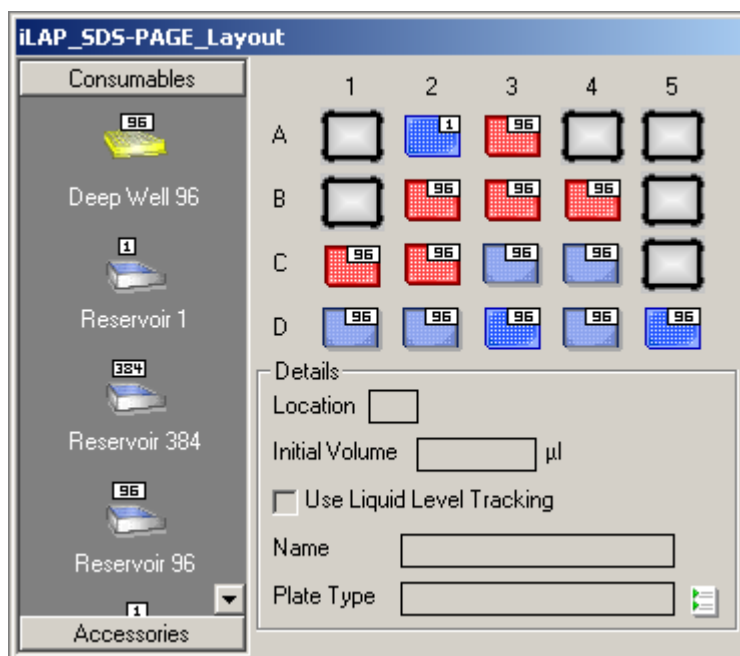
A. Reagent Preparation

1. *Cells in Medium*
 - a. If using cells grown in a flask, add the cells in medium to the 96-well reservoir located at position C3. Otherwise place a deep well plate containing cells at position C3.
2. *TBST Wash buffer*
 - a. Dissolve one packet of Tris-buffered saline with TWEEN 20 (T 9039) in water for a final volume of 1,000 ml. Mix thoroughly. Add at least 15 ml of Wash Buffer to each column of the 12-column reservoir for TBST located at position D2.

B. Protein Analysis by SDS-PAGE

1. *Elution buffer*
 - a. Prepare elution buffer containing 300 mM sodium chloride, 50 mM sodium phosphate, and 250 mM imidazole, pH 8.0. Add at least 15 ml of Elution Buffer to the 96-well reservoir for Elution buffer located at position C4.
2. *2x Laemmli buffer*
 - a. Place at least 15 ml of thawed 2x Laemmli buffer in the 96-well reservoir for Laemmli buffer at position D1.

C. Deck Layout for *iLAP_SDS-PAGE* Method



Deck Position	Equipment
A2	Plate lid
A3	200 µl tip box for water wash
B2	200 µl tip box for TBST waste
B3	200 µl tip box for Cells in medium
B4	200 µl tip box for Elution buffer
C1	200 µl tip box for 2x Laemmli buffer
C2	200 µl tip box for TBST
C3	96-well deep well plate containing cells in medium
C4	96-well reservoir for Elution buffer
D1	96-well reservoir for 2x Laemmli buffer
D2	12-column reservoir for TBST
D3	iLAP plate
D4	12-column reservoir for waste
D5	Shaker

D. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated iLAP procedures and can be customized to a variety of applications. For customized applications, see Section IX.

i. Getting Started

1. Set up deck layout: 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. Turn the plate shaker module controller to the first black line after the "off" line (optional). If using the Teleshake v1.2 software, use a setting of 550 rpm.
4. Run the method using Sciclone Software Version 3.2.
5. After running the automated method, samples are heated to 100 °C for 5 minutes and analyzed by SDS polyacrylamide gel electrophoresis. Samples reduced in Laemmli buffer may be stored frozen at -20 °C until needed.

ii. iLAP SDS-PAGE: Method Overview

Below is a brief summary of the *iLAP_SDS-PAGE* automated method. For complete program details download the automation program at www.sigmaldrich.com/automation

1. Speed of Sciclone head movement is set at 80%.
2. Cells in medium (100 µl) are dispensed into each well of the iLAP plate.
3. The iLAP plate is incubated for 2 hours at room temperature while mixing.
4. The spent lysate is aspirated from the iLAP plate and disposed.
5. TBST (200 µl) is dispensed into each well of the iLAP plate.
6. The iLAP plate is shaken for 10 seconds and the TBST is aspirated from the plate.
7. Steps 5 and 6 are repeated a total of 4 times.
8. After the final TBST wash, 200 µl of water is dispensed into the plate using the bulk dispenser and the plate is washed four times.
9. Following the final wash, contents from the wells are aspirated with the high volume head and the elution buffer (100 µl) is dispensed into the plate.
10. The iLAP plate is incubated for 1 hour with mixing using the shaker.
11. 2X Laemmli buffer (100 µl) is dispensed into each well of the iLAP plate and lidded.

VIII. Automated iLAP Protocol for Protein Determination

This section describes the setup and protocol for the *iLAP_BCA* method. This automated method includes steps for the integrated lysis and purification of histidine-tagged protein, as well as the setup of samples and standards for quantification.

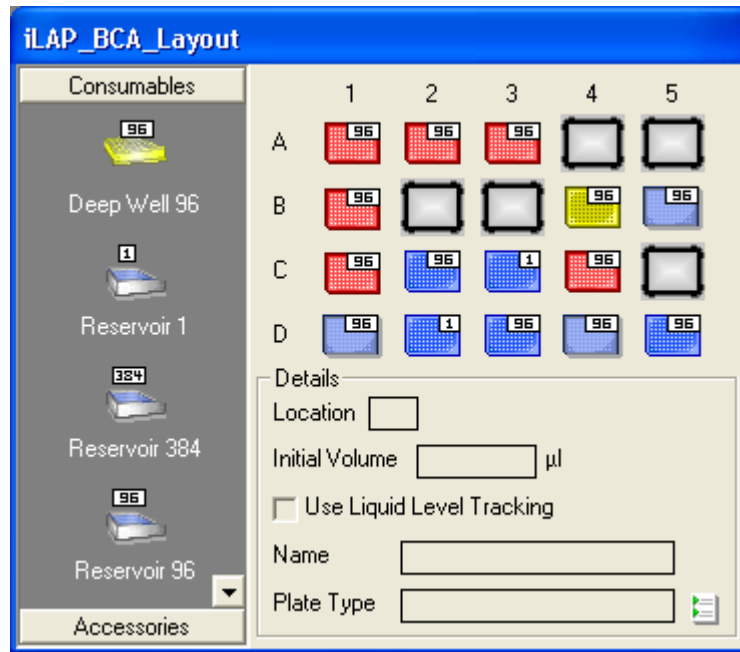
A. Reagent Preparation

1. *Cells in Medium*
 - a. If using cells grown in a flask, add the cells in medium to the 96-well reservoir located at position C3. Otherwise place a deep well plate containing cells at position C3.
2. *TBST Wash buffer*
 - a. Dissolve one packet of Tris-buffered saline with TWEEN 20 (T 9039) in water for a final volume of 1,000 ml. Mix thoroughly. Add at least 15 ml of Wash Buffer to each column of the 12-column reservoir for TBST located at position D2.

B. Protein Quantification using the BCA Method

1. *Bovine Serum Albumin Standard*
 - a. Pipette 200 μ l of Bovine Serum Albumin (BSA) standard into the column 1 wells of the 96-well polystyrene plate for the standard curve located at position C2. Place a lid on the plate.
2. *BCA Working Solution*
 - a. Add 50 ml of BCA reagent to a clean beaker. Add 1 ml of copper (II) sulfate to the BCA reagent and mix until the solution is homogenous (apple green color). Add 50 ml of the BCA Working solution to the 96-well reservoir for BCA Working solution located at position D1.

C. Layout for *iLAP_BCA* Method



Deck Position	Equipment
A1	200 µl tip box for water wash
A2	200 µl tip box for TBST dispense
A3	200 µl tip box for TBST removal
B1	100 µl tip box for preparation of standards
B4	96-well deep well plate containing cells in medium
B5	96-well reservoir for TBST
C1	200 µl tip box for dispensing of BCA Working solution
C2	96-well polystyrene plate for Standard Curve
C3	Plate lid for iLAP plate
C4	200 µl tip box for transfer of cell culture to iLAP plate
D1	96-well reservoir for BCA Working solution
D2	Plate lid for standards
D3	iLAP plate
D4	12-column reservoir for waste
D5	Shaker

D. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated iLAP procedures and can be customized to a variety of applications. For customized applications, see Section IX.

i. Getting Started

1. Set up deck layout: 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. Turn the plate shaker module controller to the first black line after the "off" line (optional). If using the Teleshake v1.2 software, use a setting of 550 rpm.
4. Run the method using Sciclone Software Version 3.2.
5. After running the automated method, protein determinations can be calculated after the 2-hour of incubation period.

ii. iLAP BCA: Method Overview

Below is a summary of the iLAP total protein quantification BCA Assay. For complete program details, download automation program from www.sigmaaldrich.com/automation

1. Speed of Sciclone head movement is set at 80%
2. Cells in medium (100 μ l) are dispensed into each well of the iLAP plate.
3. The iLAP plate is incubated for 2 hours at room temperature while mixing.
4. The spent lysate is aspirated from the plate and disposed.
5. TBST (200 μ l) is dispensed into each well of the iLAP plate.
6. The iLAP plate is shaken for 10 seconds and the TBST is removed from the plate.
7. Steps 5 and 6 are repeated for a total of 4 times.
8. After the final TBST wash, 200 μ l of water is dispensed into the iLAP plate using the bulk reagent dispenser and the plate is washed four times. Following the final wash, contents from the wells are aspirated with the high volume head.

The remaining steps of the method prepare samples and standards for quantification using BCA reagents.

9. A serial dilution of the BSA standards is performed in columns 1 – 5 of the standard plate. Prior to starting the automated method, pipette 200 μ l of BSA standard into column 1 of the standard plate and place plate on the deck of the Sciclone in position C2 (see Section VIII). Following the iLAP method, water (100 μ l) is dispensed using the bulk reagent dispenser into columns 2 through 6 of the standard plate.
10. 100 μ l of the BSA Standard is aspirated from column 1, dispensed into column 2, and mixed. 100 μ l is aspirated from column 2, dispensed to column 3, and mixed. This is repeated through column 5 of the plate. Wells in column 6 are left blank.
11. BCA working reagent (200 μ l) is dispensed into columns 7 through 12 of the standard plate.
12. BCA working reagent (200 μ l) is dispensed to the iLAP plate.
13. 25 μ l of the diluted BSA standards (from columns 1-6) are transferred to columns 7 through 12 of the 96-well plate containing standards.
14. Plate lids are placed on both the iLAP and standard plates.
15. Both the iLAP plate and standard plate are mixed on the shaker for 10 seconds.
16. After a 2-hour incubation the plates are read in a spectrophotometer.

IX. Method Customization

A. Purifying a single protein using an entire iLAP plate

If it is desired to perform the purification of one protein in all 96-wells of the iLAP plate, replace the 96-well deep well polypropylene plate with a 96-well reservoir for the cells in medium. The consumable must be changed in the deck layout of the automated program if this is performed.

B. Performing a Standard Curve in an iLAP plate

If a standard curve is to be performed on an iLAP plate, dispense medium (without cells) into the wells of the iLAP plate designated for the standard curve. During the wash steps, the lysis reagents will be removed from the iLAP plate to eliminate any interference with the BCA assay.

C. Performing an SDS-PAGE analysis and BCA assay in the same plate.

It is recommended that the steps for the BCA assay be performed first (i.e. standard curve set up). During the 2-hour incubation for the BCA assay, samples can be eluted and prepared for SDS-PAGE.

D. Using an additional bulk reagent dispense module.

The automated method described in this document was developed with a single bulk reagent dispense module for the water wash. An additional bulk reagent dispense module can be purchased from Caliper and used for the TBST wash steps.

X. Performance Characteristics

Automated Method for the iLAP SDS-PAGE Analysis

Analysis of samples picked from random wells; EZBlue™ Stain

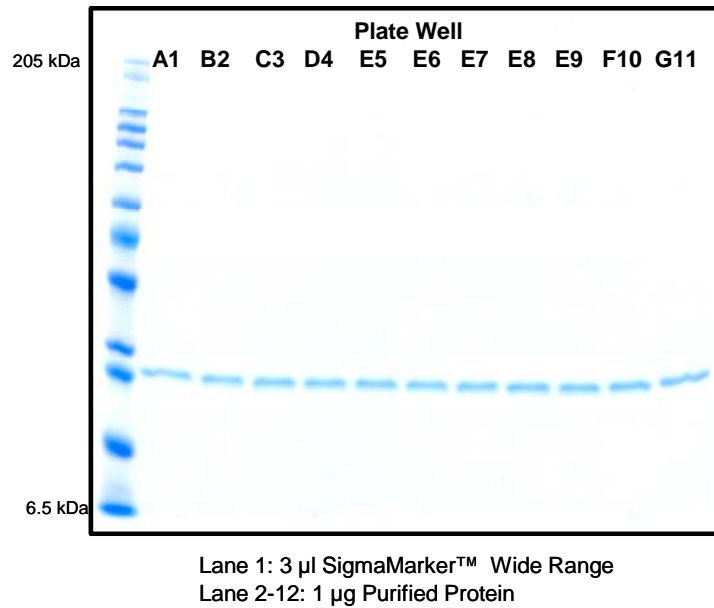


Figure 1. SDS-PAGE gel of a recombinant protein purified using iLAP plates. The automated Sciclone method was used to purify a target histidine-tagged protein from 100 μ l of an *E. coli* culture. The purified protein was eluted with 250 mM imidazole. 20 μ l samples were reduced and denatured for analysis by SDS-PAGE. The gel was then stained with EZBlue™ gel staining reagent (Product Code G 1041). Only the target protein of interest is detected on the gel.

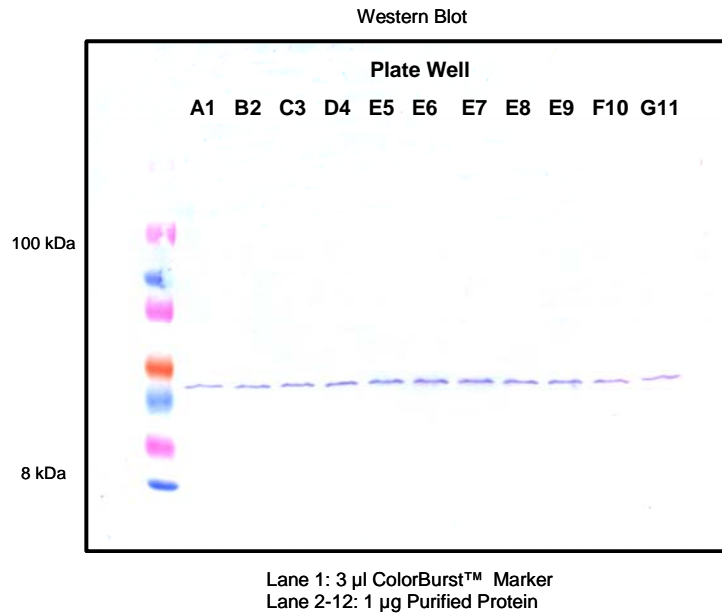


Figure 2. Western blot of a recombinant protein purified using iLAP plates. The automated Sciclone method was used to purify a target histidine-tagged protein from 100 μ l of an *E. coli* culture. 1 μ g of purified protein was transferred to the blot. Only the target protein of interest is detected.

Automated Method for the iLAP SDS-PAGE Analysis (con't)

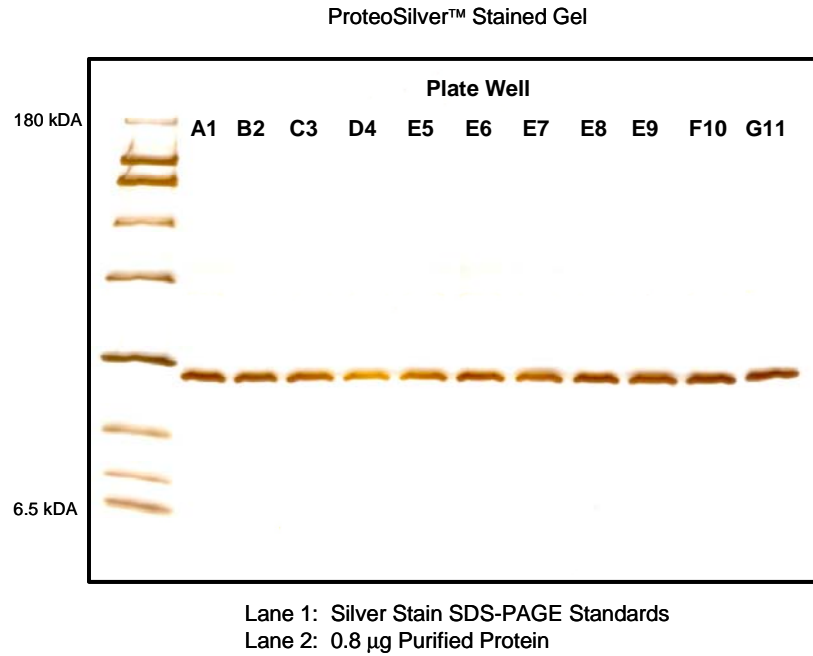


Figure 3. Silver stain of an SDS-PAGE gel of a recombinant protein purified using iLAP plates. The automated Sciclone method was used to purify a target histidine-tagged protein from 100 µl of an *E. coli* culture. The purified protein was eluted with 250 mM imidazole. 20 µl samples were reduced and denatured for analysis by SDS-PAGE. The gel was then stained with ProteoSilver™ gel staining reagent (Product Code PROT-SIL1). Only the target protein of interest is detected on the gel.

Cross-Contamination Analysis

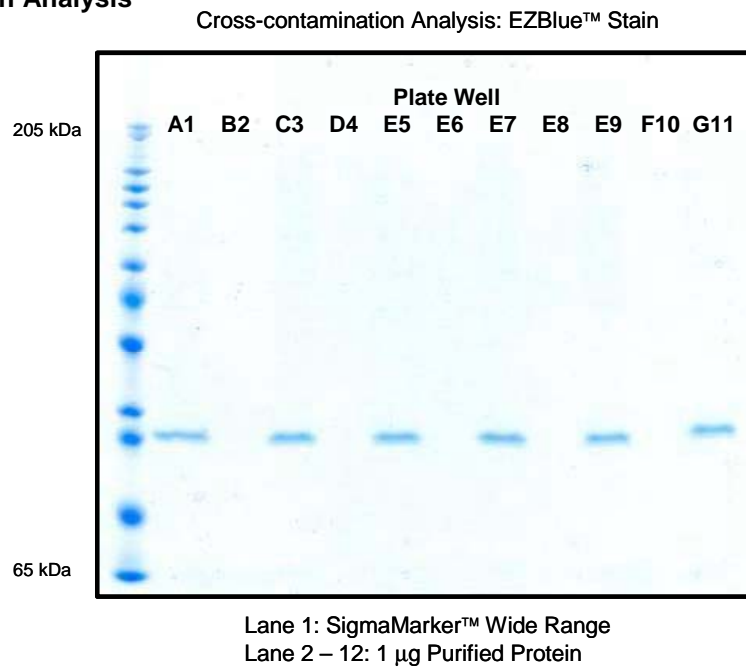


Figure 4. Cross-contamination analysis. *E. coli* cells expressing a protein of interest were dispensed into alternating columns of the iLAP plate. In Lanes 2, 4, 6, 8, 10, and 12, the purified protein (20 µl) was loaded corresponding to wells A1, C3, E5, E7, E9, and G11 on the iLAP plate. Lanes 3, 5,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

Total Protein Quantification by the BCA Method

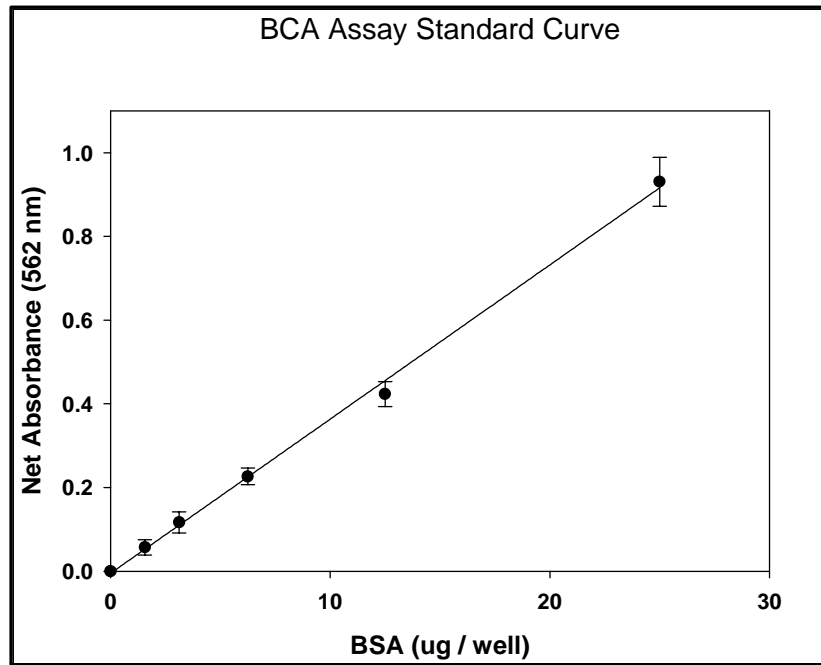
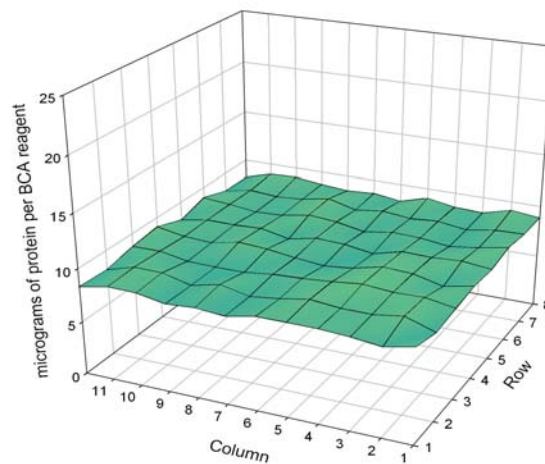


Figure 5a. Standard Curve. Albumin standards were serially diluted using the Sciclone ALH 3000 workstation. Standard curve samples were then set up for the BCA assay. Following the incubation, the absorbance was read and the standard curve was determined. Linear regression analysis was used to calculate the equation of the line and R^2 values. From this curve, concentrations were determined for the protein purified with the iLAP plate (see Figure 5b).

Total Protein Bound to each well of an iLAP plate per the BCA Reagent Assay



Mean: 8.7 $\mu\text{g/well}$
Standard Deviation: 0.3
CV = 3.3%

Figure 5b. A BCA plate map quantifying the protein of interest bound to an iLAP plate. A target protein was processed by the automated method for iLAP purification and subsequent BCA assay setup. 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 iLAP plate did not purify the protein of interest.	The protein of interest is insoluble or in an inclusion body.	The protein must be soluble to bind to the iLAP plate.
	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.
	The protein of interest is soluble and highly expressed, but the iLAP plate 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 surface of the iLAP plate.	Use a medium with a lower iron concentration.
	Others	Refer to the Technical Bulletin of the HIS-Select iLAP plate.
Negative control shows a protein of interest in the SDS-PAGE gel.	Reagents are contaminated.	Use new labware and new batch of reagents. Test the reagents in an SDS-PAGE gel.

XII. Contact Information

Technical Service Help
 (800) 325-5832
www.techserv@sial.com

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

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