

# Integrated Lysis and Affinity Purification (iLAP™) of Histidine-tagged Recombinant Proteins Suitable for Common Proteomic Techniques

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## Abstract

High-throughput functional proteomic assays require reagents and automated methods to aid in the conversion of gene sequences to purified proteins. Protein purification is the bottleneck in many of these assays due to the labor-intensive nature and largely un-automatable processes for cell lysis. HIS-Select™ iLAP™ nickel-coated 96-well plates represent a rapid and efficient one-step method for simultaneous cell lysis and affinity purification of histidine-tagged recombinant proteins. iLAP plates provide a high-capacity nickel-coated matrix in a 96-well format precoated with lysis-inducing agents, a non-ionic detergent, Benzonase®, and a protease inhibitor cocktail to ensure stability of the purified protein. The captured purified protein can be directly quantified by standard protein methods or eluted from the well and analyzed by common proteomic techniques. The iLAP plate and its downstream applications have been validated for use with robotics. Cultured *E. coli* samples expressing a metal affinity tagged (MAT) recombinant protein induced by IPTG were directly applied to the iLAP plate, incubated for 2 hours, washed, and the protein of interest eluted under mild conditions with imidazole. Specificity was demonstrated by SDS-PAGE. Confirmation of the protein was also demonstrated in a Western Blot. A peptide map was obtained by MALDI-TOF-MS analysis from a tryptic digest of the captured protein. Using this methodology, greater than 30% sequence coverage was achieved.

## iLAP Protocol

- One-step lysis and protein purification

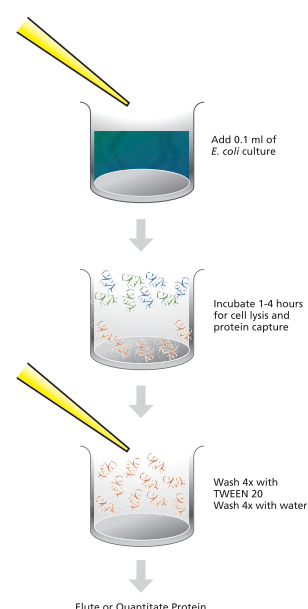
- 96-well plates coated with cell lysis reagent and the HIS-Select nickel chelate matrix

- No harvesting cells from the culture

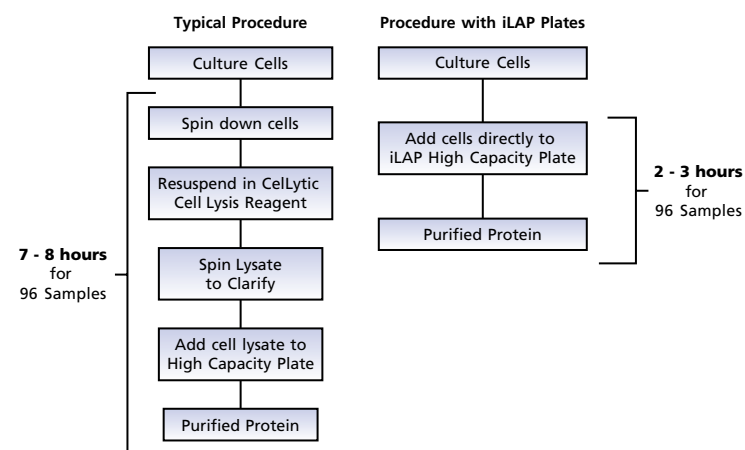
- High binding capacity and reduced non-specific binding

- Ideal for rapid colony screening and protein-protein interaction assays

- Automated protocol available

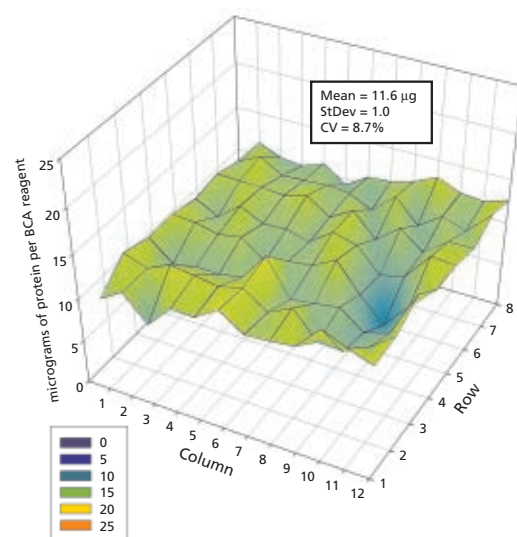


## Time Savings with iLAP



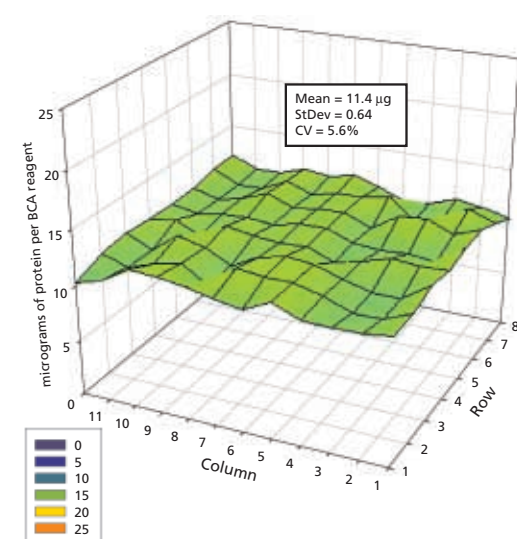
## Total Protein Quantification (Manual)

Panel A: Total Protein Bound to each well of an iLAP plate per the BCA reagent assay



## Total Protein Quantification (Automated)

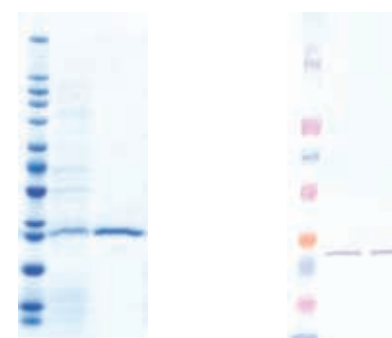
Panel B: Total Protein Bound to each well of an iLAP plate per the BCA reagent assay



**Figure 1:** Panel A: A BCA plate map quantifying the protein of interest bound to an iLAP plate. *E. coli* culture expressing the histidine-tagged protein was manually pipetted (100 µl) into each well of an iLAP plate. The plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. 200 µl of the BCA working reagent was then added to each well and the absorbance was read. 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. Panel B: A BCA plate map quantifying the protein of interest bound to an iLAP plate. A procedure was developed for the Sciclone® ALH 3000, where 100 µl of *E. coli* culture expressing histidine-tagged protein was added to each well of the iLAP plate. The plate was then incubated at room temperature for 2 hours to lyse the cells and capture the protein. 200 µl of BCA working reagent was added to each well and the absorbance was read. 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. The variability from well to well (%CV) was lower than the manually pipetted plate.

## SDS-PAGE and Western Blot Analysis (Manual Method)

Panel A



Lane 1: 3 µl Sigma Marker™ Wide Range  
Lane 2: 1 µg Extract  
Lane 3: 1 µg Purified Protein

Lane 1: 5 µl ColorBurst™ Marker  
Lane 2: 10 µl Extract  
Lane 3: 10 µl Purified Protein

## SDS-PAGE Analysis (Automated Method)

Panel B



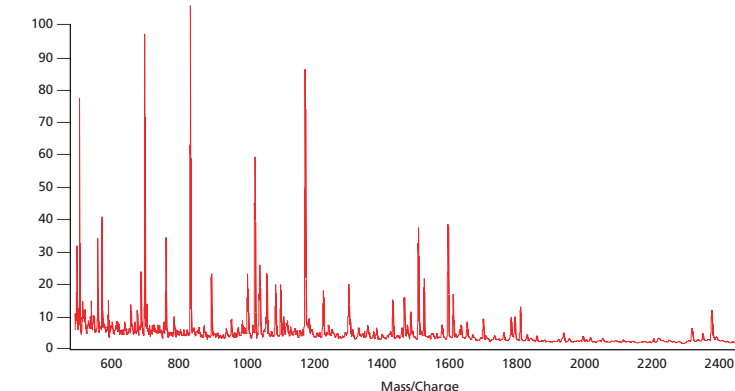
**Figure 2:** Panel A: SDS-PAGE gel and Western blot of eluted protein taken from a random well of an iLAP plate. 100 µl of an *E. coli* culture expressing the histidine-tagged protein of interest was manually pipetted into an iLAP plate. The plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. The plate was washed and the purified protein was eluted with 250 mM imidazole. A 20 µl sample was taken from one random well, reduced, and denatured for analysis by SDS-PAGE. For comparison, crude cell lysate was run also. The gel was stained with EZBlue™ stain. Only the specific purified protein of interest can be seen in lane 3. Panel B: SDS-PAGE gel of eluted protein taken from random wells of an iLAP plate. The automated Sciclone method was used to pipet 100 µl of an *E. coli* culture expressing the histidine-tagged protein of interest. The iLAP plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. The plate was then washed and purified protein was eluted with 250 mM imidazole (using automated methods). 20 µl samples were reduced and denatured for analysis by SDS-PAGE. The gel was then stained with EZBlue stain. Only the specific purified protein of interest was bound to the plate and can be seen on the gel. Comparable results to manual methods were achieved using the automated method on the Sciclone.

## Cross-contamination Study



**Figure 3:** SDS-PAGE gel evaluating cross-contamination. An automated iLAP protocol was developed for the Sciclone ALH 3000 to determine whether or not cross-contamination was taking place. *E. coli* cells expressing a protein of interest were dispensed into alternating columns of the iLAP plate. The plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. The entire plate was then washed and the protein was eluted with 250 mM imidazole for analysis. Samples from the alternating wells were reduced and denatured for analysis by SDS-PAGE. The gel was then stained with EZBlue stain. 3 µl of SigmaMarker™ Wide Range was loaded into lane 1. In Lanes 2, 4, 6, 8, 10, and 12, the purified protein (20 µl) was loaded. This corresponds to wells A1, C3, E5, E7, E9, G11 on the iLAP plate. Lanes 3, 5, 7, 9, and 11, corresponding to wells B2, D4, E6, E8, F10 on the iLAP plate were run as negative controls (i.e. no protein). No cross-contamination was observed.

## MALDI Mass Spectrum



**Figure 4:** MALDI Mass Spectrum includes both correctly cleaved tryptic peptides and missed cleavages. The data in the spectrum above generated the tryptic peptide chart. Eluted samples from an iLAP plate were reduced and alkylated and run on a 4-20% tris-glycine SDS-PAGE gel. The band containing the protein of interest was excised and trypsinised using the Trypsin Profile IGD Kit (PP0100).

## Tryptic Peptide Chart

Tryptic Peptide	[M + H] <sup>+</sup>
T <sub>1</sub>	1094.57
T <sub>3</sub>	770.45
T <sub>5-7</sub> *	2024.91
T <sub>6-7</sub> *	905.41
T <sub>9-10</sub> *	2357.21
T <sub>11</sub>	1032.59
T <sub>13</sub>	1013.47
T <sub>15</sub>	1516.80
T <sub>16</sub>	581.30
T <sub>17</sub>	581.33
T <sub>19</sub>	708.39
T <sub>21</sub>	697.30
T <sub>24</sub>	666.36
T <sub>27</sub>	1026.58
T <sub>30</sub>	2326.14
T <sub>31</sub>	686.38
T <sub>32</sub>	707.33
Sequence Coverage	79%

\*Missed Cleavages

**Figure 5:** Tryptic Peptide Chart generated from the MALDI Mass spectrum chart. Protein was purified using the HIS-Select iLAP plate. Eluted samples were reduced and alkylated. The samples were then run on a 4-20% tris-glycine SDS-PAGE gel and stained with EZBlue stain. The band containing the protein of interest was excised and trypsinized using the Trypsin Profile IGD Kit (PP0100). The sequence coverage for the protein of interest was determined to be 79%. Some Missed Cleavages were observed.

## Conclusions

- An automated method for rapidly purifying recombinant proteins has been developed using the HIS-Select iLAP plate and has been validated on the Sciclone ALH Workstation
- Significant time savings over standard methods
  - No centrifugation required to harvest cells
  - No mechanical disruption of cells required
- Binding capacities are greater than 4 µg/well
- This format is useful for common proteomic techniques such as SDS-PAGE, Western Blots, and peptide mass fingerprinting