

proteomics

HIS-Select™ iLAP™ Plate: For the Rapid Screening of Histidine-tagged Protein

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Application Notes

- One-step cell lysis and His-tagged protein purification
- Efficient cell lysis without harvesting cells from the culture
- Highly selective for His-tagged proteins, high-binding capacity, and less non-specific binding
- Ideal for rapid colony screening

Introduction

Screening large numbers of recombinant clones for the production of soluble target proteins can be time consuming and laborious. Affinity purification of recombinant protein is made easier by the introduction of small peptide tags. Tags allow for the rapid purification of the protein, but sample preparation can still require a lot of effort. Each clone must be grown and the cells induced to make the protein of interest. After induction, the bacteria must be harvested by centrifugation, protein must be extracted from the cells, and then the cellular debris removed by another centrifugation step. At this point, the tagged protein must be bound to a matrix, washed, and finally eluted for analysis. This process works well with one or two clones but can quickly become time consuming when preparing a larger number of proteins.

To meet the needs of researchers who screen large numbers of soluble His-tagged proteins, Sigma-Aldrich has developed the HIS-Select™ iLAP™ 96-well plate (integrated lysis and purification; Product Code [H 9412](#)), a novel high-capacity multiwell plate that can lyse cells directly from a culture and bind greater than 4 µg of His-tagged protein per well.

Rapid purification using the HIS-Select iLAP plate

The basic scheme for purification using the HIS-Select iLAP plate is shown in Figure 1. Bacterial cells are grown in a 2 ml deep 96-well plate and an aliquot is placed directly into the HIS-Select iLAP plate. In the plate the cells are lysed and the target protein is captured directly from this crude extract. The plate is then washed with a standard plate washer and the target protein is eluted for further analysis. Alternatively, the protein can be measured directly in the plate using the Bicinchoninic Acid (BCA) Kit for Protein (Product Code [BCA-1](#)).

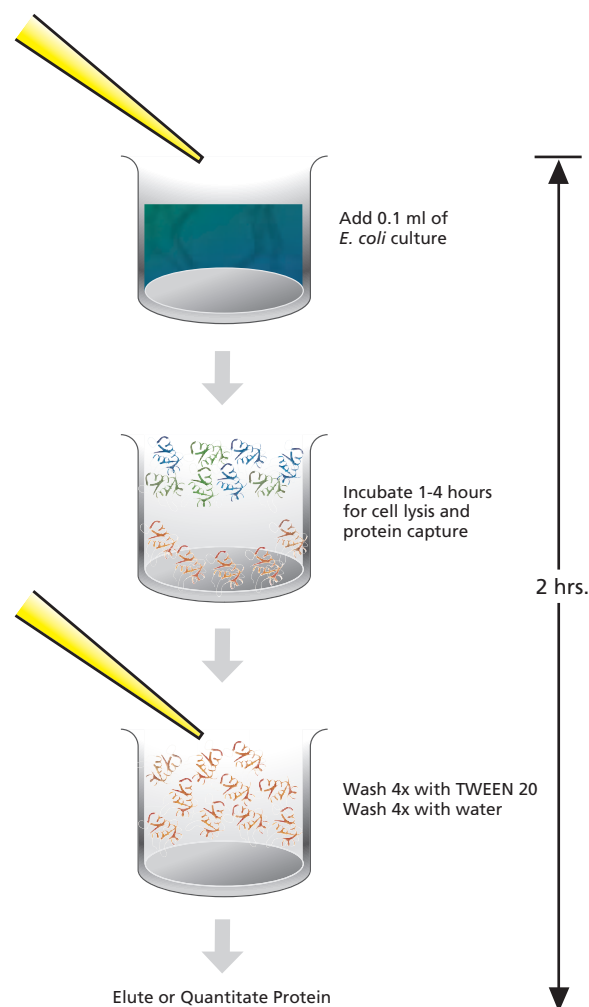


Figure 1. Protocol for the HIS-Select iLAP plate.

In order to illustrate the utility of the HIS-Select iLAP plate, a 28 kDa protein with the metal affinity tag (MAT™) was transformed into a BL21 *E. coli* strain. The cells were then plated on several LB *amp* plates. Ninety-six individual colonies were placed in 0.1 ml of Terrific Broth Media (TB; Product Code [T 9179](#)) in a 2 ml deep-well plate and incubated overnight at 37 °C. The next morning 0.9 ml of fresh TB was added to each of the wells and allowed to grow for an additional 2 hours. The cells were then induced with IPTG and grown for an additional 2 hours. At the end of the induction phase, aliquots of 0.1 ml of each of the cultures were placed into the HIS-Select iLAP plate and incubated for 2 hours. Next, the plate was washed four times with Tris-buffered saline with 0.1% TWEEN® 20 (Product Code [T 9039](#)) and then four times with purified water. The purified protein was then eluted with 0.1 ml of 50 mM sodium phosphate, 0.3 M sodium chloride and 250 mM imidazole pH 8.0. Samples of the eluted protein were analyzed by SDS-PAGE as shown in Figure 2. Each of the wells contained approximately 5 µg of target protein as determined by BCA (data not shown).

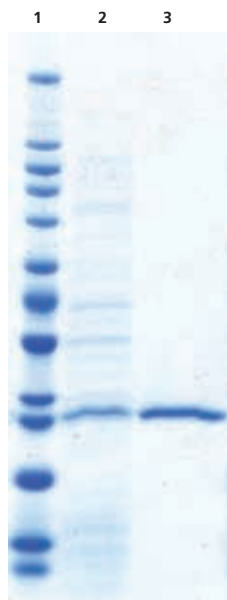


Figure 2. SDS-PAGE analysis of HIS-Select iLAP purified-protein proteins. Aliquots of the eluted protein (10 µl) were mixed with an equal volume of Laemmli sample buffer (Product Code [S 3401](#)). They were then boiled for 5 minutes and then the proteins were separated on a 4-20% tris-glycine gel. The proteins were then stained with EZBlue™ Gel Staining Reagent (Product Code [G 1401](#)). Lane 1 is wide-range molecular weight marker. Lane 2 is the crude cell lysate applied to the plate. Lane 3 is the eluted protein (5% of the eluted well).

The purity of the eluted protein is single banded, as determined by SDS-PAGE (Figure 2) and Western blot analysis using monoclonal antibody directed against the protein (Figure 3). Target protein was also eluted from the well using 0.1% TFA for direct analysis by MALDI mass spectrometry (data not shown). Other potential uses of the plate include use as an archive for long-term storage of purified histidine-tagged proteins or for generating a recombinant protein array for screening potential binding partners of the purified target protein in protein-protein interaction assays.



Figure 3. Western blot analysis of HIS-Select iLAP purified-protein proteins. The blot was detected using an antibody directed against the expressed protein and developed with a TMB substrate.

Lane 1 is wide-range molecular weight marker.
Lane 2 is the crude cell lysate applied to the plate.
Lane 3 is the eluted protein.

Summary

The HIS-Select iLAP plate is a novel tool for the parallel, integrated lysis and purification of His-tagged proteins from multiple clones of *E. coli*. The simple procedure allows for rapid screening of up to 96 clones per plate for expression. Each well can easily purify microgram quantities of very pure His-tagged target protein that subsequently can be analyzed by a variety of standard protein and proteomic methods. These novel lysis and capture plates are very powerful tools for rapid colony screening and high-throughput protein analysis.

Ordering Information

Product	Description	Unit
H 9412	HIS-Select iLAP Plate	1 each 5 x 1 each