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Product Information

HIS-Select® iLAP® 5 ml Column

Catalog Number **H9913**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Immobilized metal affinity chromatography (IMAC) is widely used for the purification and identification of recombinant fusion proteins with histidine tags. The affinity of the histidine tag for nickel chelate is sequence dependent, but is generally very high. IMAC technology allows the histidine-tagged protein to be captured on a solid support that contains a chelated nickel ion.[1-3]

The HIS-Select® iLAP® (Integrated Lysis and Affinity Purification) 5 ml Column is a single-use, disposable column designed for one-step purification of histidine-tagged proteins directly from a 5 ml bacterial culture. This single-step method uses Sigma's iLAP technology, which allows for quick and simple screening of histidine tagged recombinant proteins.

Each column contains five cell lysis/protein extraction reagent tablets as well as one HIS-Select Affinity Gel pellet.

The lysis reagent tablets include all necessary detergents and enzymes needed for efficient cell lysis and protein extraction. Each ready-to-use column is capable of purifying at least 1 mg of histidine-tagged protein in less than an hour. The procedure provided can be used to extract soluble proteins directly from growing bacterial cells without the need for a clarification step and results in highly purified fusion proteins.

Reagents and Equipment Required but Not Provided

- HIS-Select Wash Buffer – 300 mM NaCl, 50 mM sodium phosphate, and 10 mM imidazole, pH 8.0 (Catalog Number H5288)
- HIS-Select Elution Buffer – 300 mM NaCl, 50 mM sodium phosphate, and 250 mM imidazole, pH 8.0 (Catalog Number H5413)

These stand-alone reagents are also available together as a kit:

- HIS-Select Wash & Elution Buffer Kit (Catalog Number HS0100)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended that the entire Technical Bulletin be read prior to use, especially the reagent compatibility chart.

Storage/Stability

Store HIS-Select iLAP 5 ml Columns at 2–8 °C. Unopened columns are stable for at least 2 years at 2–8 °C.

Procedure

1. Grow cells expressing the recombinant histidine-tagged protein per standard procedures. HIS-Select iLAP Columns are optimized for the purification of proteins from Terrific Broth lysates; however, Luria Broth cultures may also be used with a pH adjustment of the culture (see the Troubleshooting Guide).
2. Ensure that the bacterial culture is a homogenous suspension by gently mixing to resuspend any cells that may have settled to the bottom of the container.
3. Before using the iLAP column, make sure that the bottom cap is firmly in place by pushing it snugly against the column. With the bottom cap in place, add 5 ml of the bacterial culture into the iLAP column. If less than 5 ml of culture is used, excess white lysis tablets may be removed (each tablet will lyse one ml of culture). Briefly vortex to aid in resuspending the lysis reagents and agarose. If desired, save a small aliquot (~50 µl) of the bacterial culture for SDS-PAGE analysis.
4. Cap the column and incubate with continuous mixing for at least 20 minutes at room temperature. Longer incubation times and occasional vortexing may be necessary to ensure complete lysis.
   Note: Incubating the column for longer than one hour is not recommended, as proteolytic degradation of the target protein may occur.
5. Secure the column on a ring stand or other suitable device for performing the subsequent gravity flow purification.
6. Remove the bottom cap and place an appropriate collection tube below the column. Then remove the top cap and allow the cell lysate and unbound proteins to drain completely from the column. If desired, save a sample of this column flow-through for SDS-PAGE analysis.
7. Wash the resin bed by adding 2 ml of HIS-Select Wash Buffer onto the top of the column. Be careful not to disturb the bed of resin at the bottom of column, as this may result in an ineffective wash. Allow the wash solution to completely drain from the column. If desired, save a sample of the wash for SDS-PAGE analysis.
8. For improved purity of the target protein, repeat step 7 once. If desired, save a sample of the second wash for SDS-PAGE analysis.
   Note: It is recommended to use only one wash step for screening expression of histidine-tagged proteins, unless the nature of the protein requires additional washing. Increasing the wash volume may result in decreased target protein in the elution. See the Troubleshooting Guide for more information regarding preparation of the wash solution.
9. Elute the target protein into a clean tube by adding 1 ml of HIS-Select Elution Buffer onto the top of the resin bed. Be careful not to disturb the bed, as this may result in an ineffective elution.
   Note: For a more concentrated solution of target protein, the elution volume may be as low as 600 µl. However, this smaller elution volume may not fully elute the target protein from the resin.
10. The concentration of the eluted protein may be measured by BCA or Bradford protein assay. For analysis by SDS-PAGE, mix an aliquot of each saved sample with an equal volume of 2× Laemmli Sample Buffer (Catalog Number S3401). Boil the samples for 5 minutes and load onto an appropriate gel. Following electrophoresis, the gel may be stained using EZBlue™ Gel Staining Reagent (Catalog Number G1041). Alternatively, samples may be analyzed by Western blot.

Related Products	Catalog Number
EZBlue Gel Staining Reagent	G1041
Bicinchoninic Acid Kit for Protein Determination	BCA1
Bradford Reagent	B6916
2× Laemmli Sample Buffer	S3401

References

1. Sulkowski, E., Immobilized Metal Ion Affinity Chromatography of Proteins. In Protein Purification: Micro to Macro, Burgess, R., ed., Alan R. Liss, Inc. (New York, NY: 1987), pp. 149-162.
2. Herndan, E.S., *et al.*, Surface Topography of Histidine Residues: A Facile Probe by Immobilized Metal Ion Affinity Chromatography. Proc. Natl. Acad. Sci. USA, **86**, 1811-1815 (1989).
3. Anderson, L., *et al.*, Facile Resolution of α-Fetoproteins and Serum Albumins by Immobilized Metal Affinity Chromatography. Cancer Res., **47**, 3624-3626 (1987).

Reagent Compatibility Chart

Reagent	Effect	Comments
Chelating agents (EDTA and EGTA)	Strip metal ions from IMAC resins, chelate essential Mg^{2+} ions	Chelating agents are not compatible with the HIS-Select line of products. They will chelate metal ions from the affinity gel. Also, addition of these reagents to the original cell lysis mixture will chelate metal ions essential for endonuclease activity, which will result in a viscous solution.
Protease Inhibitors	Prevent protein degradation	Protease inhibitors may be added to the bacterial cell culture extraction, if desired. Protease inhibitor cocktails containing EDTA should not be used, as they will chelate metal ions from the affinity gel.

Troubleshooting Guide

Problem	Cause	Solution
Lower than expected protein levels	Cells not completely lysed.	Ensure the lysis materials have completely dissolved into the bacterial culture. Gentle vortexing may be required.
	Target protein degraded.	Addition of protease inhibitors may help reduce target protein degradation.
	Expression level may be too low.	• Add more inducing agent. • Induce for a longer time period. • Check the construct. • Use another bacterial cell line.
	Protein has formed inclusion bodies.	HIS-Select iLAP Columns are used for the purification of soluble histidine-tagged proteins. If insoluble proteins are trapped on top of the resin bed, they may be solubilized with CelLytic™ IB Inclusion Body Solubilization Reagent (Catalog Number C5236). The purification may continue under denaturing conditions.
	Cells grown in Luria Broth.	A minor pH adjustment is necessary for optimal purification, due to the lack of a buffering component in Luria Broth. Adjust the pH of the cell lysate inside the column to 6.8±0.2 before purifying the protein.
	Target protein eluted during wash step.	Decrease the imidazole concentration of the HIS-Select Wash Buffer to 5 mM. Additionally, the wash volume may be reduced to 1 ml. These steps will aid in reducing undesirable elution of the target protein. These measures may also result in an increase in non-specific protein in the elution.

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