

HIS-Select® iLAP® HC Nickel Coated Plate, 96-well, clear

Catalog Number **H9412**
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 the nickel chelate is sequence dependent, but is generally very high. This 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) HC Nickel Coated Plates allow for combined cell lysis and capture of the histidine-tagged target protein in a single well. The plates are coated with a proprietary, patented, high-density nickel chelate matrix. This matrix provides high capacity affinity binding of histidine-tagged recombinant fusion proteins. In addition, the coating contains an optimized mixture of reagents necessary for the fast and efficient lysis of bacterial cells for the purification of recombinant proteins. The lysis components of the coating include a non-ionic detergent, lysozyme, Benzonase®, and protease inhibitors. The lysozyme ensures efficient cell lysis. Benzonase digests nucleic acids, which reduces sample viscosity and allows for greater clarification of nucleic acids. The protease inhibitors help prevent proteolytic degradation of the target protein. The procedure provided can be used to extract soluble proteins directly from growing cells and results in a nearly pure fusion protein preparation following elution from the plate.

The coated plate allows unique multi-sample application potentials including:

- Affinity purification of recombinant fusion proteins with histidine tags
- Direct quantitation of bound protein by standard protein methods (BCA)

- Elution of the fusion protein for SDS-PAGE and Western blotting, or for mass spectrometric analysis.
- Compatible with robotic equipment and protocols
- A complete product for fast and simple colony screening for histidine-tagged recombinant clones

The unique affinity matrix coating possesses the following features:

- Highly specific interaction providing single step protein purification to >90% purity
- High capacity of ≥2 µg protein per well

Binding Capacity: In saturation binding assays performed using this product, binding of ≥2 µg of protein per well is observed following an one hour incubation at 37 °C using a 30 kDa histidine-tagged recombinant protein.

Equipment and Reagents Required But Not Provided

- Wash Buffer - TBS containing 0.05% TWEEN® 20 (Catalog Number T9039) or Phosphate buffered saline containing 0.05% TWEEN 20 (Catalog Number P3563)
- Automated plate washer
- Reagents for protein determination, BCA Procedure (Catalog Number BCA1)
- Elution Buffer - 50 mM sodium phosphate, pH 8.0, with 300 mM sodium chloride and 250 mM imidazole or 0.1% trifluoroacetic acid (TFA) solution
- 2× Laemmli Sample Buffer (Catalog Number S3401).

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.

Storage/Stability

For optimal performance, the unopened product should be stored in a sealed container with desiccation at 2–8 °C. Under these storage conditions, the product is stable for two years.

Procedure

Binding of the histidine-tagged proteins is pH dependent. The suggested pH range for incubation is 7.0–7.5; however, binding can occur over the pH range of 6.5–8.0.

Binding of Recombinant Fusion Proteins with Histidine

Tags: purification from growing cells.

1. Grow cells containing the recombinant protein per standard procedures. These plates have been used successfully with cells grown with either Luria Broth or Terrific Broth media. Binding of the target protein is concentration dependent and good results will be obtained if the concentration of the target fusion protein is at least 0.1 mg/ml in the cell suspension.
2. Load up to 0.2 ml of the cell suspension (growth medium and cells) per well. Allow the samples to incubate 1–4 hours at room temperature or overnight (12 hours) at 2–8 °C. Binding of the target protein is both time and temperature dependent. Low expression levels of certain types of clones may lead to sub-saturation amounts of protein and lower yields. Shorter incubation times may lead to insufficient cell lysis and low protein yields. **Note:** It is recommended to cover the plate during incubation to minimize evaporation and contamination.
3. The cell suspension in each well of the plate should be gently mixed to ensure proper lysis of the cells. This can be done with an orbital mixer or the material in the wells can be pipetted up and down several times after ~1 hour of incubation. Avoid mixing the samples too vigorously, since this may result in cross-well contamination.
4. Wash each well a minimum of four times with 0.3 ml of TBS containing 0.05% TWEEN 20 (Catalog Number T9039). Phosphate buffered saline containing 0.05% TWEEN 20 (Catalog Number P3563) may also be used with good results.
5. Wash each well a minimum of four times with 0.3 ml of ultrapure water to remove any residual detergent. The detergent may interfere with subsequent assays of the protein.

6. The purified protein is now bound in each of the wells. The amount of protein in each well may be determined by direct assay in the well using a BCA protein assay (BCA Kit or QuantiPro BCA Assay Kit). The protein may also be eluted for analysis by SDS-PAGE and Western blot or by mass spectrometry. The protein bound in the well cannot be used for further analysis after the BCA protein assay.

Determination of Bound Protein - In-well BCA Assay

Please refer to the BCA Kit (Catalog Number BCA1) technical bulletin. Note that the bound protein cannot be eluted and analyzed by other methods if the BCA procedure is performed on the bound protein. If other procedures will be performed, then first elute the protein from the appropriate wells and then perform the BCA protein assay on an aliquot of the eluted protein solution.

96-Well Plate Assay for 5–25 µg of total protein

1. Prepare the required amount of BCA Working Reagent needed for the assays (200 µl for each well). Combine the specified volumes of Reagents A and B and mix until the BCA Working Reagent is a uniform, light green color. Prepare standards (typically BSA or other appropriate protein) of different concentrations. For the multiwell format, the protein standards (25 µl) should be added to wells of a separate non-coated plate (Catalog Number M4034).
2. Add 200 µl of the BCA working reagent to each well to be assayed.
3. Seal the plate either with a lid or sealing membrane and incubate for the following time:
60 °C for 15 minutes or
37 °C for 30 minutes or
25 °C (room temperature) for 2 hours to overnight.
4. Read the absorbance at 562 nm. For a plate reader, which does not have the exact wavelength filter, a filter in the range of 540-590 nm can be substituted.

Note: For more sensitive protein detection, the QuantiPro™ BCA Assay Kit is recommended.

Elution and Preparation for SDS-PAGE analysis

1. Add 50–200 µl of Elution Buffer, 50 mM sodium phosphate, pH 8.0, with 300 mM sodium chloride and 250 mM imidazole.
2. Incubate for 10–60 minutes at either room temperature or 37 °C.
3. Transfer the eluted protein solution into a clean container or empty multiwell plate.
4. Mix an aliquot of the eluted sample with an equal volume of 2× Laemmli Sample Buffer (Catalog Number S3401).
5. Boil the sample for 5 minutes.
6. Load onto an SDS-PAGE gel.

Note: Samples eluted from iLAP plates are also compatible with Western blotting techniques.

Elution and Preparation for MALDI-MS

1. The protein can be eluted by addition of 0.2 ml of 0.1% trifluoroacetic acid (TFA) solution to each well.
2. Incubate the solution in the well for 5–30 minutes at room temperature.
3. Mix the eluted sample with the appropriate MALDI matrix.

Note: Samples may also be analyzed by other methods such as reverse phase HPLC or HPLC electrospray mass spectrometry.

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).

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Related Products	Catalog Number
Sodium Chloride	S9625
Sodium Phosphate, Dibasic	S0876
Sodium Phosphate, Monobasic	S8282
Imidazole	I2399
ProteoSilver™ Plus Staining Kit	PROTSIL2
BCA Kit for Protein Determination, recommended for 200-1,000 µg of protein/ml	BCA1
QuantiPro™ BCA Assay Kit, recommended for 0.5-30 µg of protein/ml	QPBCA
EZBlue™ Gel Staining Reagent	G1041

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