High Capacity Capture of Histidine-Tagged Recombinant Proteins in a 96-Well Format for Purification and Characterization

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Abstract

A high capacity (HC) nickel chelate 96-well plate was developed for the selective, single-step capture and purification of histidine-tagged recombinant proteins in a high throughput format. Elution of the bound material was achieved using solutions amenable to characterization by a variety of techniques including SDS-PAGE, Western blotting, and MALDI-MS. Two histidine-tagged recombinant proteins were used to demonstrate the utility of the plate. In either case, microgram quantities of material was captured in a single well providing sufficient material for characterization. SDS-PAGE demonstrated the eluted material was easily detected with Coomassie staining alone. Identity and specificity of the eluted material was demonstrated by Western blotting. Finally, the target protein, eluted in 0.1% trifluoroacetic acid (TFA), was compatible for direct analysis by MALDI-MS.

Introduction

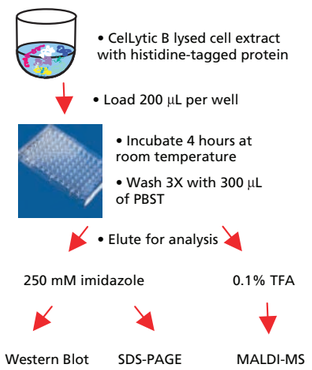
Immobilized metal affinity chromatography (IMAC) is widely used for the purification and identification of recombinant fusion proteins with histidine-containing tags. The affinity of the histidine tags for the nickel chelate is sequence dependent but is generally very high. This allows for the capture of histidine-containing proteins on a solid support (agarose, multiwell plate, magnetic beads, etc.) that contains a chelated nickel ion.[1-3]

The advantage of using the multiwell plate is that it offers high throughput methodology, while resins and magnetic beads do not. HIS-Select HC Nickel Coated 96-Well Plates were utilized for the purification and characterization of recombinant histidine-tagged proteins. The plates were evaluated for capacity, specificity, selectivity, and compatibility with various analysis techniques.

Materials

- Histidine-tagged protein, proprietary
- HAT-BAP, a Bacterial Alkaline Phosphatase with a HAT-tag (HAT is a trademark of Clonetech)
- CelLytic B (B3553)
- HIS-Select HC Nickel Coated 96-Well Plates (S5563)
- HIS-Select HC Nickel Affinity Gel (P6611)
- BCA Protein Determination Kit (BCA-1)
- ColorBurst Marker (C4105)
- EZBlue Gel Staining Reagent (G1041)
- ProteoSilver Kit (PROT-SIL1)
- ProteoQuest Colorimetric Western Blotting Kit (PQ 0101)
- All other reagents used were obtained from Sigma-Aldrich

Methods



- The crude *E. coli*, containing the expressed histidine-tagged proteins, were lysed using CelLytic B. Purified histidine-tagged protein was obtained by purification on the HIS-Select HC Nickel Affinity Gel.
- Purified histidine-tagged proteins were used at saturating amounts for the capacity study.
- The *E. coli* lysate, containing the expressed histidine-tagged protein, was loaded into the wells of the Sigma and competitor plates. For a negative control, cells were lysed in CelLytic B and imidazole was added at a final concentration of 250 mM as a competitive inhibitor.
- SDS-PAGE analysis was performed using 4-20% Tris-Glycine gels. The samples were diluted 1:1 in Laemmli loading buffer (S3401) and 20 µL was loaded in each lane, representing 5% of the elution. 5 µL of the ColorBurst Marker was loaded undiluted. Coomassie staining was performed using EZBlue Gel Staining Reagent. Silver staining was performed using a ProteoSilver Kit.
- After separation by SDS-PAGE, the proteins were transferred to a nitrocellulose membrane. The membranes containing the histidine-tagged proteins were blotted and detected with monoclonal antibodies directed to the proteins. Anti-Mouse HRP (A9044) was the secondary antibody and the membrane was developed using 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (T0565).
- The histidine-tagged protein was eluted from the plate in 200 µL of 0.1% TFA. The samples were mixed 1:1 with sinapinic acid (10 mg/mL in 70% acetonitrile, 0.1% TFA) for analysis by MALDI-MS.
- MALDI-MS data was acquired in the linear positive ion mode using an Axima-CFR mass spectrometer (Shimazdu Biotech).

Results

Binding Capacity Comparison

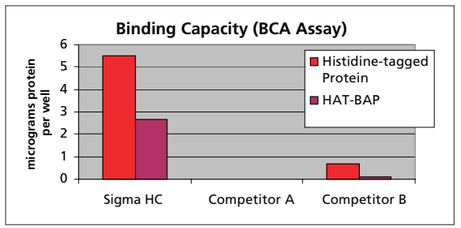


Fig. 1. Binding capacity comparison of the Sigma HC plate to commercially available nickel chelate plates using two different histidine-tagged proteins. Binding capacities were determined using a BCA protein assay in the plate. Sigma HC nickel chelate plate binds several micrograms per well of protein while the competitor plates bind at a level below the detection limit of the assay.

Purification and Characterization of Histidine-tagged Protein

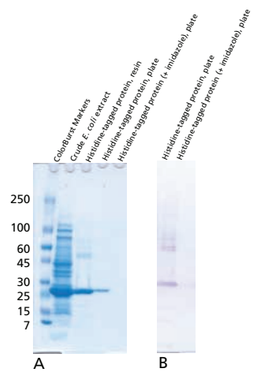


Fig. 2. A) **SDS-PAGE gel** stained with EZBlue. B) **Western blot** of the histidine-tagged protein eluted from the plate. The competitive inhibition of binding by 300 mM imidazole demonstrated the specificity of binding. The protein can be purified from a crude extract on a plate with the same selectivity as the resin.

Comparison of Competitor Plates by SDS-PAGE

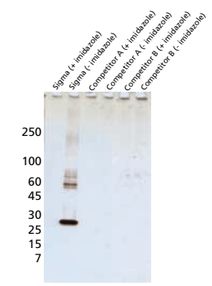


Fig. 3. Silver stained gel comparing the Sigma HC plates to competitor plates for purification of a histidine-tagged protein. No target protein was detected from competitor plates, even with silver staining which is sensitive down to 0.5 ng.

Characterization by MALDI-MS

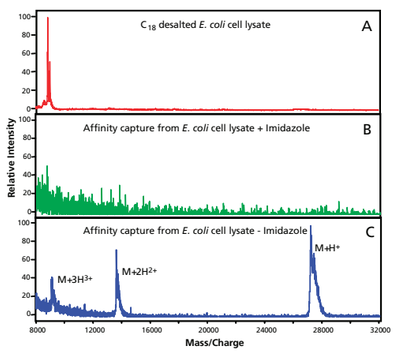


Fig. 4. MALDI mass spectrum of A) a C_{18} desalted *E. coli* cell lysate positive for the expression of the histidine-tagged protein. B) an affinity purification of the histidine-tagged protein from *E. coli* cell lysate in the presence of 300 mM imidazole. C) an affinity purification of the histidine-tagged protein from *E. coli* cell lysate in the absence of imidazole on Sigma HC nickel chelate. The plate purification from the crude extract allows for direct MALDI-MS analysis (C), although it was not detected in the total lysate after a desalt step (A).

Analysis of Captured HAT-BAP

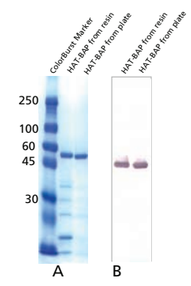


Fig. 5. A) **SDS-PAGE gel** stained with EZBlue. B) **Western blot** of the HAT-BAP eluted from the plate. The gel indicates that the plate has greater specificity than the resin for the tagged protein. The Western blot demonstrates that the approximately 55 kDa protein captured was HAT-BAP.

Conclusions

- Microgram quantities of histidine-tagged protein are captured per well, which is sufficient protein for standard characterization techniques such as SDS-PAGE, Western blotting, and MALDI-MS.
- HIS-Select HC plates offer at least a 10-fold greater capacity than other commercially available nickel chelate multiwell plates.
- HIS-Select HC plates selectively capture histidine-tagged proteins from cell lysates.
- The specificity of binding to the histidine-tagged proteins was illustrated by inhibition of binding with imidazole.
- HIS-Select plates can be eluted under conditions compatible with MALDI-MS.

References

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2. Hemdan, 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. Andersson, L., et al., Facile Resolution of α-Fetoproteins and Serum Albumins by Immobilized Metal Affinity Chromatography. Cancer Res., **47,** 3624-3626 (1987).