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Expression, Purification and Detection of Recombinant Fusion Proteins Using the MAT™ Tag System

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Abstract

We recently developed the MAT™ tag system (metal affinity tag) for the expression, purification, and detection of recombinant fusion proteins. The system utilizes a small, novel, seven amino acid histidine-based affinity tag that allows simple, one-step purification of target fusion proteins by Immobilized Metal Affinity Chromatography (IMAC). In addition, we have developed a very sensitive detection system utilizing a highly specific monoclonal antibody that can detect both N- and C-terminal MAT-tagged proteins.

In this work we describe use of the MAT tag system for *Escherichia coli* colony fusion protein expression screening and for standard methods of expression, purification, and detection of a variety of different bacterial and mammalian recombinant fusion proteins. In addition, we demonstrate the utility of the MAT tag for fusion protein purification using different IMAC formats. This system provides additional flexibility for studying protein expression, structure, modification, function, and protein-protein interactions.

Introduction

Goal

- Test the utility of a new IMAC-compatible fusion protein tagging system

Approach

- Screen and test expression of different MAT-tagged fusion proteins
- Purify MAT-tagged fusion proteins using different IMAC platforms

Background

Short peptide tags containing histidine residues allow easy, one-step purification of tagged fusion proteins by IMAC. The HIS-Select™ nickel chelate IMAC affinity gels and plates were recently developed at Sigma-Aldrich. They contain a proprietary tetradentate nitriloacetic acid (NTA) analog chelate attached to the support matrix by an uncharged, hydrophilic spacer arm (Figure 1). This chemistry results in a stable, highly selective affinity capture system with low non-specific protein binding.

We wished to demonstrate the utility of this novel fusion protein tagging system for the expression and purification of a variety of different fusion proteins using different IMAC formats.

HIS-Select™ Nickel Chelate

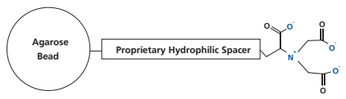

Figure 1. Depiction of the HIS-Select nickel chelate IMAC affinity resin structure. A quadradentate NTA chelate group is attached to agarose beads through a proprietary uncharged, hydrophilic spacer arm. This IMAC affinity resin displays less non-specific protein binding than conventional IMAC resins because the spacer arm is uncharged. The atoms of the chelate group that bind the nickel ions are shown in blue.

The MAT Tag

Creation of fusion tag pool for screening for a unique metal affinity tag
A 27 kD fusion protein coding sequence was cloned with a partially randomized C-terminal synthetic primer sequence containing the coding sequence for $_N$His-X-His-X-His-X-His$_C$.

The codon positions marked X were randomized to code for all 20 amino acids except histidine, proline, and glutamine.

Primary Screen
E. coli clones transformed with expression plasmid DNA containing the randomized sequence were screened for expression by immunostaining colony lifts. Clones that showed robust staining were screened by an ELISA assay using HIS-Select HS (high sensitivity) Nickel-Coated 96-Well Plates.

Secondary Screen

Twenty-one *E. coli* clones showing high expression by the ELISA assay were screened for expression by SDS-PAGE analysis of tagged protein captured in wells of HIS-Select HC (high capacity) Nickel-Coated 96-Well Plates (Figure 2).

HIS-Select HC Plate Screening for MAT Tag

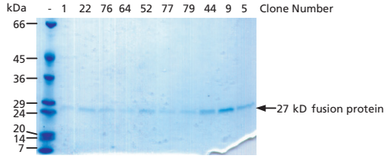

Figure 2. Individual clones showed differential binding and recovery when screened on high capacity HIS-Select plates. Twenty one clones with the highest ELISA assay values were screened for binding and elution on HIS-Select HC Nickel-Coated 96-Well Plates. Lysates of the clones were incubated in wells with 5 mM imadazole, washed and subsequently eluted with 200 mM imidazole elution buffer. The eluates were analyzed by SDS-PAGE and visualized by EZBlue™ staining. Clone 9 showed the highest amount of target protein captured and eluted.

MAT Tag Sequence

The plasmid from one of the clones showing high expression in the secondary screen was partially sequenced. The **m**etal **a**ffinity **t**ag amino acid sequence encoded in that plasmid DNA, designated as the **MAT tag**, is shown:

N-His-Asn-His-Arg-His-Lys-His-C

Anti-MAT™ Monoclonal Antibody

The Anti-MAT monoclonal antibody was purified from ascites fluid generated by a hybridoma (MAT 1-87), which was produced by fusion of NS1 mouse myeloma cells (NS1) and splenocytes from BALB/c mice immunized with synthetic MAT peptide (HNHRHKHGGGC) conjugated to KLH via the C-terminal cysteine.

Expression Screening with Anti-MAT mAb

The open reading frames of twelve different *E. coli* proteins were cloned into an expression vector in frame with an N-terminal FLAG tag and a C-terminal MAT tag.

Transformant colonies were screened for expression of C-terminal MAT-tagged proteins by using the anti-MAT monoclonal antibody to immunostain filter lifts of the induced colonies. Differential expression can be seen on the immunostained filter (Figure 3).

Colony Lift Screening with Anti-MAT Monoclonal Antibody

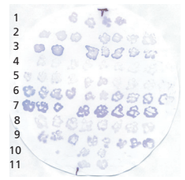

Figure 3. Differential expression of MAT-tagged proteins can be detected by Anti-MAT antibody immunostaining of *E. coli* colony lifts. Sets of different open-reading frame *E. coli* transformant colonies **(rows 1–11)** were grown on an LB + amp plate overnight. The colonies were lifted with a nitrocellulose filter to a plate containing 0.1 M IPTG and induced for 4 hours. The induced colonies were lysed, and the filter immunostained using Anti-MAT monoclonal antibody (0.5 µg/ml), followed by Rabbit-Anti-Mouse IgG-HRP conjugate. The filter was developed and visualized with TMB Substrate.

Western Blot Immunostaining with Anti-MAT mAb

The coding region for bacterial alkaline phosphatase (BAP) was cloned into an expression vector to generate an N-terminal FLAG-tagged and a C-terminal MAT-tagged fusion protein.

ANTI-FLAG and Anti-MAT monoclonal antibodies specifically recognized full-length FLAG-BAP-MAT fusion protein by immunostaining of Western blots of samples from induced *E. coli* cell lysates (Figure 4, **Lanes 3 and 6)**. No protein was detected in samples of lysates of uninduced cells (Figure 4, **Lanes 2 and 5)**.

Tag-Specific Immunostaining of FLAG-BAP-MAT

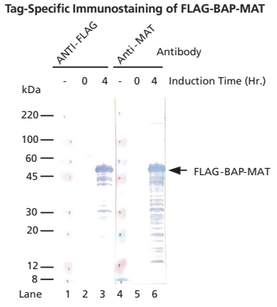

Figure 4. Full-length FLAG-BAP-MAT fusion protein is detected on Western blots by monoclonal antibodies to the FLAG and MAT tags. Lysates from uninduced **(Lanes 2 and 5)** and induced **(Lanes 3 and 6)** *E. coli* cultures and ColorBurst Markers **(Lanes 1 and 4)** were separated by SDS-PAGE and blotted to nitrocellulose. The resulting blot was immunostained using either ANTI-FLAG M2-HRP conjugate **(Lanes 1–3)** or Anti-MAT monoclonal antibody (0.5 µg/ml) followed by Rabbit-Anti-Mouse IgG-HRP conjugate **(Lanes 4–6)**. The blots were developed and visualized with TMB Substrate.

Expression Screening with the Anti-MAT Antibody

The expression of ten different *E. coli* open reading frame clones was tested by immunostaining a Western blot of small-scale expression trial lysates. Expression of the induced proteins and fragments could be detected with the anti-MAT monoclonal antibody (Figure 5, **right panel, Lanes 2-11)**.

Expression Screen Western Blot Immunosaining

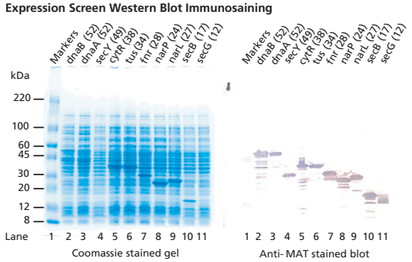

Figure 5. Screening of small-scale expression trials by Western blot immunostaining. Open reading frames of ten different *E. coli* proteins **(name and predicted molecular weight above each lane)** were cloned into an *E. coli* expression vector in frame with a C-terminal MAT tag. Small cultures were induced for expression and CellLytic B lysates were screened for fusion protein expression by SDS-PAGE, followed by EZBlue staining **(left panel)** or Western blot immunostaining with Anti-MAT monoclonal antibody (0.5 µg/ml), followed by Rabbit-Anti-Mouse IgG-HRP conjugate **(right panel)**. The blot was developed and visualized with TMB Substrate.

Affinity Spin Column Screening

The expression of five different *E. coli* open reading frame clones was tested by affinity spin column purification of clarified small-scale expression trial lysates. The eluted, soluble, expressed proteins were detected by Coomassie staining of gels (Figure 6, **Lanes 2, 6 and 8)**.

Affinity Spin Column Screening for Soluble Expressed Protein

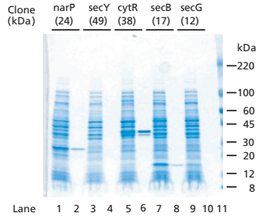

Figure 6. Soluble fusion protein expression can be determined by rapid, small-scale purification using IMAC spin columns. Positive clones identified by filter lift and Western blot immunostaining were grown in small cultures and induced. Lysates were made in CellLytic B and applied to HIS-Select Nickel Spin Columns. After washing, the bound proteins were eluted with 250 mM imidazole. Samples of the lysates **(Lanes 1,3,5,7, and 9)** and eluted proteins **(Lanes 2,4,6,8, and 10)** were seperated by SDS-PAGE and visualized by EZBlue staining.

Purification of FLAG-GrpE-MAT by IMAC

The coding region for an *E. coli* chaperone protein, GrpE, was cloned into an expression vector to generate an N-terminal FLAG-tagged and a C-terminal MAT-tagged fusion protein.

FLAG-GrpE-MAT fusion protein expression was induced in *E. coli* and the fusion protein purified from a cell lysate on a 1 ml HIS-Select HF (high flow) Nickel Affinity Gel column. The FLAG-GrpE-MAT purification was analyzed by SDS-PAGE analysis of purification fractions (Figure 7). About 7 mg of purified FLAG-GrpE-MAT was recovered in the elution fractions from a 500 ml culture.

IMAC Purification of FLAG-GrpE-MAT

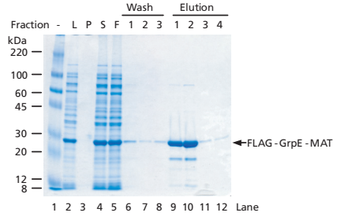

Figure 7. A MAT-tagged fusion protein expressed *E. coli* can be affinity purified by IMAC. The coding region for GrpE was cloned into an *E. coli* expression plasmid, pFLAG-MAC, with an N-terminal FLAG tag and a C-terminal MAT tag. The fusion protein was expressed in *E. coli* strain BL21. A lysate **(L, lane 2)** was made in CellLytic B and separated by centrifugation into a pellet **(P, Lane 3)** and a supernatant **(S, Lane 4)** fraction. The supernatant was applied to a 1.0 ml HIS-Select HF Nickel Affinity Gel column and the flow through **(F, Lane 5)**, wash **(1–3, Lanes 6–8)** and elution **(1–4, Lanes 9–12)** fractions were collected. Samples of the fractions and ColorBurst Markers **(Lane 1)** were separated by SDS-PAGE and visualized by EZBlue staining.

Direct Lysis and Capture of MAT-tagged Proteins

Induced *E. coli* cultures expressing two different MAT-tagged proteins were applied to wells of an integrated lysis and purification (iLAP™) plate. The purified, eluted MAT-tagged proteins could be detected after SDS-PAGE by silver staining (Figure 8, **Lanes 3 and 5)**.

iLAP Plate Purification

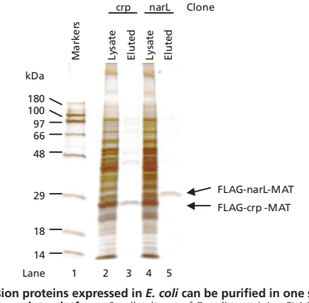

Figure 8. MAT-tagged fusion proteins expressed in *E. coli* can be purified in one step using an integrated lysis and capture plate platform. Small cultures of *E. coli* containing FLAG-crp-MAT (25 kDa) or FLAG-narL-MAT (28 kDa) expression plasmids were grown and induced. Samples (0.1 ml) were transferred manually to wells of a HIS-Select iLAP HC Nickel Coated 96-Well Plate. After 2 hours of incubation at room temperature, the wells were washed repeatedly. The bound protein was eluted in 250 mM imidazole elution buffer and the lysate **(Lanes 2 and 4)** and eluted proteins **(Lanes 3 and 5)** separated by SDS-PAGE. The proteins in the gel were visualized by silver staining.

Automated Lysis and Capture

Cultures of *E. coli* expressing a MAT-tagged fusion protein were robotically directly lysed and purified by IMAC in wells of iLAP plates. The captured and eluted MAT-tagged protein was efficiently and reproducibly purified in different wells, as shown by SDS-PAGE analysis (Figure 9).

iLAP Plate Automated Purification

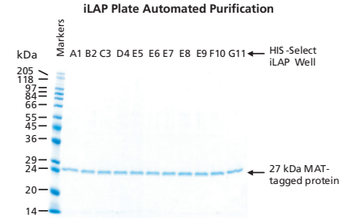

Figure 9. A 27 kDa MAT-tagged mammalian fusion protein expressed in *E. coli* and purified robotically using an integrated lysis and capture plate platform. Small cultures of *E. coli* containing a 27 kDa MAT-tagged protein were grown and induced robotically in a deep well 96-well plate. Samples (0.1 ml) were transferred robotically to wells of a HIS-Select iLAP HC Nickel Coated 96-Well Plate. After 2 hours of incubation at room temperature, the wells were repeatedly robotically washed. The wells were eluted with 250 mM imidazole elution buffer and the proteins eluted from eleven of the wells were separated by SDS-PAGE. The eluted proteins on the gel were visualized by EZBlue staining. Automated processing was performed on a Sciclone ALH 3000 Workstation (Caliper Life Sciences).

Discussion

1. A novel metal affinity tag, the MAT tag, was discovered by screening a fusion tag expression library using immunological assays and by SDS-PAGE analysis of fusion proteins captured with HIS Select HC Nickel-Coated 96-Well Plates.
2. An Anti-MAT mouse monoclonal antibody was developed which was used to specifically detect MAT-tagged fusion proteins on colony filter lifts and Western blot immunostaining. The Anti-MAT antibody was also used to detect MAT-tagged proteins in cells by immunostaining and capture MAT-tagged target proteins by immunoprecipitation (data not shown).
3. We demonstrate here the utility of the MAT tag system to express, screen, characterize, and purify a variety of MAT-tagged fusion proteins.

Conclusion

We have developed a new metal affinity tag, the MAT tag, MAT-fusion protein expression vectors, and the Anti-MAT monoclonal antibody as a complete protein expression system. We have demonstrated its utility for expression, purification, and detection of a variety of recombinant fusion proteins.

The novel MAT tag system allows investigators additional flexibility for studying protein expression, structure, modification, function, and protein-protein interactions.

Acknowledgements

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Materials

Product Name	Product Number
HIS-Select™ HS Nickel-Coated 96-Well Plates	S 5688
HIS-Select HC Nickel-Coated 96-Well Plates	S 5563
HIS-Select HF Nickel Affinity Gel	H 0537
HIS-Select Spin Columns	H7787
HIS-Select iLAP™ HC Nickel-Coated 96 Well Plate (Clear)	H 9412
HIS-Select Wash and Elution Buffer Kit	HS 0100
CelLytic™ B Cell Lysis Reagent	B 3553
pT7-FLAG-MAT™-1 Expression Vector	E 5280
pFLAG-MAC™ Expression Vector	E 5644
ANTI-FLAG® M2 mAb-HRP Conjugate	A 8592
Anti-MAT™ Monoclonal Antibody	M 6693
Rabbit Anti-Mouse IgG-HRP Conjugate	A 9044
ColorBurst™ Marker	C 4105
EZBlue™ Gel Staining Reagent	G 1041
ProteoSilver™ Silver Stain Kit	PROT-SILI
3,3′,5,5′ Tetramethylbenzidine (TMB) Liquid Substrate for Membranes	T 0565

All other reagents were obtained from Sigma-Aldrich.
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