

Simplified purification of secreted histidine-tagged proteins 分泌型组氨酸标签蛋白的简单纯化



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Outline

Introduction 介绍

Applications 应用

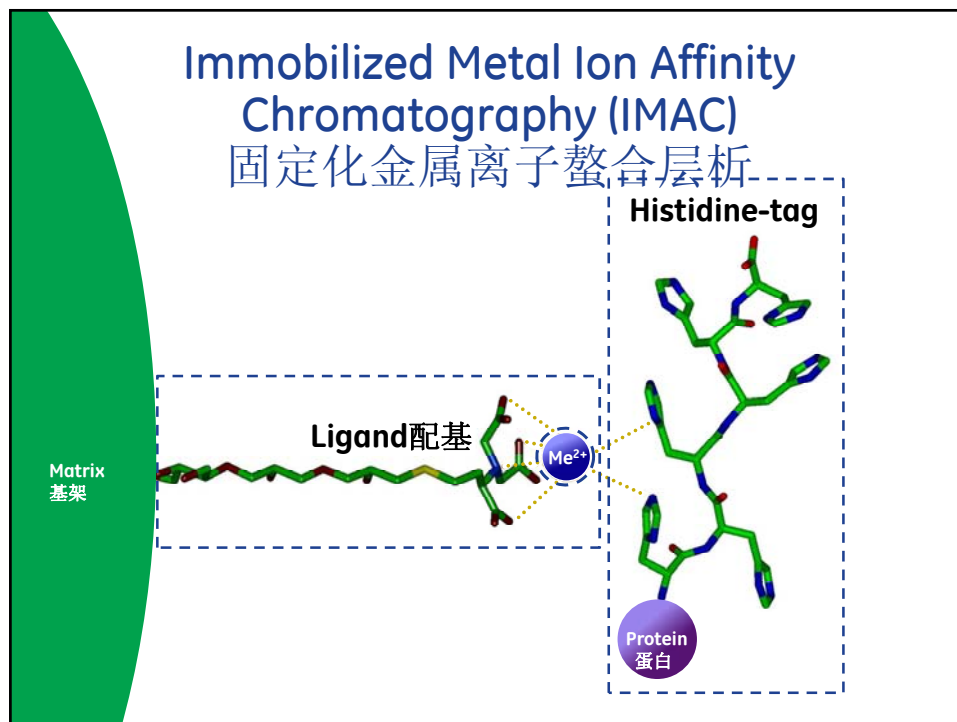
Summary 总结



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Advantages of IMAC

IMAC的优点

Histidine-tag 组氨酸标签

- Small with low charge 小且带电荷少**
- Stable 稳定**
- Compatible with many chemicals 与多种化学试剂相兼容**
- Compatible with denaturing conditions 兼容变性条件**

Chromatography medium 层析填料

- High binding capacity 高结合载量**
- Controllable selectivity 可控的选择性**
- Mild elution conditions 温和的洗脱条件**

The diagram shows a protein with a histidine tag (green chain) binding to a metal ion (blue sphere) immobilized on a green **Matrix** surface. Dashed lines represent the coordination between the metal ion and the histidine tag.

Matrix

A challenge in IMAC purification IMAC纯化的一个挑战

Secretion into cultivation media for eukaryotic cells 真核细胞分泌到培养基中

- **Interfering substances:** Chelators (e.g. EDTA), histidine, arginine, etc.
干扰物质: 螯合剂 (如EDTA), 组氨酸, 精氨酸等
- **Low target protein concentration** → Large sample volumes (> 5 L)
目标蛋白浓度低 样品体积大(> 5 L)

Issue 问题:

→ Stripping of immobilized Me^{2+} from conventional IMAC resins 从传统的IMAC螯合介质上剥离金属离子

No or low binding of histidine-tagged protein

弱结合或不结合组氨酸标签蛋白

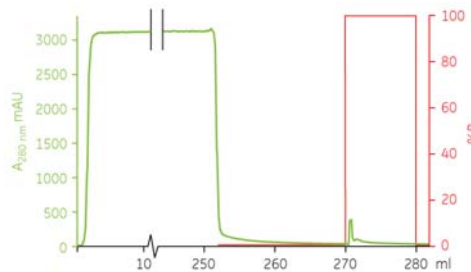


ima₂

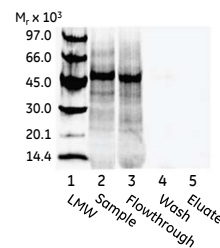
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Conventional IMAC 传统的IMAC Protein secreted into CHO cell culture medium 分泌到CHO细胞培养基中的蛋白

Resin: Ni Sepharose™ 6 Fast Flow (FF), 1 ml (column Ø=5 mm)
Sample: 250 ml of mPAI-1-(his)₈ secreted into GIBCO™ CD CHO medium, pH 7.0
System: ÄKTA™ avant 25



Equilibration buffer: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer: 20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



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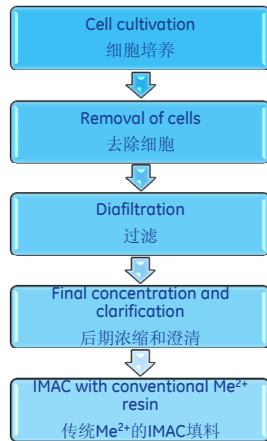
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Remedy改进

Common workflow 一般流程



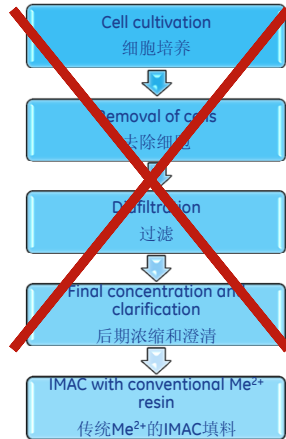
Remedy改进

Common workflow 一般流程

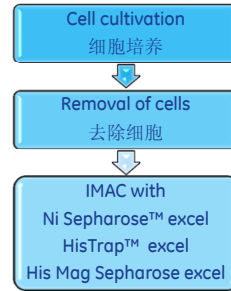


Remedy改进

Common workflow 一般流程



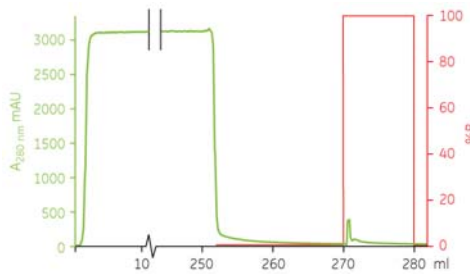
Simplified workflow 简化流程2



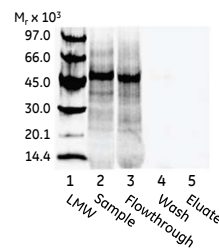
Conventional IMAC传统的IMAC

Protein secreted into CHO cell culture medium
分泌到CHO细胞培养基中的蛋白

Resin: Ni Sepharose™ 6 Fast Flow (FF), 1 ml (column Ø=5 mm)
Sample: 250 ml of mPAI-1-(his)₆ secreted into GIBCO™ CD CHO medium, pH 7.0
System: ÄKTA™ avant 25



Equilibration buffer: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4
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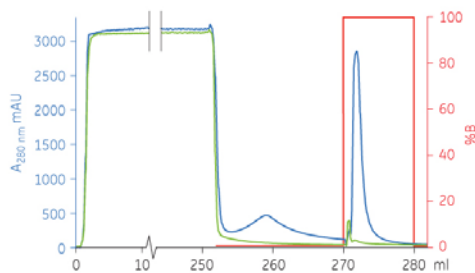


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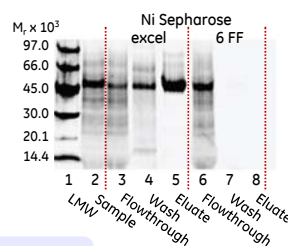
Simplified IMAC简化的IMAC

Protein secreted into CHO cell culture medium 分泌到CHO细胞培养基中的蛋白

Resins: Ni Sepharose™ excel (blue) and Ni Sepharose 6 FF (green), 1 ml (column Ø=5 mm)
Sample: 250 ml of mPAI-1-(his)₈ secreted into GIBCO™ CD CHO medium, pH 7.0
System: ÄKTA™ avant 25

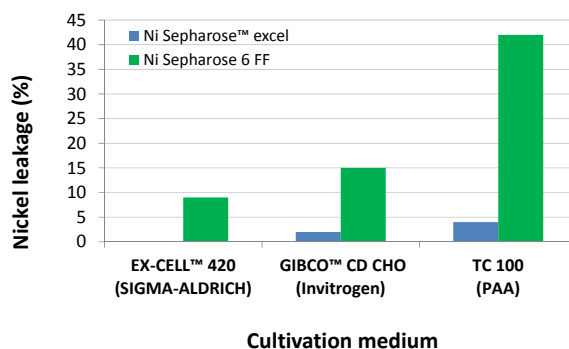


Equilibration buffer: 20 mM sodium phosphate, 500 mM NaCl, 0 mM (excel) or 20 mM (FF) imidazole, pH 7.4
Wash buffer: 20 mM sodium phosphate, 500 mM NaCl, 10 mM (excel) or 20 mM (FF) imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



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Nickel leakage 镍离子脱落



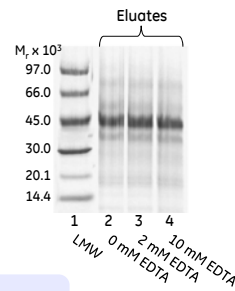
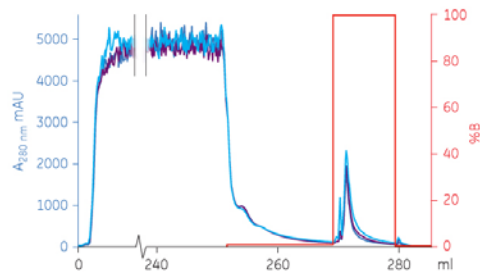
Test: 1 ml of each resin, incubated in 5 ml cultivation media for 24h at room temperature.
检测: 每种介质1ml, 室温条件下在5ml培养基中孵育24小时.
Analysis: Nickel leakage was determined by elemental analysis.
分析: 通过元素分析来检测Ni脱落



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Compatibility with EDTA EDTA兼容性

Column: HisTrap™ excel 1 ml
Sample: 250 ml of proCPU-(his)₆ secreted into GIBCO™ Sf-900 II insect cell medium, pH 6.8, later supplemented with 0 mM (dark blue), 2 mM (purple), or 10 mM (pale blue) EDTA
System: ÄKTA™ avant 25



Equilibration buffer: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer: 20 mM sodium phosphate, 500 mM NaCl, 15 mM imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



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Introduction介绍

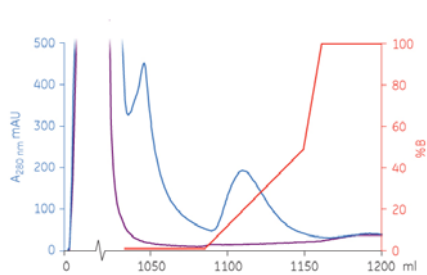
Applications应用

Summary总结

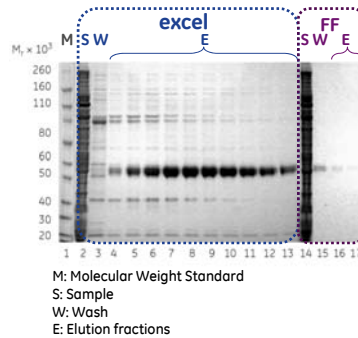


Protein secreted into insect cell culture medium 分泌到昆虫细胞培养基中的蛋白

Columns: HisTrap™ excel 5 ml (*blue*) and HisTrap FF crude 5 ml (*purple*)
 Sample: (his)₆-HA secreted into GIBCO™ SF-900 II SFM insect cell culture medium, pH 6.6
 Sample volumes: 1020 ml and 1012 ml, respectively
 System: ÄKTA™



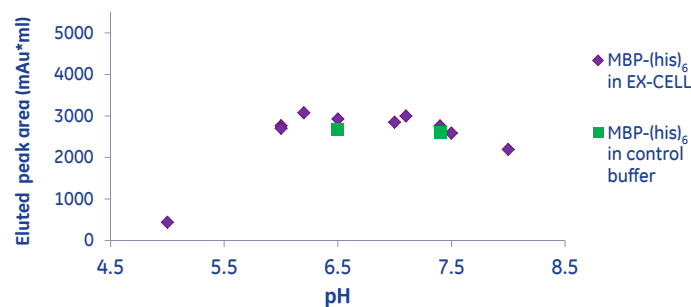
HisTrap excel 5 ml: Yield 8.9 mg of target protein
 HisTrap FF crude 5 ml: Yield too low to be quantified



Data from Dr. Linda Lua et al., UQ Protein Expression Facility, the University of Queensland, Brisbane, Australia.

Effect of sample pH 样品pH对收率的影响 on yield

Resin: Ni Sepharose™ excel, 0.25 ml (column Ø=5 mm)
 Sample: 35 ml of 0.1 mg/ml MBP-(his)₆ added to SAFC EX-CELL™ 405 culture medium, pH 5 to 8
 System: ÄKTA™ avant 25



→ Yields are equal at sample pH values ranging from 6.0-7.5
 样品pH从6.0-7.5范围内基本相同



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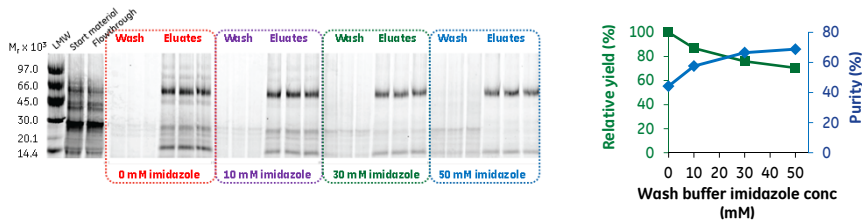
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GE /

Impact of imidazole on purity and yield 咪唑对收率及纯度的影响

Resin: His Mag Sepharose™ excel, 200 µl 10% medium slurry
Sample: 10 ml PRCP-(his)₆ secreted into SAFC EX-CELL™ 405 culture medium, pH 6.9

Imidazole conc was varied in the wash buffer: 0 mM (red), 10 mM (purple), 30 mM (green) and 50 mM (blue)



Higher imidazole concentration → higher purity & lower yield

高咪唑浓度 → 高纯度 & 低收率



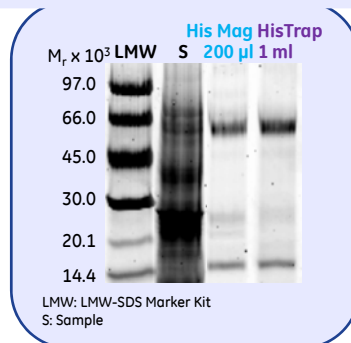
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50-fold scale-up 50倍放大 His Mag Sepharose™ excel → HisTrap™ excel column

Resin/column: His Mag Sepharose excel, 200 µl 10% medium slurry (blue) scaled up to HisTrap excel, 1 ml column (purple)

Sample: 10 and 500 ml PRCP-(his)₆ secreted into SAFC EX-CELL™ 405 culture medium, pH 6.9

30 mM imidazole was used in wash buffer for scale-up experiment
放大实验在冲洗缓冲液中使用了30 mM咪唑



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Summary总结

IMAC purification of proteins secreted into eukaryotic cell culture media is simplified using Nickel Sepharose™ excel
使用Nickel Sepharose™ excel简化了分泌在真核细胞培养基中的蛋白的固定化金属离子螯合层析

- Exceptionally strong binding of Ni^{2+} 特别强的结合 Ni^{2+}
- Minimal sample pretreatment 最少的样品预处理
 - No dialfiltration or other buffer-exchange procedure needed
无需超滤或其它缓冲液置换的过程
 - Broad pH range广泛的pH范围
- Flexibility灵活



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Thank you!



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Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent numbers 5,284,933 and 5,310,663, and equivalent patents and patent applications in other countries assignee: Hoffman La Roche, Inc.).

Ni Sepharose 6 Fast Flow products are sold under a license from Sigma-Aldrich under patent number EP 1277616 (Metal chelating compositions) and equivalent patents and patent applications in other countries.

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