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

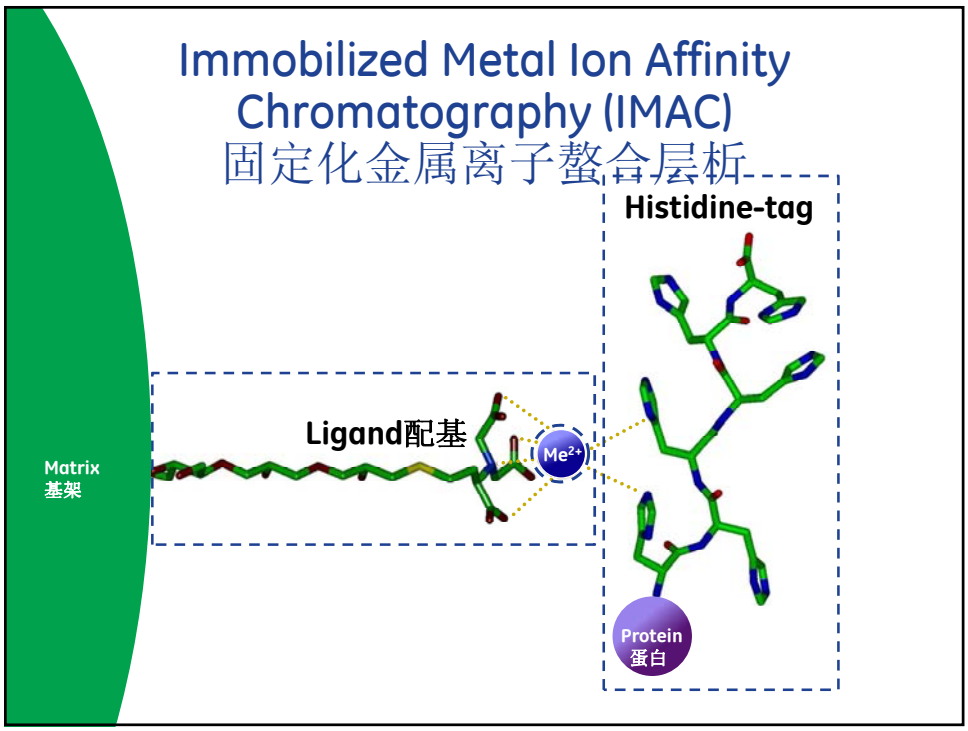
Introduction介绍

Applications应用

Summary总结

GE imagination at work

Immobilized Metal Ion Affinity Chromatography (IMAC)
固定化金属离子螯合层析



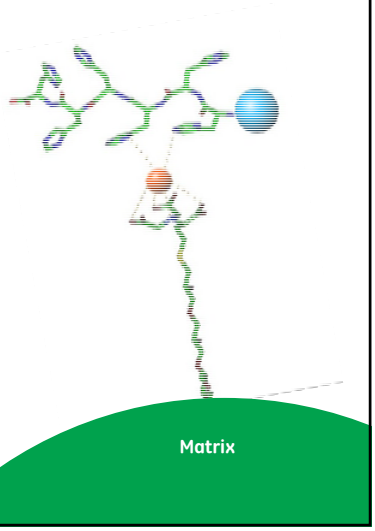
Advantages of IMAC
IMAC的优点

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



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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:

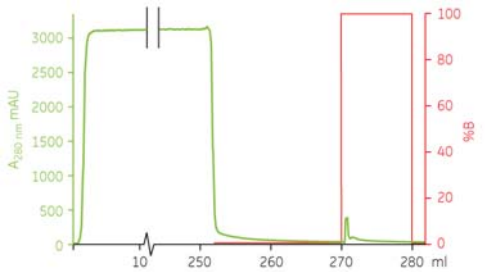
→ Stripping of immobilized Me^{2+} from conventional IMAC resins从传统的IMAC螯合介质上剥离金属离子

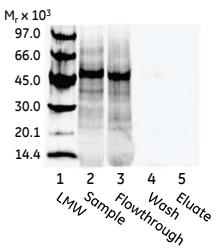
No or low binding of histidine-tagged protein

弱结合或不结合组氨酸标签蛋白

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*

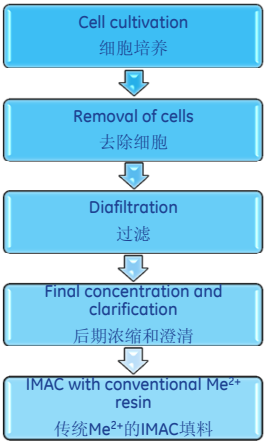




Equilibration buffer:	*20 mM sodium phosphate, 500 mM NaCl, pH 7.4*
Wash buffer:	*20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4*
Elution buffer:	*20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4*

Remedy改进

Common workflow
一般流程



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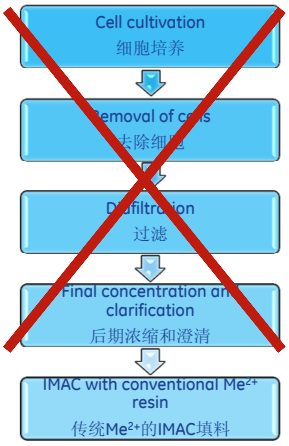
Remedy改进

Common workflow
一般流程



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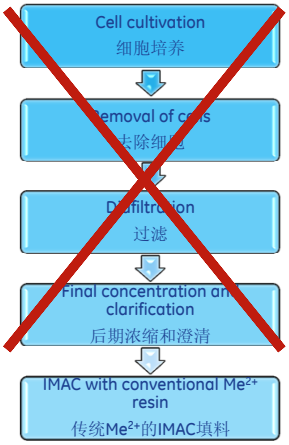
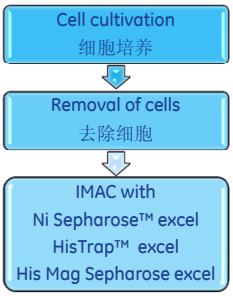
Remedy改进

Common workflow
一般流程



Simplified workflow
简化流程2



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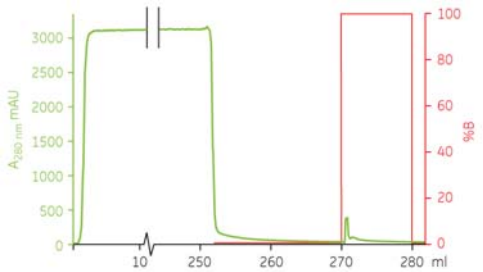
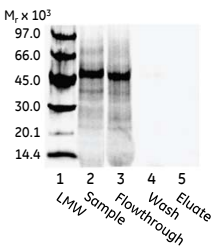
Conventional IMAC传统的IMAC

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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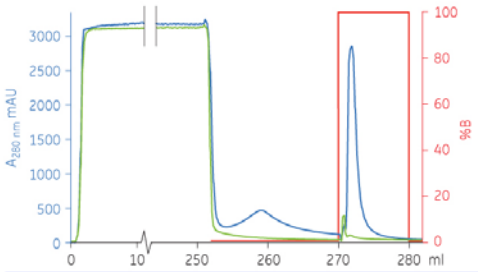
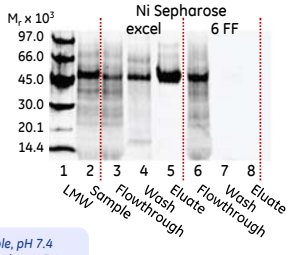
Simplified IMAC简化的IMAC

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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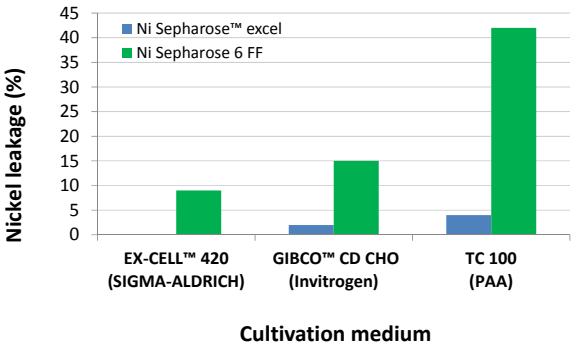
Nickel leakage

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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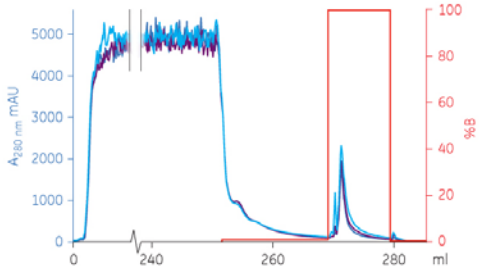
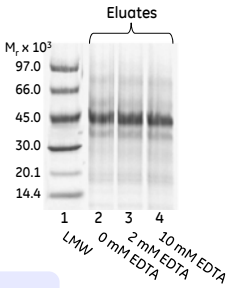
Compatibility with EDTA
EDTA兼容性

Column:	*HisTrap™ excel 1 ml*
Sample:	*250 ml of proCPU-(his)$_8$ 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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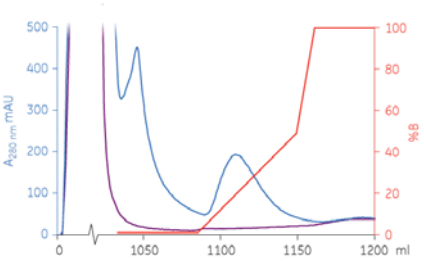
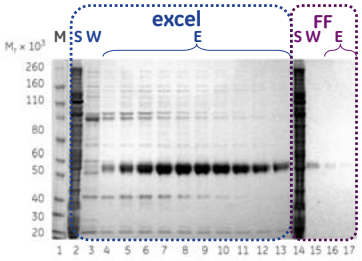
Protein secreted into insect cell culture medium
分泌到昆虫细胞培养基中的蛋白

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

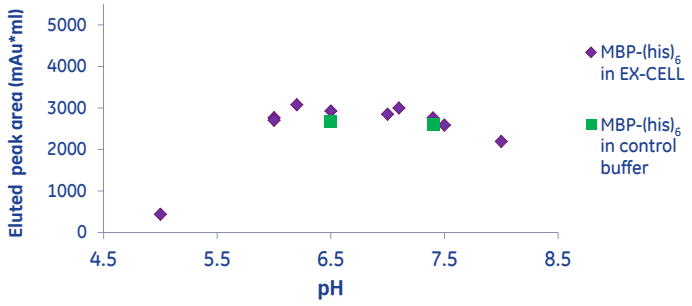


M: Molecular Weight Standard
S: Sample
W: Wash
E: Elution fractions

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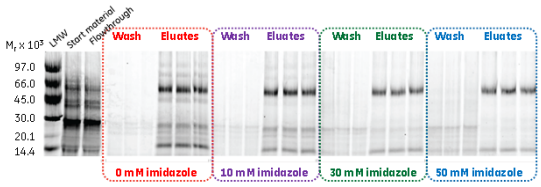
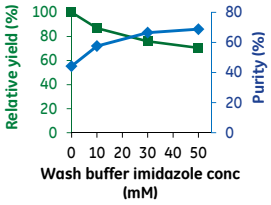
Impact of imidazole on purity and yield
咪唑对收率及纯度的影响

Resin: *His Mag Sepharose™ excel, 200 µl 10% medium slurry*
Sample: *10 ml PRCP-(his)$_9$ 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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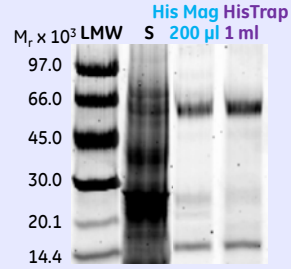
50-fold scale-up 50倍放大
His Mag Sepharose™ excel → HisTrap™ excel column

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)$_9$ 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



LMW: LMW-SDS Marker Kit
S: Sample

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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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