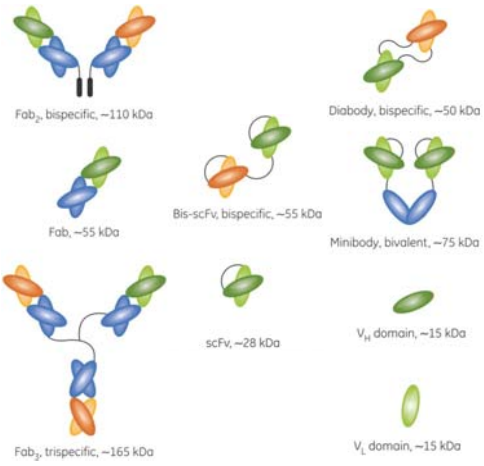
Purification of Antibody Fragments
抗体片段的纯化



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Outline 概况

Introduction 介绍

Applications 应用

- Fab
- Dab
- scFv

Summary 总结

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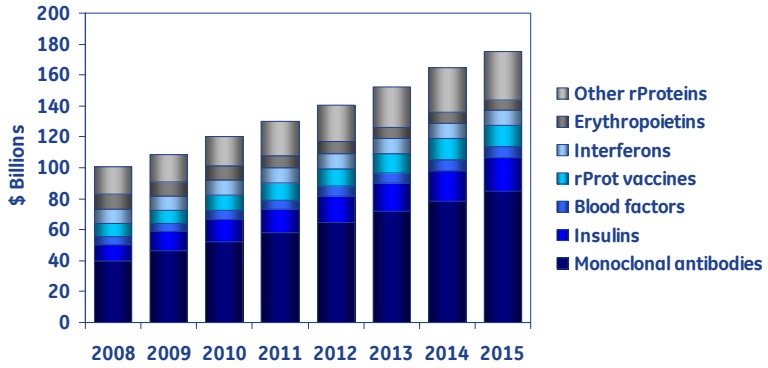
Development of MAb market
单抗市场的发展
Estimated sales of biologics, 2007-2014



What comes beyond MAb's?
哪种产品将超越单抗？

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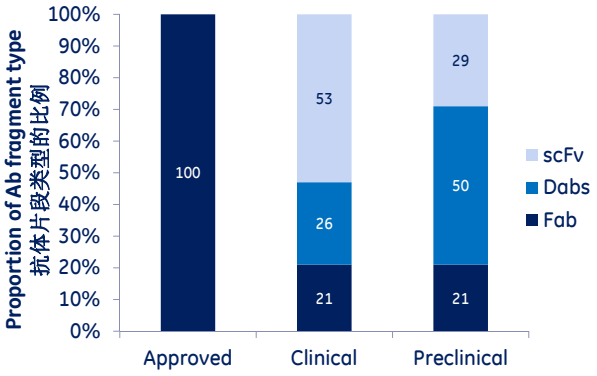
Source: GE Healthcare estimations based on market analysis and company annual reports

Antibody fragment market
抗体片段市场

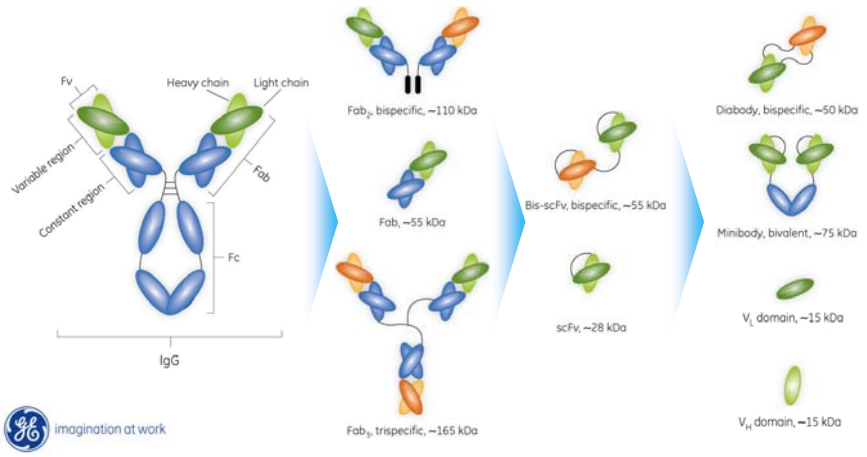


- Fab market already existing抗体片段市场已经存在
- Shift towards smaller antibody fragments in early phases
  早期转向更小的抗体片段市场

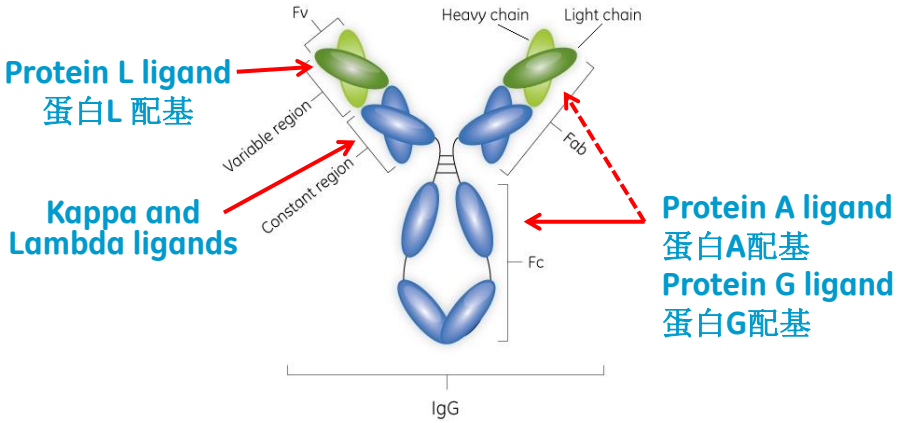
Source: Development trends for therapeutic antibody fragments, Nature Biotechnology Vol 27, No 4, April 2009

Antibodies and antibody fragments
抗体和抗体片段

Mabs	1G Fabs	2G ScFv	3G Dabs



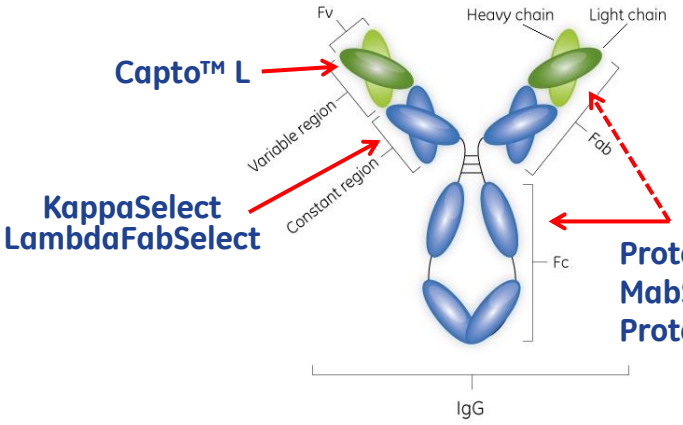
Affinity ligands for antibody fragments
针对抗体片段的亲和配基



Two heavy chains 两条重链

Two light chains两条轻链 Kappa or Lambda

Chromatography media for fragments
针对抗体片段的层析填料



Two heavy chains
两条重链

Two light chains两条轻链
Kappa or Lambda

Potential and challenges with antibody fragments
抗体片段的机遇和挑战

Potential 机遇

- **Improved kinetics** in solid tumors
  实体瘤中提高药物动力学
- **Enables binding** of targets not accessible to Ab
  可结合靶点而不影响抗体
- **Higher diversity** in specific binding structures
  特殊结合位点上具有更高的可变性
- **Advantages in manufacturing –** yeast and bacterial systems can be used
  工业生产上的优势——可使用大肠杆菌和酵母系统

Challenges挑战

- **Lower stability** and **solubility**
  可溶性和稳定性差
- Sometimes **low target retention**
  某些片段靶向性差
- **Varying expression levels**
  表达水平不同
- **Lack of generic purification protocols**
  缺少通用的纯化方案

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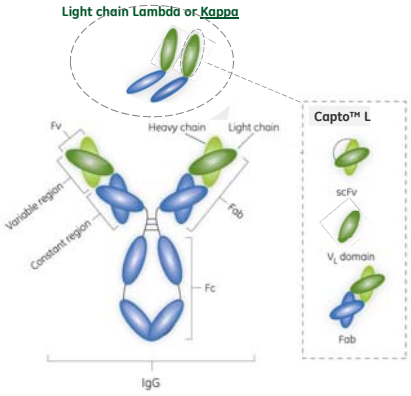
Properties of Protein L
蛋白L的属性

Protein L binds:结合

- to the variable region of most subtypes of Ab kappa light chain
  结合抗体kappa轻链可变区
- a wider range of Ab classes than Protein A or G
  比蛋白A或蛋白G有更广的抗体种类
- Ab fragments抗体片段 ; Fabs, Dabs and scFv
- without interfering with antigen binding 对于抗原的结合没有干扰



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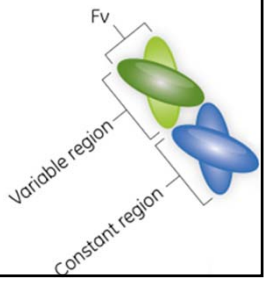
Purification of human Fab
纯化人源Fab

Sample: Fab kappa derived from IgG_1 in *E. coli* cell culture supernatant
样品：源于大肠杆菌细胞培养表达的IgG1 的kappa片段

- Concentration in feed: 1 mg/ml 上样浓度： 1 mg/ml
- pI: 8.5 等电点：8.5
- Mw: 48 kDa 分子量：48kDa



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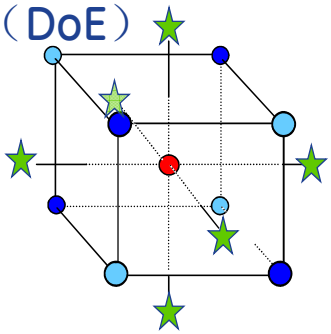
Capto™ L: Design of Experiments (DoE)
Capto™ L实验设计（DoE）

DoE:

- Wash pH: 4.5-7.5
- Wash [NaCl]: 40-460 mM
- Elution pH: 2.9-3.1



CCC design

Constants: 非变量

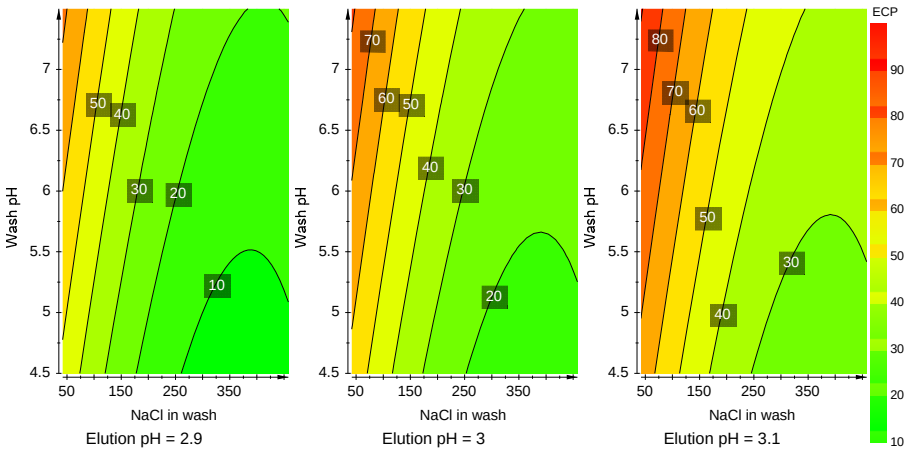
Load 15 mg/ml (70% of DBC), residence time 4 minutes, wash volume 7 CV
15mg/ml上样（动态载量的70%），保留时间4min，冲洗7倍柱体积

Responses: 响应值

Yield and *E. coli* protein (ECP) content
收率及大肠杆菌蛋白含量

Capto™ L DoE results: ECP

Capto™ L实验设计结果：大肠杆菌蛋白



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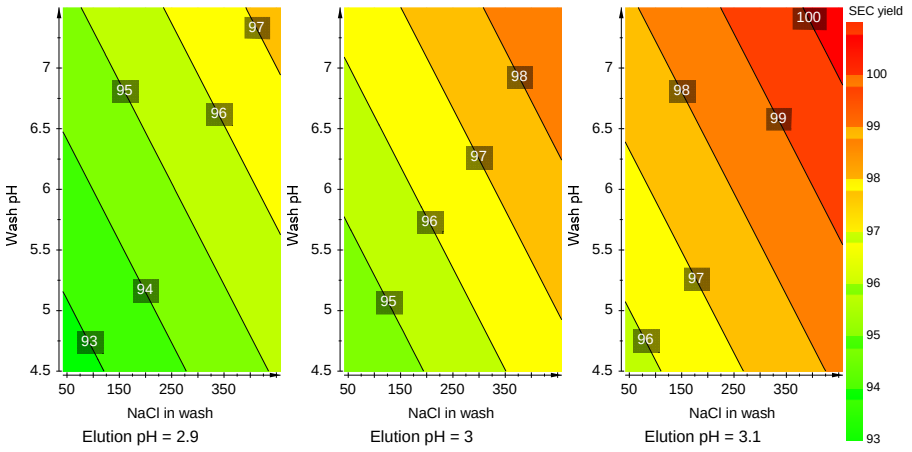
Start: 300 000 ppm

Capto™ L DoE results: Yield

Capto™ L实验设计结果：收率



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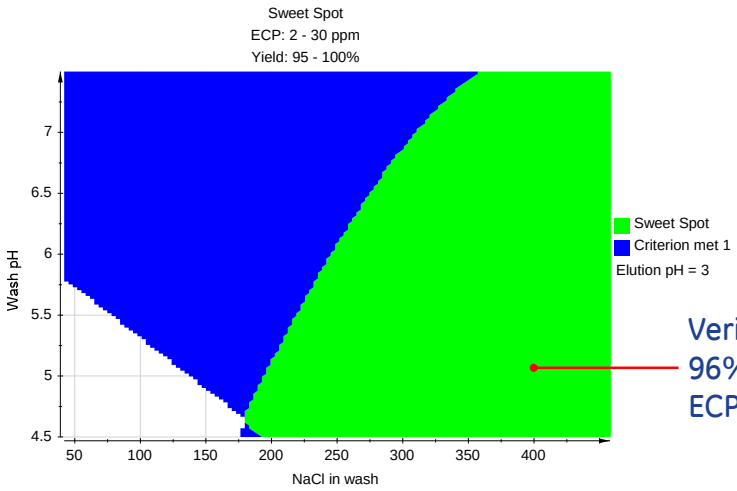
19/09/2012

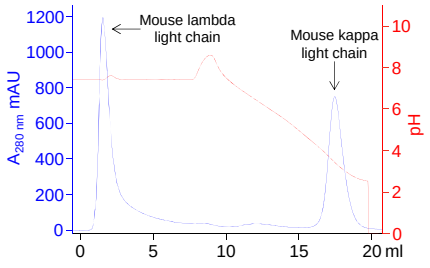
Capto™ L: Sweet spot plot



Verification run:
96% yield
ECP 12 ppm

Purification of mouse Fab
鼠源Fab的纯化

Column层析柱: *HiTrap™ Protein L, 1 ml*
Sample样品: *2 mg mouse polyclonal Fab containing kappa light chain (Jackson ImmunoResearch laboratories) 2mg含有kappa轻链的鼠源多克隆Fab*
Elution洗脱: *pH 7.4-2.3 in 10 column volumes*
System系统: *ÄKTA™ avant 25*



Binding buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl, pH 7.4)*
Wash buffer: *25 mM sodium citrate, 25 mM sodium phosphate, pH 7.4*
Elution buffer: *25 mM sodium citrate, 25 mM sodium phosphate, pH 2.3*

Flow rates: *1 ml/min (equilibration, wash and elution), 0.25 ml/min (sample load)*

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Outline概况

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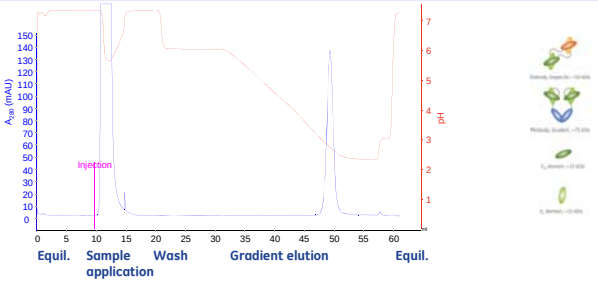
Purification of a human Dab人源Dab的纯化

Determination of optimal elution pH 优化洗脱pH

Column层析柱: *HiTrap™ Protein L, 1 ml*
Sample样品: *1 ml extract of E. coli expressing a domain antibody (Dab) (D115)*
1ml大肠杆菌表达的Dab(D115)提取物
Elution洗脱: *pH 6.0-2.3 in 20 column volumes*
System系统: *ÄKTA™ pure*



Binding buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl, pH 7.4)*
Wash buffer: *50 mM citrate, pH 6.0*
Elution buffer: *50 mM citrate, pH 2.3*

Flow rates: *1 ml/min (equilibration, wash and elution), 0.5 ml/min (sample load)*

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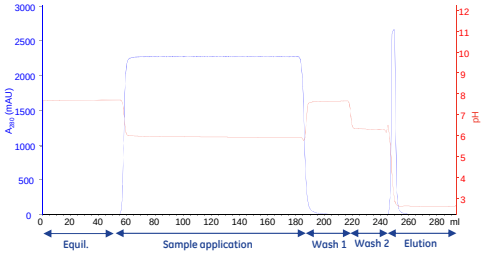
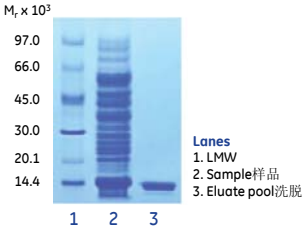
Purification of a human Dab人源Dab的纯化

Scaling up using step elution使用步级洗脱来放大

Column层析柱: *HiTrap™ Protein L, 5 ml*
Sample样品: *127.5 ml extract of E. coli expressing a domain antibody (Dab) (D115)*
Elution洗脱: *pH step gradient; pH 6.0 and pH 2.6*
System系统: *ÄKTA™ pure*





Binding/wash 1 buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl, pH 7.4)*
Wash 2 buffer: *50 mM citrate, pH 6.0*
Elution buffer: *50 mM citrate, pH 2.6*

Flow rates: *5 ml/min (equilibration, wash and elution), 2.5 ml/min (sample load)*

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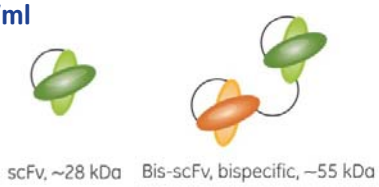
Challenging customer application
客户应用的挑战

BIBITEC GmbH is a German CMO, specialized in the production of recombinant proteins and MAbs for use in clinical trials up to phase III using mammalian cells.
BIBITEC GmbH是一家德国的CMO公司，专业生产用哺乳动物细胞培养、用于三期临床试验的重组蛋白和单克隆抗体。

In this application, the challenge was to **purify scFv fusion protein (57 kDa) from transgenic animal plasma**.
从转基因动物中纯化**scFv**融合蛋白（57kDab）的应用挑战

The sample had high content of albumin, IgG as well as many other plasma proteins
样品中含有大量的白蛋白、IgG及其它血浆蛋白
Dynamic binding capacity ($Q_{b,\ 10\%}$): 23 mg/ml
动态结合载量： 23 mg/ml



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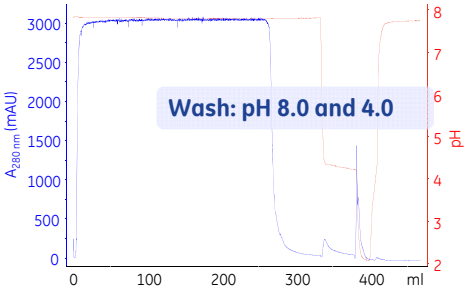
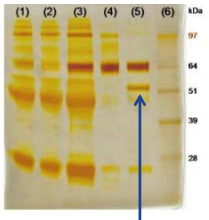
First screening: Promising results
首先筛选： 理想结果

Column层析柱: *HiScreen™ Capto L, 4.7 ml*
Sample样品: *Precipitated plasma containing scFv fusion protein*
Elution洗脱: *pH step gradient; pH 6.0 and pH 2.0*
System系统: *ÄKTA™*





Lanes
1. Feed (1/10)上样
2. Flowthrough (1/10)流穿
3. Wash pH 8（pH8冲洗）
4. Wash pH 4（pH4冲洗）
5. Elution (1/4) 洗脱
6. Mw-marker分子量Mark

Target protein目标蛋白

Binding/wash 1 buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl), 100 mM glycine pH 8.0*
Wash 2 buffer: *50 mM glycine, 50 mM citrate, pH 4.0*
Elution buffer: *50 mM glycine, 50 mM citrate, pH 2.0*
Flow rate: *1 ml/min*

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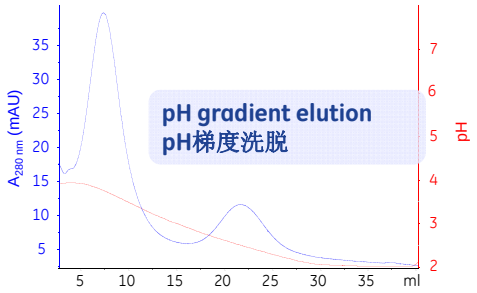
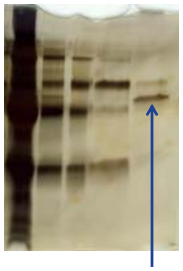
pH gradient: Potential for optimization
pH梯度：优化潜力

Medium: *Capto™ L, 2.4 ml (column Ø=10 mm)*
Sample: *Precipitated plasma containing scFv fusion protein*
Elution: *pH 4.0-2.0 in 10 column volumes*
System: *ÄKTA™*





Lanes
1. Flowthrough 流穿
2. Wash pH 8（pH8冲洗）
3. Wash pH 4（pH4冲洗）
4. Gradient peak 1梯度峰1
5. Gradient peak 2梯度峰2

Target protein目标蛋白

Binding/wash 1 buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl), 100 mM glycine pH 8.0*
Wash 2 buffer: *50 mM glycine, 50 mM citrate, pH 4.0*
Elution buffer: *50 mM glycine, 50 mM citrate, pH 2.0*
Flow rate: *1 ml/min*

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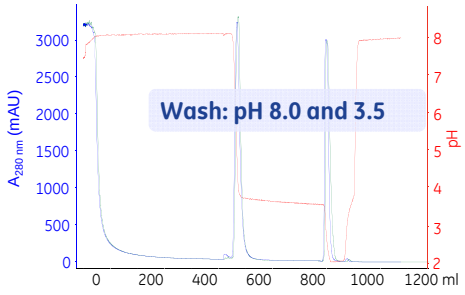
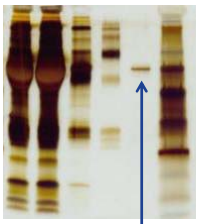
Scale-up study放大研究

Column层析柱: *Capto™ L, 34 ml (column Ø=26 mm)*
Sample样品: *Precipitated plasma containing scFv fusion protein*
Elution洗脱: *pH step gradient; pH 3.5 and pH 2.0*
System系统: *ÄKTA™*





Lanes
1. Feed上样
2. Flowthrough流穿
3. Wash pH 8（pH8冲洗）
4. Wash pH 3.5（pH3.5冲洗
5. Elution洗脱
6. Mw-marker分子量Marker

Target protein目标蛋白

Binding/wash 1 buffer: *PBS (10 mM Na-phosphate, 140 mM NaCl, 2.7 KCl), 100 mM glycine pH 8.0*
Wash 2 buffer: *50 mM glycine, 50 mM citrate, pH 3.5*
Elution buffer: *50 mM glycine, 50 mM citrate, pH 2.0*
Flow rate: *7.4 ml/min*

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Scale-up study放大研究

Contaminant levels	Plasma	Run 1	Run 2	Reduction
Albumin (ppm)	3.7×10^7	2.8×10^4	2.8×10^4	3 log
IgG (ppm)	1.8×10^8	7.6×10^3	7.9×10^3	4.5 log
Plasma proteins (ppm)	1.3×10^9	1.6×10^4	1.8×10^4	5 log
Pr L leakage (ppm)		≤2	≤2	

The yield was at least 89% for both runs
两次实验的收率至少达到89%

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Conclusions: scFv application example
结论：scFv应用实例

- Dynamic binding capacity: 23 mg/ml
  动态结合载量：23mg/ml
- Challenging sample
  具有挑战性的样品
- Effective wash step developed
  开发有效的清洗步骤
- High yields obtained: >89%
  高回收率：>89%

Bis-scFv, bispecific, ~55 kDa

scFv, ~28 kDa

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Summary总结

- Capto™ L is suitable for purification of antibody fragments containing kappa light chain;

  Capto™ L适于含有kappa轻链的抗体片段的纯化
  - Fab
  - Dab
  - ScFv
- Capto L purification show high yields and purity

  Capto™ L纯化表现出较高的收率和纯度

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GE Healthcare Bio-Sciences AB, a General Electric Company.

www.gelifesciences.com

GE Healthcare Bio-Sciences AB
Björkgatan 30
SE-751 84 Uppsala
Sweden

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