Convenient purification of monoclonal antibodies using HiTrap rProtein A FF

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Convenient Purification of Monoclonal Antibodies using HiTrap rProtein A FF

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Abstract

Purification of monoclonal antibodies can be achieved by a number of methods, protein A affinity chromatography being especially powerful. HiTrap™ rProtein A FF is a prepacked column for the purification of monoclonal antibodies in a quick, easy, and convenient way. The work presented shows different applications using HiTrap rProtein A FF 1 ml and 5 ml columns. Mouse monoclonal antibodies were purified from cell cultures and ascitic fluids. The purifications were performed using either a syringe or chromatography system.

Humanized IgG_4 was purified directly from a myeloma cell culture resulting in a highly purified antibody at a yield of 93%. The capacity for a mouse IgG_{2a} purified from cell culture supernatant was 18 mg/ml medium at a flow rate of 624 cm/h (4 ml/min on a 1 ml column), showing the high capacity of HiTrap rProtein A FF at high flow rates. All purified monoclonals were >95% pure according to analysis by SDS-PAGE and silver staining. The optimum binding conditions for a mouse IgG_1 were investigated in a series of scouting experiments performed on ÄKTAexplorer™. This particular antibody was efficiently bound to HiTrap rProtein A FF in a phosphate buffer containing 0.15 M NaCl at neutral pH.

Introduction

Purification of monoclonal antibodies is required for many applications in numerous fields of science and technology. Protein A affinity chromatography is well known as one of the most efficient techniques for the purification of monoclonal antibodies. Due to the high specificity of protein A for immunoglobulins, high product purity is achieved within one chromatographic step.

A rapid and convenient way to purify monoclonal antibodies is to use HiTrap rProtein FF columns. This prepacked, ready to use column, 1 ml or 5 ml, contains rProtein A Sepharose™ Fast Flow. The ligand, recombinant protein A, has been specially engineered with a C-terminal cysteine to allow the formation of a stable thioether link between the ligand and the base matrix. Terminal coupling allows the ligand to exert maximum binding capacity for IgG.

Using HiTrap rProtein A FF, monoclonal antibodies from cell cultures and ascitic fluids can easily be prepared to high purity in a single step. The column can either be operated by a simple syringe, or by coupling it to a chromatographic system. To achieve even higher binding capacitiesin one run, several HiTrap rProtein A FF columns can be connected together.

Conclusions

- HiTrap rProtein A FF provides rapid and convenient preparative purification of monoclonal antibodies from cell cultures and ascitic fluids.
- High product purity (>95%) is achieved within one chromatographic step.
- High binding capacities are obtained even at high flow rates.
- HiTrap rProtein A FF can be operated with a simple syringe or by connecting it to a chromatographic system, such as ÄKTAexplorer.

Material & Methods

Columns:	HiTrap rProtein A FF, 1 ml or 5 ml
Samples:	Different cell culture supernatants or ascitic fluids containing different subclasses of IgG
Sample pre-treatment:	Filtered through a Millipore Millex® 0.45 µm filter
Binding buffer:	0.02 M sodium phosphate, pH 7.0 (incl. 3 M NaCl for mouse IgG_1)
Elution buffer:	0.1 M sodium citrate, pH 3.0
Neutralizing buffer:	1 M Tris-HCl, pH 9.0
Fraction treatment:	200 µl neutralizing buffer/ml collected eluted fraction

Characteristics of HiTrap rProtein A FF

Column dimensions, i.d. x h:	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Ligand:	recombinant protein A, *(E. coli)*
Coupling chemistry:	epoxy
Degree of substitution:	≈ 6 mg rProtein A/ml medium
Total binding capacity:	≈ 50 mg human IgG/ml medium
Dynamic binding capacity for some monoclonal antibodies*:	23 mg mouse IgG_{2a}/ml medium 12 mg mouse IgG_1/ml medium 11 mg humanised IgG_4/ml medium
Average particle size:	90 µm
Bead structure:	highly cross-linked 4% agarose
Maximum backpressure:	3 bar, 0.3 MPa
Maximum flow rate at room temperature:	4 ml/min (624 cm/h) and 20 ml/min (600 cm/h) for 1 and 5 ml columns, respectively
Recommended flow rate:	1 ml/min (156 cm/h) and 5 ml/min (150 cm/h) for 1 and 5 ml columns, respectively
Chemical stability:	All commonly used buffers
pH stability:	
Working	3–10
Cleaning	2**–11
Temperature stability:	
Working	4°C–40°C
Storage	4°C–8°C
Storage buffer:	20% ethanol

* Running conditions:

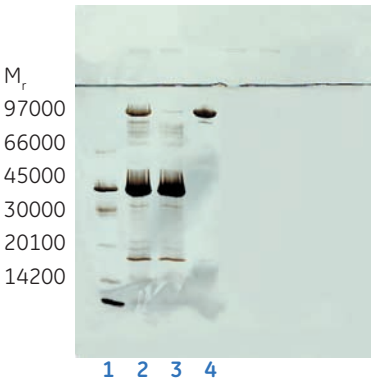
Column:	HiTrap rProtein A FF 1 ml
Sample:	cell culture supernatants
Binding buffer:	20 mM sodium phosphate (incl. 3 M NaCl for IgG1), pH 7.0.
Elution buffer:	0.1 M sodium citrate, pH 3.0
Flow rate:	1 ml/min.

** pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyze at very low pH.

Applications

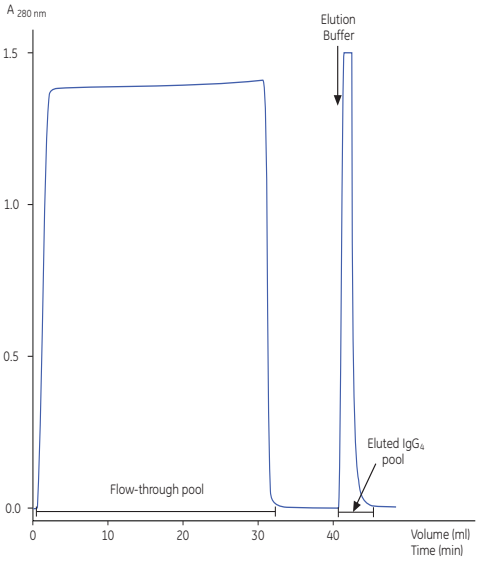
Purification of humanized IgG_4 from a myeloma cell culture

Column:	HiTrap rProtein A FF 1 ml
Sample volume:	30 ml containing 12 mg IgG_4 The sample was a kind gift from Dr. J. Bonnerjea, LONZA Biologics plc, UK
Binding buffer:	0.02 M sodium phosphate, pH 7.0
Elution buffer:	0.1 M sodium citrate, pH 3.0
Flow rate:	1 ml/min (156 cm/h)



SDS-PAGE

Lane 1:	Low Molecular Markers
Lane 2:	Starting material
Lane 3:	Flow-through pool
Lane 4:	Eluted IgG_4 poo



Result

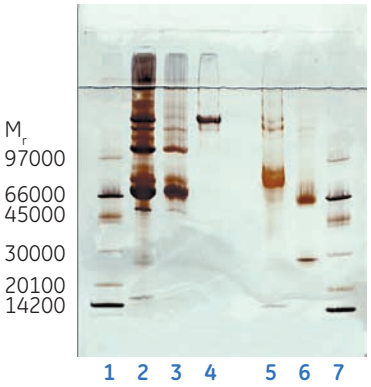
Eluted amount of IgG_4: 11.2 mg

Yield: 93%

Purity: > 95% according to SDS-PAGE

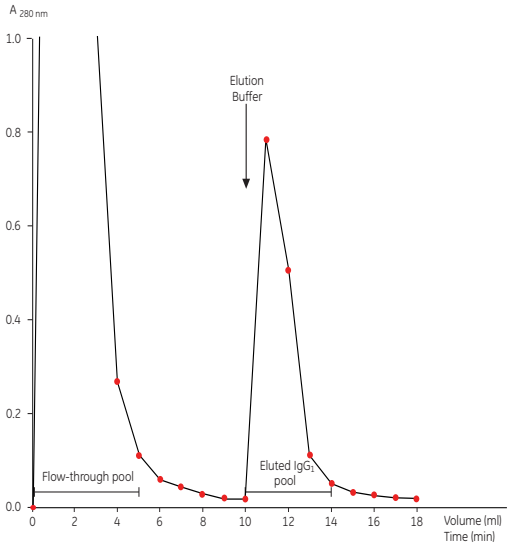
Purification of mouse IgG_1 from ascitic fluid using a syringe

Column: HiTrap rProtein A FF 1 ml
Sample volume: 1 ml (0.5 ml ascites diluted with 0.5 ml binding buffer)
The sample was a kind gift from
Dr. N. Linde, EC Diagnostics, Sweden
Binding buffer: 0.02 M sodium phosphate, 3 M NaCl, pH 7.0
Elution buffer: 0.1 M sodium citrate, pH 3.0
Flow rate: ≈ 1 ml/min
Instrumentation: Syringe



SDS-PAGE

Lane 1: Low Molecular Markers
Lane 2: Starting material, dil. 20×
Lane 3: Flow-through pool, dil. 5×
Lane 4: Eluted IgG_1 pool
Lane 5: Flow-through pool, dil. 5×, reduced
Lane 6: Eluted IgG1 pool, reduced
Lane 7: Low Molecular Markers

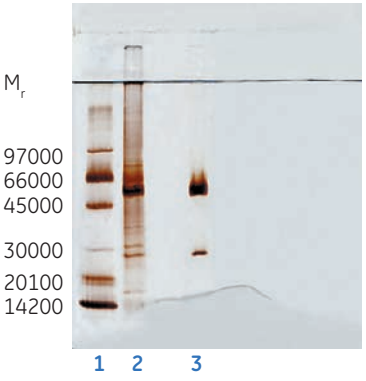


Result

Eluted amount of IgG_1: 1.1 mg
Purity: > 95% according to SDS-PAGE

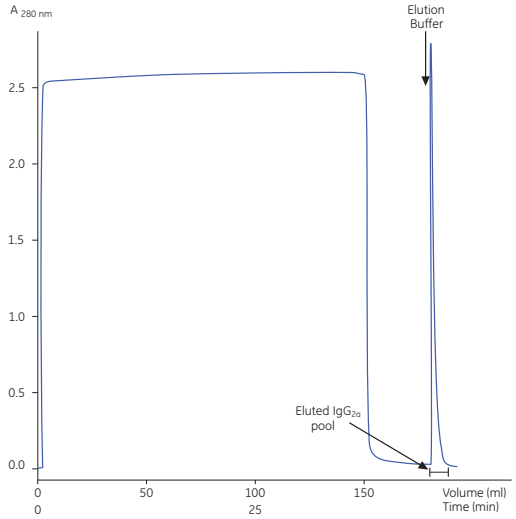
Purification of mouse IgG_{2a} from cell culture supernatant

Column: HiTrap rProtein A FF 1 ml
Sample volume: 150 ml
Binding buffer: 0.02 M sodium phosphate, pH 7.0
Elution buffer: 0.1 M sodium citrate, pH 3.0
Flow rate: 4 ml/min (624 cm/h)



SDS-PAGE

Lane 1: Low Molecular Markers reduced
Lane 2: Starting material, reduced
Lane 3: Eluted IgG_{2a} pool, dil. 3×, ♦ reduced

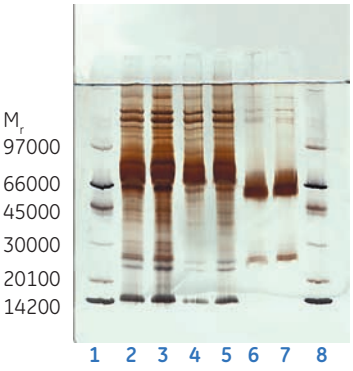


Result

Eluted amount of IgG_{2a}: 18.2 mg
Purity: > 95% according to SDS-PAGE

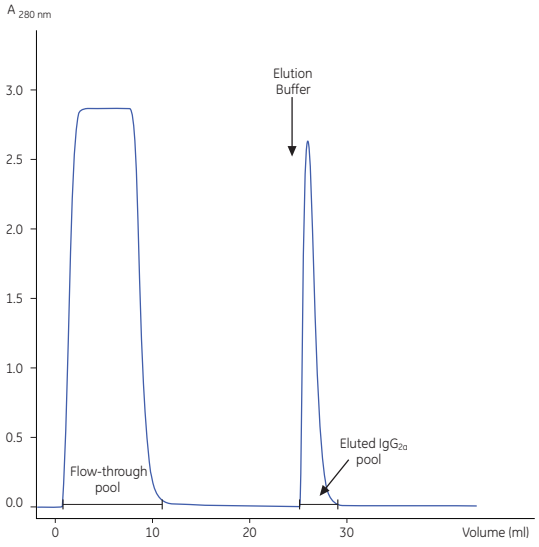
Purification of mouse IgG_{2a} from ascitic fluid

Column: HiTrap rProtein A FF 1 ml
Sample volume: 7 ml (3.5 ml ascites diluted with 3.5 ml binding buffer)
Binding buffer: 0.02 M sodium phosphate, 3 M NaCl, pH 7.0
Elution buffer: 0.1 M sodium citrate, pH 3.0
Flow rates:
sample application 0.5 ml/min (78 cm/h)
wash and elution 1 ml/min (156 cm/h)



SDS-PAGE

Lane 1: Low Molecular Markers, reduced
Lane 2: Starting material, dil. 40×, reduced
Lane 3: Starting material, dil. 20×, reduced
Lane 4: Flow-through pool, dil. 20×, reduced
Lane 5: Flow-through pool, dil. 10×, reduced
Lane 6: Eluted IgG_{2a} pool, dil. 10×, reduced
Lane 7: Eluted IgG_{2a} pool, dil. 5×, reduced
Lane 8: Low Molecular Markers, reduced



Result

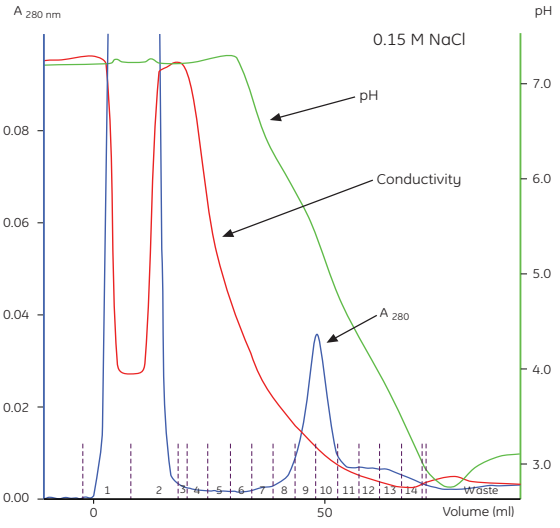
Eluted amount of IgG_{2a}: 14.5 mg
Purity: > 95% according to SDS-PAGE

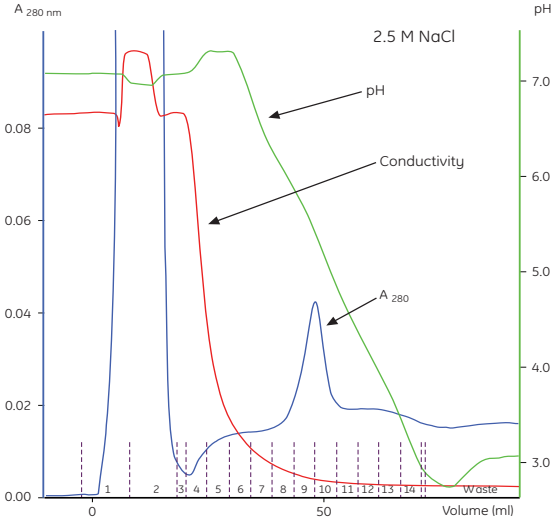
Scouting for binding conditions using ÄKTAexplorer with BufferPrep*

The concentration of NaCl in the binding buffer was varied over the range 0.15 M to 2.5 M at constant pH.

Column: HiTrap rProtein A FF 5 ml
Sample: Cell culture supernatant containing mouse IgG_1
Sample volume: 8 ml
Flow rate: 2 ml/min (60 cm/h)
Instrumentation: ÄKTAexplorer
BufferPrep solutions:
1. Buffer stock 0.1 M sodium phosphate, 0.1 M sodium citrate
2. Acid stock 0.2 M HCl
3. Salt stock 5 M NaCl
4. Water
Salt concentrations in the binding buffers: 0.15, 0.5, 1.0, 1.5, 2.0 and 2.5 M. pH of binding buffer 7.4

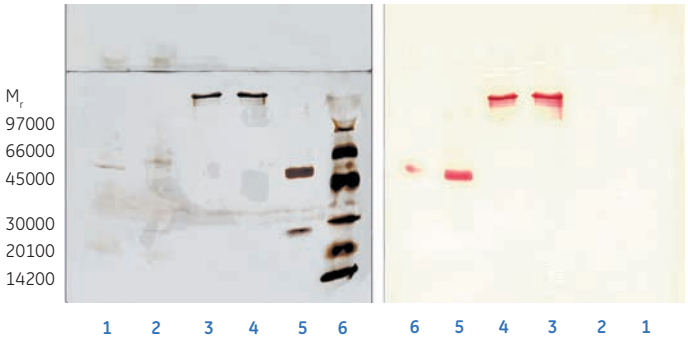
* A. Holtz, Phadia, Sweden is gratefully acknowledged for performing these experiments.





SDS-PAGE and Western Blotting from the 0.15 M NaCl run

Lane 1: Flow-through fraction 2
Lane 2: Flow-through fraction 2, reduced
Lane 3: Eluate, fraction 9
Lane 4: Eluate, fraction 10
Lane 5: Eluate, fraction 10, reduced
Lane 6: Low Molecular Markers



Result

The same amount of this particular IgG_1 bound at all NaCl concentrations tested.

SDS-PAGE analysis of the eluted fractions from the run, using 0.15 M NaCl, shows that the purified monoclonal is over 95% pure. Western blotting confirms that no antibody is in the flow-through material.

Analysis

Concentration determination

Absorbance measurement at 280 nm using Ultrospec Plus:

$A_{280}^{1\%} = 14$

Purity check:

SDS-PAGE electrophoresis

Gel:	PhastGel™ Gradient 10–15,
Sample pretreatment:	Dilution 1:5 with 15% SDS, 60 mM Tris, 6 mM EDTA, 0.06% Bromophenol Blue, pH 8.0 2-mercaptoethanol (final conc. 6%) was added when run under reducing conditions
	Heating, 5 min., 90°C
Sample volume:	1 µl
Molecular weight standard:	Low Molecular Markers
Staining:	Silver, according to the standard protocol for PhastSystem™
Instrumentation:	PhastSystem

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