GE Healthcare
Life Sciences

Application note 29-0643-02 AB

Chromatography systems

Purification of antibodies using ÄKTA™ start and HiTrap™ Protein G HP column

Polyclonal and monoclonal antibodies (MAbs) from many species can be purified using HiTrap Protein G Sepharose™ affinity columns. In this Application note, Keisha uses ÄKTA start system and a HiTrap Protein G HP column to reduce the time taken for one-step purification of MAbs from culture supernatant to high purity (> 95%).

Introduction

The diversity of antibody-antigen interactions has created many uses for antibodies. They are used for therapeutic and diagnostic applications as well as for immunochemical techniques within general research.

Keisha is a researcher at a start-up biotech company focusing on characterizing MAbs at a preclinical phase of development. Obtaining highly purified antibodies is of prime importance for her work. Keisha was often challenged by the manual purification methods for purifying MAbs. Too much effort was being spent on time-consuming work such as loading large sample volumes manually. As a result, she was able to purify only a single batch of MAb per day, and was obtaining inconsistent yields and varying degrees of purity across batches.

Keisha purifies MAbs in a single step using Protein G affinity chromatography. The basis for antibody affinity purification using protein G is the high affinity and specificity for the Fc region of IgG from a variety of species. Protein G media bind IgG from a broader range of eukaryotic species and bind more subclasses of IgG than Protein A. Protein G has been immobilized to several different matrixes, resulting in an excellent means of isolating IgG and IgG subclasses from ascites, cell culture supernatants, and serum.

Keisha switched to ÄKTA start system (comprising the ÄKTA start instrument, UNICORN™ start 1.0 control software and the Frac30 fraction collector) and selected a HiTrap Protein G HP column for purifying the MAb. HiTrap Protein G HP is a convenient, ready-to-use column prepacked with Protein G Sepharose High Performance chromatography medium (resin).

Methods

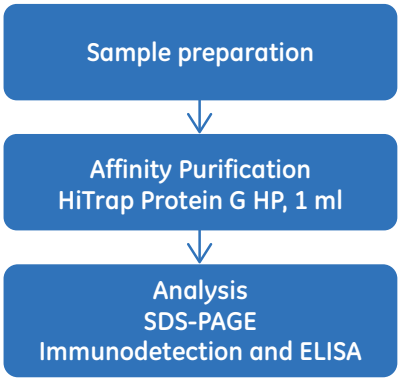


Fig 1. Workflow illustrating the steps involved in purifying MAbs.

Keisha followed the workflow described in Figure 1. The sample was prepared by diluting the culture supernatant two-fold using binding buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0). The diluted sample was kept at room temperature for 15 to 20 min to ensure solubility of the protein.

Large sample volumes can be loaded on ÄKTA start using Superloop™.

Keisha used UNICORN start to modify the predefined affinity template, as described in Table 1, and used the System Control module of the software to run the method.

The ÄKTA start pump was used to load 20 ml of diluted culture supernatant onto a HiTrap Protein G HP 1 ml column. After the sample was loaded, the column was washed with 10 column volumes (CV) of binding buffer.

Create custom methods using drag-and-drop functionality in UNICORN start Method Editor.

The bound proteins were eluted by step gradient using elution buffer (100 mM glycine-HCl, pH 2.7). The first step was 5 CV of 70% B to remove nonspecific contaminants and the second step was 10 CV of 100% B to elute the protein of interest. Peak fractions were collected using Frac30 fraction collector and pooled following the purification run. The purity and specificity of the purified MAb was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, using a 12.5% polyacrylamide gel) and immunodetection.

Table 1. UNICORN start method overview

Method flow	Method settings
Method settings	CV[1] Pressure limit 0.3 MPa[2] Flow rate 1 ml/min
Prime and equilibration	5 CV[1]
Sample application	Apply sample using pump Sample volume 20 ml
Wash out unbound	10 CV[1]
Elution and fractionation	Gradient elution: Step 1: 70% B 5 CV[1] Step 2: 100% B 10 CV[1] Fractionation: Peak fractionation level based (start/end: 5 mAU)
Prime and equilibration	5 CV[1]

[1] CV = Column volumes
[2] 0.3 MPa = 3 bar (43.5 psi)

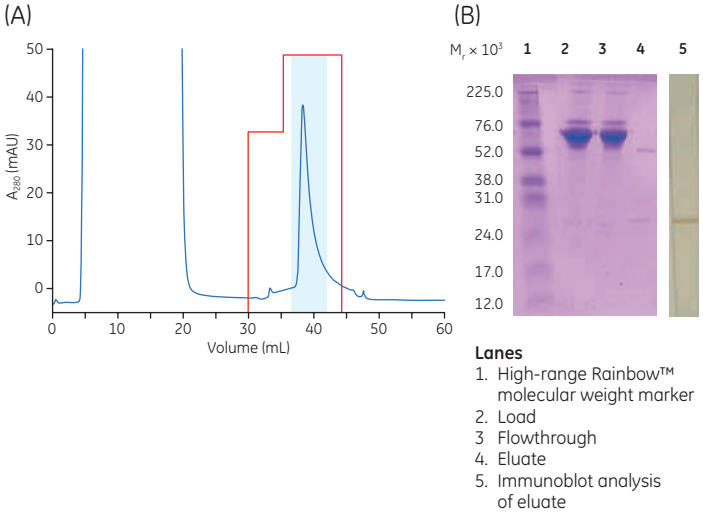
Results

Using ÄKTA start with UNICORN start software and Frac30 fraction collector, Keisha was able to purify MAb from culture supernatant in a single purification step, and she could identify and pool the peak fractions (Fig 2A). ÄKTA start system with the affinity method gave high yields and > 95% purity of the target protein. SDS-PAGE and immunoblot analysis of the purified sample with protein-specific antibodies confirmed the purity and specificity of the MAb, with no contaminating protein bands (Fig 2B). ELISA experiments confirmed that the purified MAb reacted specifically with the antigen, and activity was detected at concentrations as low as 6.25 ng/ml (data not shown).

Ready-to-use methods are available for column preparation and system cleaning.

ÄKTA start system reduced the manual intervention required for MAb purification. The time saved allowed Keisha to conduct several purification runs per day, and she was able to achieve consistent results across runs.

Column:	HiTrap Protein G HP, 1 ml
Sample:	Culture supernatant diluted in 2× binding buffer, pH 7
Sample load:	20 ml
Binding buffer:	20 mM sodium phosphate, 150 mM NaCl, pH 7.0
Elution buffer:	100 mM glycine-HCl, pH 2.7
Flow rate:	1 ml/min
Gradient:	Step 1: 70% B, 5CV Step 2: 100% B, 10 CV
System:	ÄKTA start, UNICORN start and Frac30 fraction collector
Detection	UV (280 nm)



Lanes
1. High-range Rainbow™ molecular weight marker
2. Load
3. Flowthrough
4. Eluate
5. Immunoblot analysis of eluate

Fig 2. (A) Chromatogram showing the purification profile of MAb from culture supernatant on ÄKTA start. The area highlighted in blue represents the pooled fractions containing purified MAbs. The blue line represents absorbance at 280 nm and the red line represents gradient concentration.
(B) SDS-PAGE profile (12.5% polyacrylamide gel) of the purified protein.

Summary

Keisha used ÄKTA start system with a prepacked HiTrap Protein G HP column to obtain high yields and reproducible results while purifying MAb. ÄKTA start reduced the need for manual intervention and saved Keisha time.

Acknowledgments

We thank Dr. Shama Bhat, Chairman & Managing Director, Bhat Bio-Tech India Pvt. Ltd., Bangalore, India, for kindly providing us with antibody samples and supporting us with analytical data for the purified recombinant proteins. We wish to extend our appreciation for the insights Bhat Bio-Tech team has shared about the challenges faced while purifying recombinant proteins manually.

Ordering information

Product	Quantity	Code number
ÄKTA start	1	29-0220-94
UNICORN start 1.0 control software	1	29-0187-51
Frac30 fraction collector	1	29-0230-51
HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
High-Range Rainbow Molecular Weight Markers	1 × 250 µl	RPN756E

Related literature

Product	Code number
Purification of N-terminal histidine-tagged protein using ÄKTA start, Application note	29-0642-77
Depletion of albumin from serum samples using ÄKTA start, Application note	29-0642-95
Purification of GST-tagged protein using ÄKTA start, Application note	29-0642-98

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