

APPLICATION NOTE

Purification of monoclonal antibody using MAbsorbent® A2P.

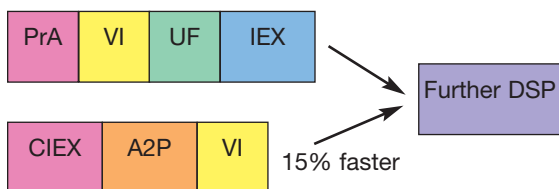
Keith Watson PhD, Manager, Technical Support, ProMetic BioSciences Ltd.

Introduction

Due to increasing process economic pressures, monoclonal antibody producers are now reconsidering their manufacturing processes, especially the chromatographic operations, with the aim of embracing new purification technologies that are more cost-effective while also providing other down-stream processing benefits. At the forefront of these new technologies are high-performance, synthetic affinity ligand adsorbents like MAbsorbent® A2P.

MAbsorbent® A2P binds all sub-classes of IgG at capacities similar to standard industry adsorbents such as Protein A (Kd= 60mM and Bmax = 38 g/L). Using simple elution conditions, excellent IgG recovery, purity and quality can be maintained. In addition, 100 cycles of consecutive IgG purification using MAbsorbent® A2P has demonstrated reproducible IgG binding capacities, purity and recovery as well as absence of MAbsorbent® A2P ligand in both the elution and sanitization fractions. Cleaning and sanitization can be performed using up to 1M NaOH. Re-thinking the chromatography cascade can remove the need for Protein A and therefore substantially reduce cost of goods as well as improving cleaning and sanitization processes.

A generic two-column process to capture and purify monoclonal antibodies is illustrated.



Chromatography Conditions

CIEX XK26/20 column (CV=100 ml)

Linear flow rate = 200 cm/hr

Equilibration Buffer: 50 mM Ammonium Acetate
pH 5.0 ± 0.1

Load: (1:3 Dilution Eqb: Buffer, pH 5.0 ± 0.1).
Capacity >30g/L

Post Load Wash: Equilibration Buffer, pH 5.0 ± 0.1

Elution: 50 mM Ammonium Acetate; 0.5M NaCl
pH 5.0 ± 0.1

Sanitisation: 0.5M NaOH

MAbsorbent® A2P XK26/20 column (CV=100 ml)

Linear flow rate = 150 cm/hr

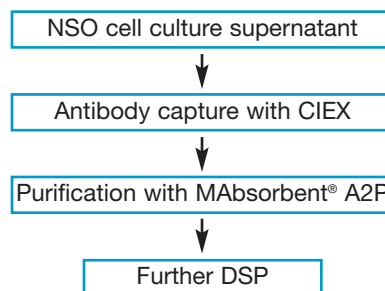
Equilibration: 50 mM Ammonium Acetate; 0.5M
NaCl pH 5.0 ± 0.1

Load: (CIEX elution fraction pH 5.0 ± 0.1).
Capacity >20g/L

Post Load Wash: Equilibration Buffer, pH 5.0 ± 0.1

Elution: 10mM Citrate, pH 3.0 ± 0.10

Sanitisation: 0.5M NaOH



Product Quality Analysis

IgG product quality was assessed by reduced SDS-PAGE.

APPLICATION NOTE

Results

Figure 1 – CIEX capture of NS0 cell derived monoclonal antibody

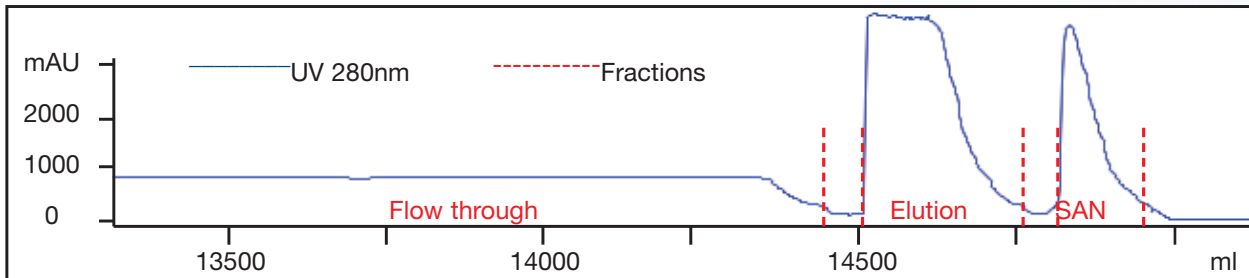


Figure 2 – High-resolution purification of mAb using MAbsorbent® A2P

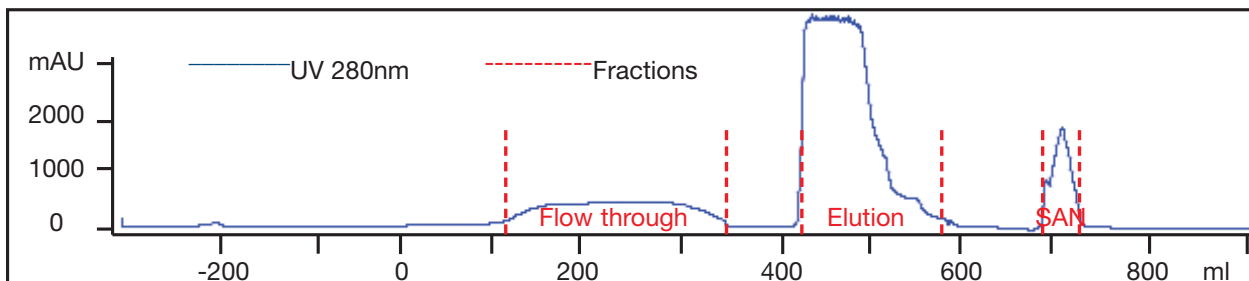
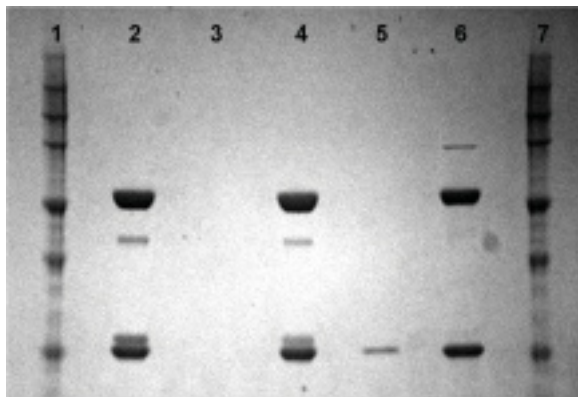


Table 1 – Recovery table for two-step purification of NS0 cell derived mAb

Fraction	mAb (g/L)	Vol. (L)	Tot. mAb (g)	Yield (%)
CIEX Load	0.21	14.29	3.00	100
CIEX NB	0.00	14.85	NA	NA
CIEX Eln	11.05	0.31	3.43	>100
A2P Load	11.05	0.18	1.99	100
A2P NB	0.72	0.24	0.17	8.5
A2P Eln	11.51	0.17	1.96	98.5

Overall two-step process recovery = 98%
 Host cell DNA < 1pg/mg protein
 Host cell protein (NS0) is comparable to PrA-VI-UF-IEX process.

Figure 3 – Reduced SDS-PAGE gel analysis of mAb purification process fractions



Lane 1: MW Marker
 Lane 2: CIEX Load
 Lane 3: CIEX NB
 Lane 4: CIEX Elution

Lane 5: A2P NB
 Lane 6: A2P Elution
 Lane 7: MW Marker

International Sales & Technical Support:

ProMetic BioSciences (USA) Inc.
 155 Willowbrook Boulevard,
 Suite 460
 Wayne, NJ 07470, USA
 Tel: +1.973.812.9880
 Fax: +1.973.812.9881
 E-mail: sales@prometic.com
 techsupport@prometic.com

www.prometic.com