

# A Novel High Performance Adsorbent as a Platform Process for the Capture & Purification of Albumin-Fusion Proteins

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## Introduction

Albumin-fusion protein technology represents an increasingly important alternative platform for the production of therapeutically significant proteins with extended *in vivo* half-lives. A number of proteins and bioactive peptides fused to albumin are currently being investigated for use in therapeutic applications.

In collaboration with Novozymes, ProMetic BioSciences Ltd (PBL) have developed **AlbuPure**<sup>®</sup> - a novel adsorbent which is highly selective, stable, robust and non-toxic which provides a high binding capacity platform technology for the purification of genetically engineered albumin-fusion proteins.

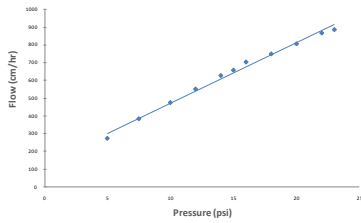
**AlbuPure**<sup>®</sup> is an affinity matrix comprising a novel colorless synthetic triazine ligand derived using PBL's Mimetic Ligand<sup>™</sup> technology and coupled to our proprietary PuraBead<sup>®</sup> 6HF base matrix (highly cross-linked 6% near-monodisperse beaded agarose).

The adsorbent demonstrates a high dynamic binding capacity, recovery and purification efficiency. In combination with Novozymes' Albufuse<sup>®</sup> albumin fusion technology (including the new Albufuse<sup>®</sup>Flex technology), **AlbuPure**<sup>®</sup> can form part of a platform process for a range of different active pharmaceutical ingredients (APIs) to reduce development costs and increase speed to market. In addition, the ability of **AlbuPure**<sup>®</sup> to be run at flow rates of up to 1000 cm/hr coupled with the ability to clean-in-place and re-use over many process cycles can significantly reduce manufacturing costs.

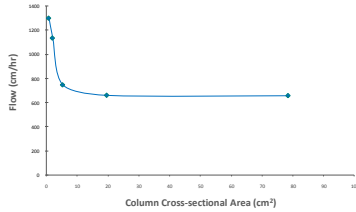
The use of **AlbuPure**<sup>®</sup> for the capture and purification of recombinant albumin-fusion proteins from a yeast based high expression system (Albufuse<sup>®</sup>) is described.

## Flow Properties

**FIGURE 1**  
Pressure flow curve for **AlbuPure**<sup>®</sup> using a 10 cm diameter column.



**FIGURE 2**  
Operating pressure (1 bar) measured for **AlbuPure**<sup>®</sup> as a function of column dimension, column bed length was ~20 cm for all columns.

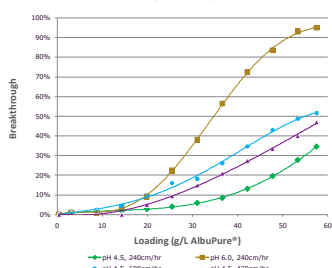


## Dynamic Binding Capacity

Platform	Automated chromatography workstation
Column parameter	15 cm bed height
Load	scFv Albumin-fusion fermentation supernatant
Conditions	Binding Capacity at 10% Breakthrough
pH 4.5, 240 cm/hr (3.8 min RT*)	38.4 g/L of adsorbent
pH 4.5, 420 cm/hr (2.1 min RT)	26.2 g/L of adsorbent
pH 4.5, 600 cm/hr (1.5 min RT)	21.4 g/L of adsorbent
pH 6.0, 240 cm/hr (3.8 min RT)	19.9 g/L of adsorbent

(\* RT - Residence time)

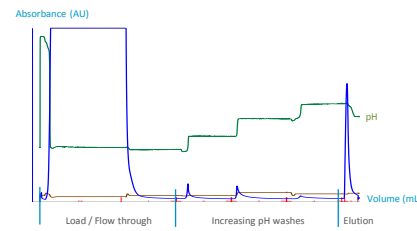
**FIGURE 3**  
**AlbuPure**<sup>®</sup> breakthrough profiles and binding capacity results (measured by GP-HPLC), using a 15 cm bed height column, loading scFv albumin-fusion fermentation supernatant at various flow rates at pH 4.5 and pH 6.0.



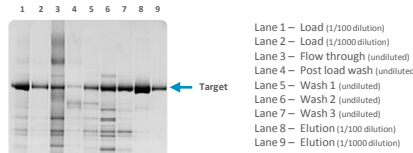
## IL-1ra Albumin-Fusion Purification

Platform	Automated chromatography workstation
Column parameters	22.1 mL CV (1.6 cm diameter, 11 cm bed height)
Equilibration buffer	50 mM sodium acetate, pH 5.3
Load	20 mg target /mL adsorbent of IL-1ra albumin-fusion protein from a yeast derived cell culture supernatant (pH adjusted to pH 5.3 with diluted acetic acid)
Wash buffers	Wash 1: 50 mM sodium phosphate, pH 6.0 Wash 2: 50 mM sodium phosphate, pH 7.0 Wash 3: 50 mM ammonium acetate, pH 8.0
Elution buffer	50 mM ammonium acetate, 10 mM sodium octanoate (caprylate), pH 7.0
Clean-in-Place (CIP)	0.5 M NaOH

**FIGURE 4**  
Chromatogram of the capture, purification (increasing pH washes) and recovery of IL-1ra albumin-fusion protein from a cell culture supernatant using **AlbuPure**<sup>®</sup>.



**FIGURE 5**  
Non-Reduced SDS-PAGE of the process fractions from the purification (increasing pH washes) of IL-1ra albumin-fusion protein from a cell culture supernatant using **AlbuPure**<sup>®</sup>.

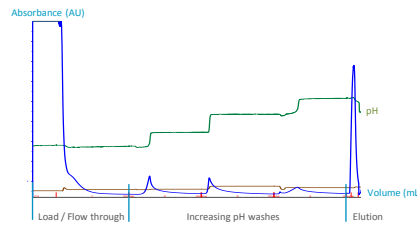


## Anti-HIV peptide (T20) Albumin-Fusion Purification

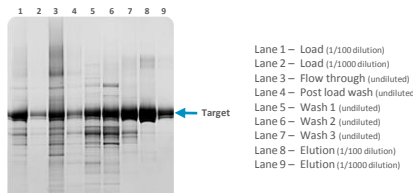
**NB:** process conditions were the same as those used for the IL-1ra albumin-fusion purification

Load	20 mg target /mL adsorbent of anti-HIV peptide (T20) albumin-fusion protein from a yeast derived cell culture supernatant (pH adjusted to pH 5.3 with diluted acetic acid)
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**FIGURE 6**  
Chromatogram of the capture, purification (increasing pH washes) and recovery of anti-HIV peptide (T20) albumin-fusion protein from a cell culture supernatant using **AlbuPure**<sup>®</sup>.



**FIGURE 7**  
Non-Reduced SDS-PAGE of the process fractions from the purification (increasing pH washes) of anti-HIV peptide (T20) albumin-fusion protein from a cell culture supernatant using **AlbuPure**<sup>®</sup>.



## AlbuPure<sup>®</sup> Applications

**AlbuPure**<sup>®</sup> has been shown to successfully capture and purify a range of albumin-fusion proteins with different fusion partners (varying molecular weight and N- or C-terminal coupling), the effects of which are compared to the binding of albumin in the table below. Depending on the specific fusion partner, the strength of binding varies from similar to very strong.

Fusion Partner	Approximate Mw of fusion partner	Fusion tested	Binding strength*
HIV Peptides	5 kDa	C & N Terminal	Similar
IL-1ra	18 kDa	C & N Terminal	Similar
Endostatin	20 kDa	C & N Terminal	Stronger
Prosaptide	2.5 kDa	C Terminal	Similar
Kunitz Domain	7 kDa	C Terminal	Stronger
scFv	30 kDa	C & N Terminal and Bivalent	Slightly Stronger
dAb	13 kDa	N Terminal	Similar
Nanobody	14 kDa	N Terminal	Very Strong
vNAR	13 kDa	N Terminal	Stronger

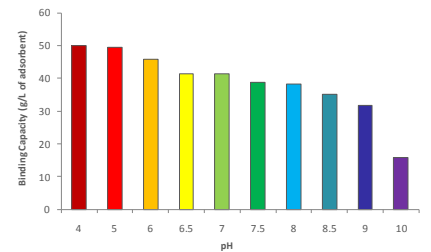
\* In comparison to the binding of albumin.

## pH Effect

The effect of pH on **AlbuPure**<sup>®</sup> binding capacity was investigated. The load material was prepared by pH adjustment of partially purified anti-FITC scFv albumin-fusion protein feedstock with acetic acid or sodium hydroxide. Atoll MediaScout<sup>®</sup> MiniColumns containing 200 µL of **AlbuPure**<sup>®</sup> were loaded to 50 g/L of adsorbent.

Figure 8 below shows that **AlbuPure**<sup>®</sup> can be operated between pH 4 to pH 9 maintaining a binding capacity of >30 g/L.

**FIGURE 8**  
Binding capacity versus pH using an Atoll MediaScout<sup>®</sup> MiniColumns containing 200 µL of **AlbuPure**<sup>®</sup> per column.



## Conclusions

- **AlbuPure**<sup>®</sup> enables a linear flow rate of up to 1000 cm/hr at 1.5 bar (~22 psi) using a 10 cm diameter column packed to a bed height of 20 cm.
- **AlbuPure**<sup>®</sup> demonstrates a high dynamic binding capacity of >35 g/L for scFv albumin-fusion fermentation supernatant loading to 10% breakthrough at pH 4.5 (240 cm/hr, 3.8 minute residence time).
- **AlbuPure**<sup>®</sup> gives good recovery and high purity results for the purification of albumin-fusion proteins using non-complex buffers.
- **AlbuPure**<sup>®</sup> can be used to capture and purify a large range of albumin-fusion proteins irrespective of size of fusion partner or fusion coupling orientation (N- or C-termini).
- **AlbuPure**<sup>®</sup> can bind albumin-fusion proteins over a wide pH range (from pH 4 to pH 9) whilst maintaining a high binding capacity >30 g/L.
- Combined with **Albufuse**<sup>®</sup> albumin fusion technology, **AlbuPure**<sup>®</sup> provides a complete platform technology for the purification of albumin-fusion proteins with superior flow properties providing reduced process times.

## Acknowledgements

The help and data provided by Novozymes Biopharma has been greatly appreciated for the successful completion of this work.

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