Instructions For Use

# Praesto™ Jetted A50 HipH

ECOLAB®

# Praesto Jetted A50 HipH

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# Introduction

The first protein A resins were based on the wild type of protein A, expressed by a pathogenic ***Staphylococcus aureus*** strain. Since then, the manufacturing of monoclonal antibodies has grown tremendously, and they are now the most important group of molecules in the pharma industry. Along with this development, protein A resins have been significantly improved, both with respect to capacity, productivity and alkaline stability, resulting in a dramatic enhancement of process performance.

Current trends in antibody-based therapeutics include the development of a variety of different atypical IgG constructs that, in many cases, are prone to aggregation, are expressed at very high titre and contain several product variants. Therefore, there is a growing interest in resins capable of resolving product variants at the protein A step and eluting IgG at milder pH conditions when compared to other protein A resins on the market.

**Praesto Jetted A50 HipH has been designed to meet these challenges.**

Herein, we describe basic screening and development conditions for protein A chromatography and recommendations for use with Praesto Jetted A50 HipH.

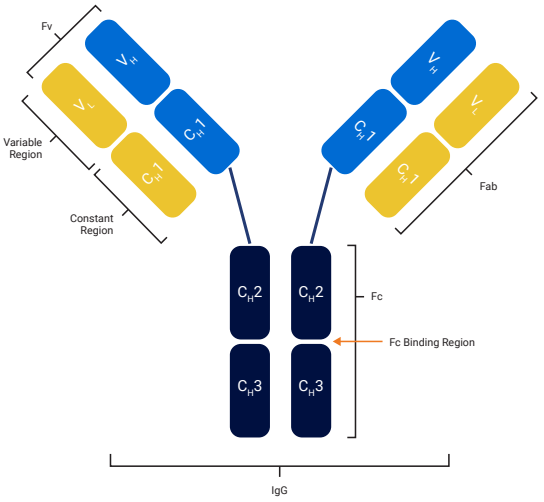
With regards to mAb purification, there are several different buffer and process conditions for protein A capture presented within scientific literature. However, the outcome with respect to yield and purity is, in general, similar for most published protocols.

It is important to acknowledge that all molecules, whether they be mAb, Fab or fragments, are unique, and will differ both in chemical and physical characteristics. Optimization of washing and elution steps are important in the development stage.

# Features

## FIGURE 1

**Schematic representation of immunoglobulin G antibody structure**




A typical antibody is Y-shaped (Figure 1) and could be divided into two identical antigen binding (Fab) regions and one Fc (fragment crystallizable) region. All protein A resins have high affinity to the Fc region of the immunoglobulin G antibody. However, native protein A, and most recombinant protein A ligands, can also bind to the $V_H$ domain of antibodies that belong to the $V_H$ 3 family. $V_H$ 3 binding resins typically require a pH well below 3.8 for complete elution of the antibody. Praesto™ Jetted A50 HipH was specifically designed to bind to the Fc region, with negligible affinity for the $V_H$ 3 domain.

## TABLE 1 Typical physical and chemical characteristics of Praesto Jetted A50 HipH

| Product Characteristics | Praesto Jetted A50 HipH |
|---|---|
| Polymer Structure | Highly cross-linked agarose |
| Dynamic Binding Capacity | Up to ~60 mg hIgG/ml resin, 6 minutes RT |
| Average Particle Size | 50 µm |
| Particle Size Range | 95% between 35-90 µm |
| Pressure / Flow Specifications | Up to 200 cm/h (30 x 20 cm) |
| pH Stability (Working Range) | 3-12 |
| pH Stability (CIP) | 2-13 |
| Recommended Storage Conditions | 2-8°C in 20% ethanol |

# Purification Protocol

The protein A step is followed by two (sometimes one) additional chromatography steps to achieve sufficient purity and virus clearance before final formulation.

Table 2 summarizes the suggested buffers and process steps in a "generic" mAb purification protocol. Ideally, the elution buffer should be designed allowing a simple titration to condition the sample for the subsequent step.

The suggested buffer volumes are dimensioned for large columns. In a small-scale lab system, the column to system ratio is typically less optimal, which is why it is recommended to increase the wash and equilibration volumes.

Adsorption and desorption in a bead is mainly a diffusion-driven process. Thus, a high flow rate would have to be compensated with larger buffer volumes to achieve the same contaminant clearance, compared to what would be the result at a lower flow rate. We recommend a flow rate corresponding to a residence time (RT) of 6 - 8 minutes for 50 µm Praesto beads when using a 20 cm bed height. Higher residence times can be used with shorter bed heights; see operational flow diagram for more information.

**N.B.:** Before cycling a chromatography column and after storage, it is important to run a blank cycle including CIP to wash out storage buffer and minimize the risk of bioburden.

**TABLE 2 Generic purification protocol for use with Praesto Jetted A50 HipH***

| Step | Buffer | Column Volume** |
|---|---|---|
| **Equilibration** | 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0-7.4 | 3 |
| **Sample Load** | 70-90% of the dynamic binding capacity (DBC) | N/A |
| **Intermediate Wash 1** | Equilibration buffer | 3 |
| **Intermediate Wash 2** | 20 mM sodium phosphate, 1.0 M NaCl, pH 8.0 | 3 |
| **Intermediate Wash 3** | 20 mM sodium phosphate, pH 7.0 | 3 |
| **Elution** | 100 mM sodium acetate, pH 4.5 | 3 |
| **Regeneration** | 100 mM acetic acid | 3 |
| **CIP** | 0.1 M NaOH | 3 |
| **Equilibration** | 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0-7.4 | 3 |

**Column volumes for laboratory-scale columns

## Loading

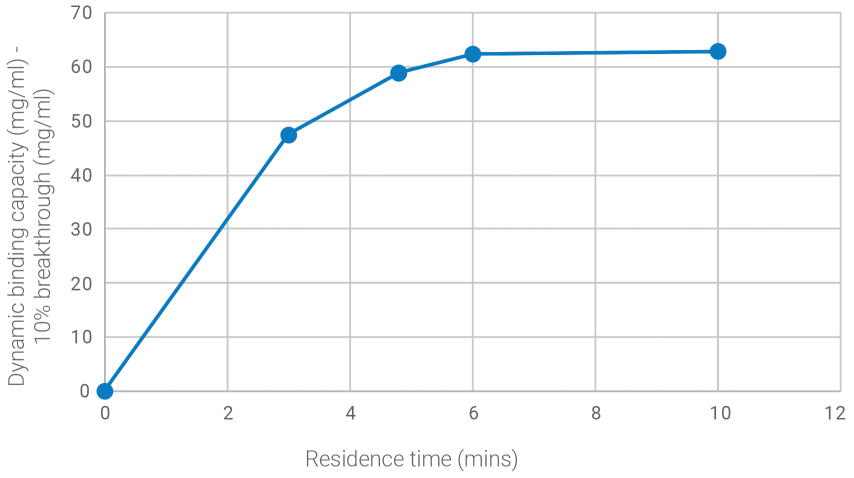
In designing loading parameters for the study, it is important to perform a screening process to determine the capacity of the target antibody, and choose the appropriate conditions for optimal process performance, economy and facility fit.

During process development, it is important to align the pressure flow properties of the resin with the dimensions of the process column and capabilities of the chromatography system intended for manufacture.

Figure 3 below shows operational flows at a range of bed heights with Praesto Jetted A50 HipH*. The flows have been determined using a column diameter of 30 cm packed by pressure and manual compression. The relationship between residence time and linear velocity detailed here can be used as a guide in conjunction with capacity data to determine the most suitable process conditions.

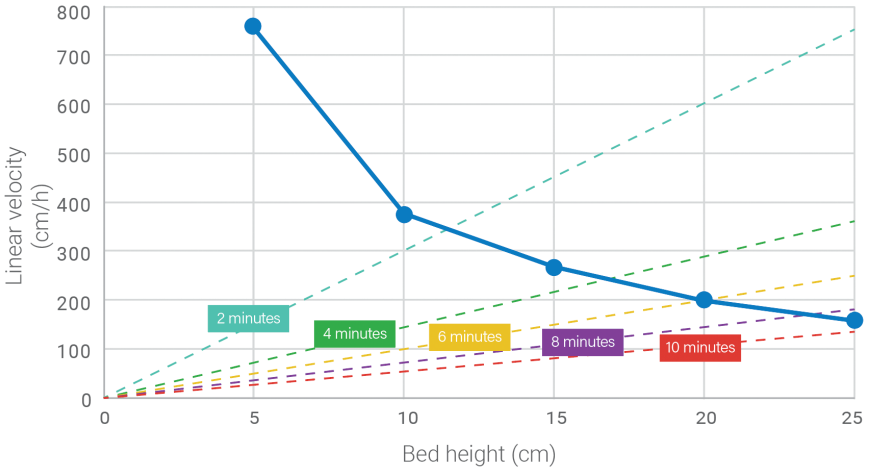
### FIGURE 2

**Dynamic binding capacity at 10% breakthrough for polyclonal human immunoglobulin G on Praesto Jetted A50 HipH at a range of residence times**




### FIGURE 3

**Expected operational window for Praesto Jetted A50 HipH***



## Elution

There is no clear rationale in choosing between acetate, citrate or glycine buffers. Acetate and citrate are the most commonly used buffers. Acetic acid has little buffer capacity at the elution pH and is easy to titrate without significantly increasing conductivity for the following step that commonly is cation exchange. Citrate has buffering capacity over a wider pH range (3-7) and is preferable if it is important to have a very specific elution buffer pH (but be aware that the preceding buffer and the elution pool volume will impact the pH of the eluate pool). Typical concentrations used are 10-100 mM.

The unique properties of Praesto Jetted A50 HipH allow for efficient elution at milder pH levels to conventional protein A chromatography. It is therefore important to screen the optimum elution pH for the target molecule with regards to optimal elution CV, purity and process-related impurity removal.

When optimizing elution, it is important to understand the highest pH possible to desorb the target antibody by loading a small amount of the antibody under neutral conditions and performing an elution gradient over 10-20 CV at a residence time greater than 6 minutes.

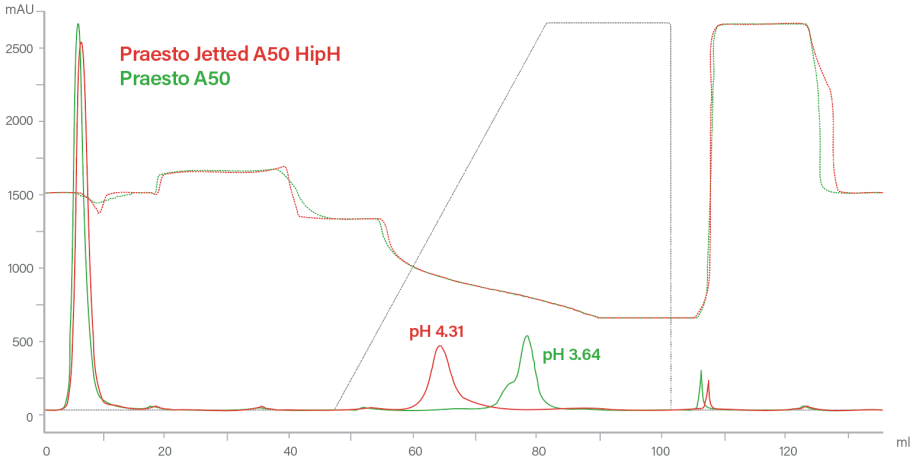
Buffers containing A (50 mM citrate; pH 7.0) and B (50 mM citrate; pH 3.0) can be used for gradient evaluation. If running a gradient is not possible, pH 4-4.5 is a good starting point for screening.

**TABLE 3** Suggested protocol for determining maximum pH elution for target molecule

| Step | Buffer | Column Volume |
|---|---|---|
| Equilibration | 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0-7.4 | 5 |
| Sample Load | 70-90 % of the dynamic binding capacity (DBC) | N/A |
| Chase | Equilibration buffer | 5 |
| Column Wash | 100 mM sodium citrate, pH 7.0 | 5 |
| Elution | 100 mM sodium citrate, pH 3.0 | 0-100% 10 CV 100% B 5 CV |
| Regeneration | 100 mM acetic acid | 3 |
| CIP | 0.1 M NaOH | 3 |
| Equilibration | 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0-7.4 | 3 |

## FIGURE 4

**Elution chromatogram for an IgG1 subclass monoclonal antibody captured on Praesto Jetted A50 HipH & Praesto Jetted A50**




## Leached Protein A

The protein ligand from Praesto Jetted A50 HipH can be analyzed using the commercially available ELISA kit for the detection of NGL-Impact® A HipH Ligand – 9777-1.

It is important to use the appropriate kit when determining leached protein A levels; use of alternative ligand calibration standards can lead to anomalous results.

## Regeneration

0.1 M acetic acid or low pH (3.0) for 2-3 CVs post elution is sufficient for regeneration of the resin.

## Intermediate Wash (Wash 2)

While the mAb is bound to the protein A resin, it is common practice to introduce an intermediate wash step. There are different strategies, but in principle, any shift from the sample loading conditions with respect to conductivity and pH will lower HCP levels in the elution pool. There are published methods including solvents or detergents; however, such additives must be assayed to show the removal, which can be difficult. Due to the unique characteristics of Praesto™ Jetted A50 HipH, the pH range of the intermediate wash is narrowed when compared to conventional protein A purification. It is recommended to be working within the pH range of 7-9 for any intermediary washes.

## Storage

It is recommended that Praesto Jetted A50 HipH should be stored between 2-8°C. After storage, you should equilibrate your resin with the starting buffer and perform a blank run, including CIP. This will ensure that there is no contamination on your resin.

## Cleaning-in-Place (CIP)

A regular cleaning-in-place (CIP) procedure is recommended to be performed after each cycle. Sodium hydroxide (NaOH) is commonly used in bioprocessing as an industry standard for CIP. Sodium hydroxide exhibits high efficiency in removing bound proteins, nucleic acids, and lipids from bioprocess resins, alleviating the risk of fouling on heavily burdened protein A columns.

To maximize column lifetime, use the recommended CIP protocol of 0.1 M NaOH with a 15-minute contact time. For more aggressive feeds, consider the use of 0.1 M NaOH every cycle with a higher NaOH (0.2-0.5 M) concentration every tenth cycle and between campaigns. Exposure to NaOH is cumulative and will impact the overall lifetime of the resin and the maximum number of cycles.

**Performance data at scale was executed with an uncoupled, 50 µm base-bead to demonstrate packing, pressure and flow parameters for the following resins: Praesto Jetted A50, Praesto Jetted A50 HipH, Praesto AP+50 and DurA Cycle A50.*

# Notes

# Notes

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