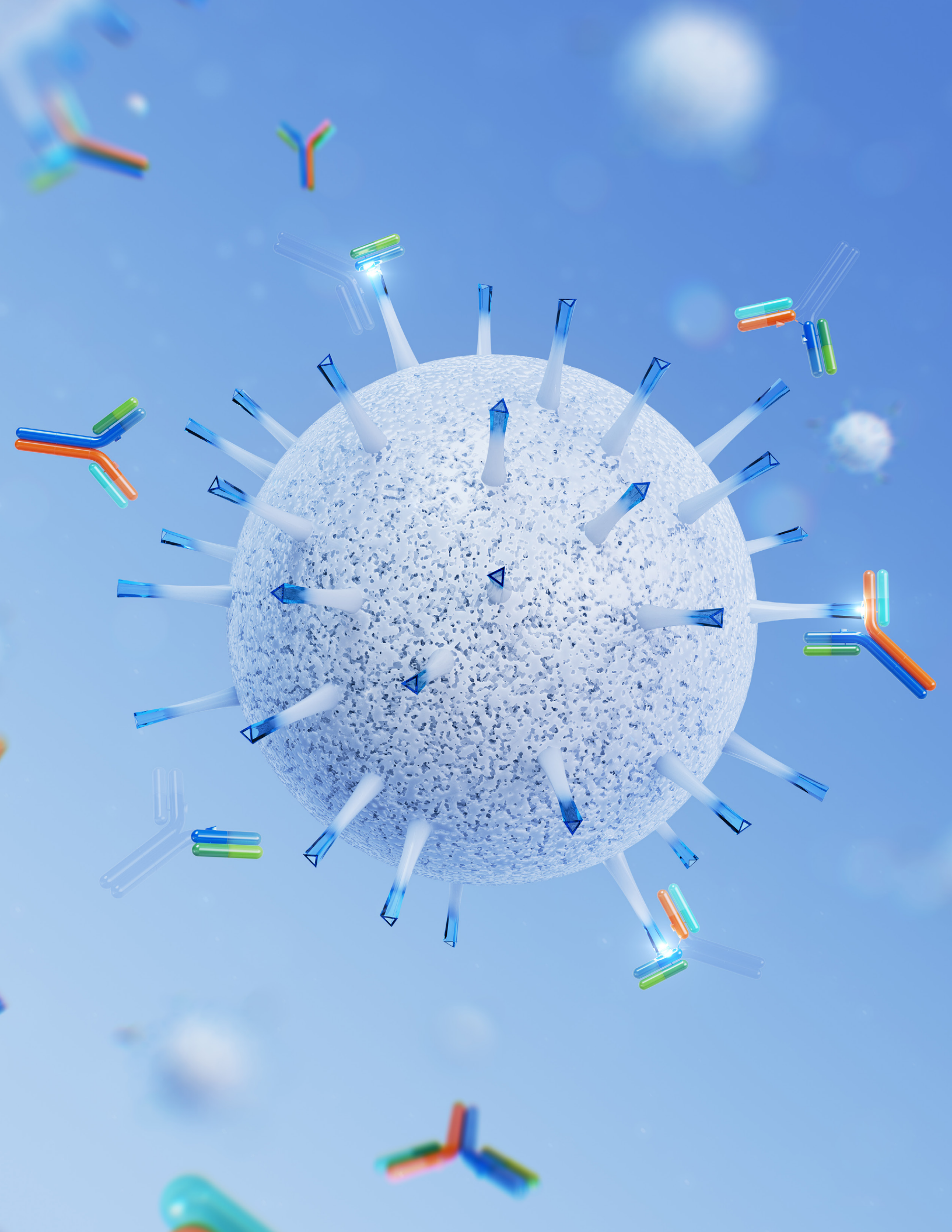
Instructions For Use

Praesto® 70 CH1

Purolite®
An Ecolab Company



Praesto® 70 CH1

Contents

Introduction

Praesto® 70 CH1 is a cross-linked agarose-based affinity resin designed for the purification of antigen binding fragments (Fabs) from human immunoglobulins (IgGs) and monoclonal antibodies (mAbs).

Praesto 70 CH1 binds to molecules that contain the CH1 domain of the heavy chain of human IgG. The resin has high dynamic capacity for human mABs and Fabs of IgG1 subclass. Praesto 70 CH1 resin is available in loose resin formats and in OPUS® pre-packed chromatography columns for rapid implementation.

All Praesto chromatography resins are manufactured using our patented Jetting technology, which produces consistent agarose beads with a uniform particle size distribution. Jetting means supply chain security, reduced lead times, and faster mAb processing, all whilst contributing towards your sustainability goals.

This guide provides the user recommended conditions and parameters for the use of Praesto 70 CH1. For further optimization or troubleshooting support please contact us via purolite.com or by using the contact details on the back cover of this document.

Physical and Chemical Characteristics

Praesto 70 CH1 comprises an engineered affinity ligand immobilized to a highly crosslinked agarose support matrix. Praesto 70 CH1 enables a high purity capture step which decreases process time and improves overall yield in the production of mAbs and Fabs.

It offers high selectivity for antibodies and antigen binding fragments. The combination of high binding capacity and high flow capability affords excellent resin productivity.

TABLE 1 **Product Specifications**

Product Characteristics	Praesto® 70 CH1
Matrix	Highly cross-linked agarose
Ligand	Alkali-tolerant recombinant protein
Dynamic Binding Capacity	40 g/L mAb (IgG1) Fab* at 6 minute residence time
Jetted Particle Size	70 µm
Particle Size Range	> 95% between 43–122 µm
Pressure/Flow	400 cm/h at 1.8 bar (30 x 20 cm)
pH stability (Working Range)	2–13
pH stability (CIP)	2–13
Recommended Storage Conditions	2–8 °C in 20% ethanol

* Fragment of Trastuzumab monoclonal antibody (IgG1)

Affinity Step Optimization

The primary aim of optimizing the affinity step (or, optimizing the binding buffer) is to establish the conditions that bind the highest amount of target molecule, in the shortest time, with the highest product recovery and purity. The degree to which Praesto 70 CH1 binds to Fabs and IgG varies with respect to both the origin and antibody subclass.

There might even be a substantial diversity in binding characteristics between different CH1 containing proteins. This is an important consideration when developing the purification protocol.

- Increase pH to reduce electrostatic repulsion between the CH1 ligand and IgG and allow an uninhibited affinity interaction.
- Increase salt concentration to reduce electrostatic repulsion and increase hydrophobic interactions.

Purification Protocol

The affinity of mAb affinity resins like Praesto® 70 CH1 varies for antibodies of different species, classes and subclasses. As such, initial screening should be conducted under a broader range of conditions that will bind the largest diversity of antibodies and highlight potential interference between the target antibody and possible contaminating antibodies. This can be optimized by changing pH and salt conditions (Table 2).

TABLE 2 Generic Purification Protocol for Use with Praesto 70 CH1

Step	Buffer	Column Volume*
Equilibration	Phosphate Buffered Saline pH 7.4	5
Sample Application	Load 85% of 10% Breakthrough	N/A
Chase	Phosphate Buffered Saline pH 7.4	5
Wash 1	50 mM Tris, 1 M NaCl, pH 8.0	5
Wash 2	50 mM Tris, pH 7.5	5
Elution	100 mM Sodium Citrate pH 2.8	5
CIP	0.1 M NaOH	5
Equilibration	Phosphate Buffered Saline pH 7.4	5

**Column volumes for laboratory scale columns.*

Cleaning-in-Place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated, or denatured substances from the resin and hardware. The accumulation of these contaminants may affect subsequent performance of the purification system or allow unwanted, potentially immunogenic, contaminants into the bulk active pharmaceutical ingredient (API). If the fouling is severe, it may block the column, increase back pressure, and reduce flow rate. Regular CIP prevents the build-up of these contaminants in the packed bed, and helps to maintain the capacity, flow properties, and general performance of Praesto 70 CH1.

Typically, CIP is conducted every 5 cycles or prior to storage; however, the frequency of CIP will ultimately depend on the nature of the feed material. It may be necessary to run more than one CIP protocol if the resin is contaminated with a diverse range of contaminants. Severe fouling will require specific protocol development.

CIP Protocol:

- Wash with 3–5 column volumes of 0.05–0.1 M NaOH with a total of 30 minutes contact time.
- Wash immediately with at least 5 column volumes of water or equilibration buffer.

Innovative Solutions for Bioprocessing

In partnership with Repligen, Purolite® develops and supplies innovative solutions for the bioprocessing industry, working with many of the top pharmaceutical companies to deliver the next-generation of healthcare. Our resins are used across the globe to deliver lifesaving medicines.

Global Support Network

No matter the location, our expert field application team members are positioned to help you solve your technical and downstream purification challenges, together. We provide the guidance necessary to develop robust, scalable, high productivity purification processes for mAbs and recombinant processes using Praesto® Jetted chromatography resins. For wherever you are in your biomanufacturing journey, we are here to help.

Purolite Affinity Resin Toolbox

Purolite's diverse toolbox offers Protein A resins, Praesto A50 and APc+, designed for high performance and increased sustainability, as well as novel resins, Praesto A50 HipH and Praesto 70 CH1, designed to enable cost-effective and reliable purification of bispecifics and Fc fusion proteins.

Purolite Ion Exchange Toolbox

Purolite's ion exchange toolbox consists of Praesto SP and Praesto Q resins in four particle sizes to ensure predictable selectivity across particle sizes, allowing for rapid performance screening.

Purolite, an Ecolab company, is a leading manufacturer of quality ion exchange, catalyst, adsorbent and specialty high-performance resins with global sales support.

www.purolite.com

We're ready to solve your process challenges.

For further information on Purolite products and services,
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