



# Ca<sup>++</sup>PURE-HA<sup>®</sup> HYDROXYAPATITE MEDIA

## INTRODUCTION

Ca<sup>++</sup>Pure-HA is a hydroxyapatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH)) chromatography packing material composed of calcium and phosphate and is used in the separation of biomolecules. Ca<sup>++</sup>Pure-HA media offer unparalleled selectivity and resolution for process scale operations.

Ca<sup>++</sup>Pure-HA is specifically developed for the purification of monoclonal and polyclonal antibodies, antibody isoforms, fusion- and phosphoproteins, and the isolation of single-stranded from double-stranded DNA.

Ca<sup>++</sup>Pure-HA media is a spherical, macroporous form of the hexagonal crystalline structure of hydroxyapatite (Figure 1). It has been sintered at high temperatures for increased mechanical and chemical stability, allowing it to withstand the rigors of industrial-scale applications. The robust nature of Ca<sup>++</sup>Pure-HA allows for it to be used reproducibly for many cycles at high flow rates and in large columns. In large-scale processes, Ca<sup>++</sup>Pure-HA is easy to pack and unpack.

## PRODUCT ATTRIBUTES

Particle size (mean):	39 μm
Max. flow pressure:	10 MPa
Shipped as:	dry powder
pH stability:	6.5-14
Shelf life (estimated):	10 years

## FEATURES

Ca<sup>++</sup>Pure-HA offers a dynamic binding capacity (DBC), at 5% breakthrough, of greater than 30 g/L human IgG at residence times as low as 2 minutes, and even DBC greater than 50 g/L at 5-minute residence time (Figure 2).

Ca<sup>++</sup>Pure-HA is effective at removing aggregates and degradation products from mAbs with an elution buffer such as potassium chloride (Figure 3). The separation profile shows high resolution between the monomer peak and the aggregate peaks.

## Ca<sup>++</sup>PURE-HA CRYSTALLINE STRUCTURE

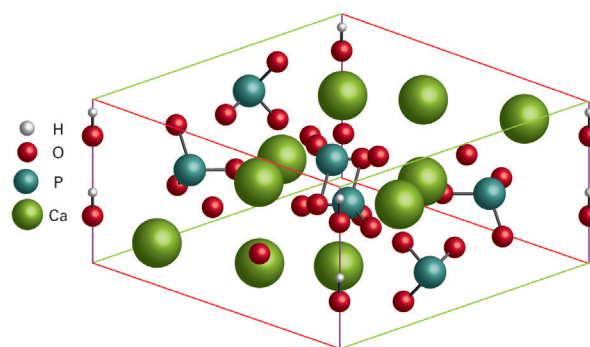


Figure 1

## Ca<sup>++</sup>PURE-HA DYNAMIC BINDING CAPACITY

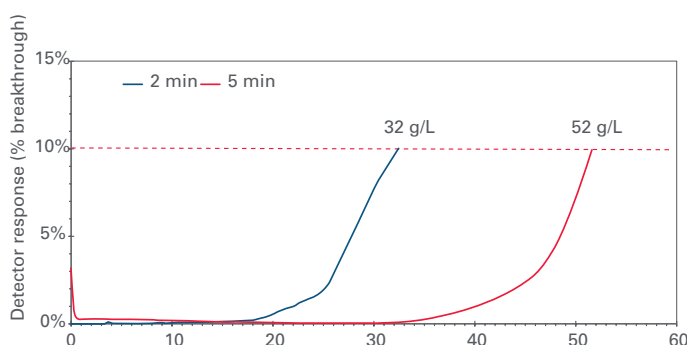
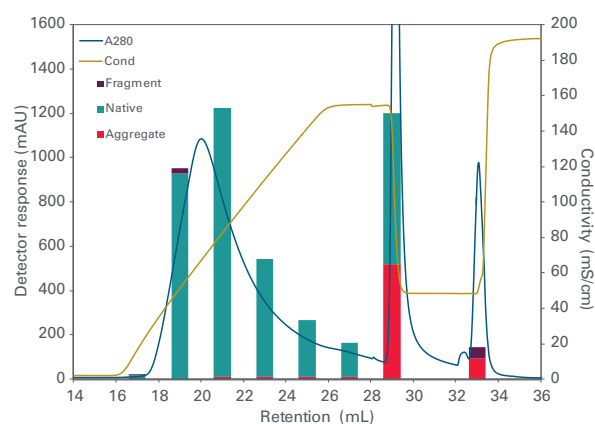


Figure 2

## POLISHED IgG SAMPLE (POOLED FRACTIONS FROM POST-PROTEIN A PURIFICATION) USING Ca<sup>++</sup>PURE-HA



Media: Ca<sup>++</sup>Pure-HA  
 Column: 5 mm × 5 cm (1.0 mL)  
 Equilibration: 20 mmol/L MES, 5 mmol/L KPO<sub>4</sub>, pH 6.5  
 Elution/Strip: 500 mmol/L KPO<sub>4</sub>, pH 6.5  
 Sanitization: 1.0 mol/L KOH  
 Flow rate: 75 cm/hr (4 min residence time)  
 Detection: UV @ 280 nm (mAU), conductivity (mS/cm)  
 Temperature: ambient  
 Sample: IgG @ 2.00 g/L

Figure 3

High molecular weight aggregates and degradation products are reduced significantly (Figure 4).

Ca<sup>++</sup>Pure-HA is alkaline stable in 0.5 mol/L NaOH for greater than 65 CIP cycles with no appreciable loss of dynamic binding capacity (Figure 5). Its high mechanical stability allows packing of large process columns (Figure 6). Further details about packing and usage of Ca<sup>++</sup>Pure-HA can be found in the dedicated Instruction Manual.

**Ca<sup>++</sup>Pure-HA media offer chromatographers the combination of exceptional separation properties and unequalled selectivity and resolution for multiple classes of biomolecules. The highly selective and robust nature of Ca<sup>++</sup>Pure-HA provides the flexibility to use this media at any stage in a process from capture to final polishing.**

SEC ANALYSIS OF POOLED MONOMER IgG ELUTED PEAK

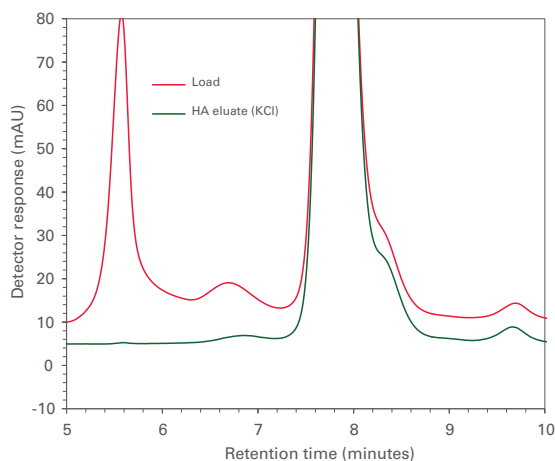
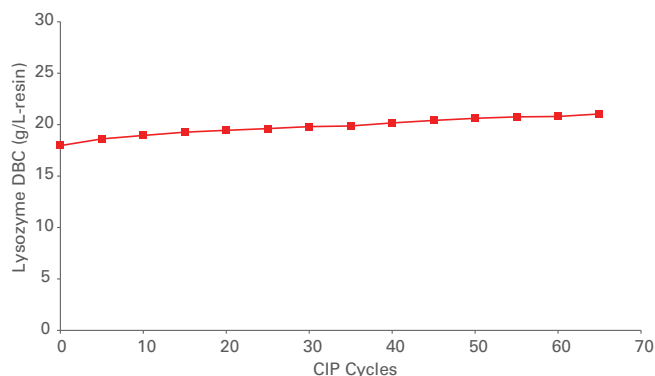


Figure 4

MECHANICAL STABILITY OF CA<sup>++</sup>PURE-HA



- DBC of lysozyme was measured after every 5th CIP cycle with 1.0 mol/L NaOH
- Preliminary study of Ca<sup>++</sup>Pure-HA resin shows that it is stable over 65 CIP cycles

Figure 5

MECHANICAL STABILITY OF CA<sup>++</sup>PURE-HA

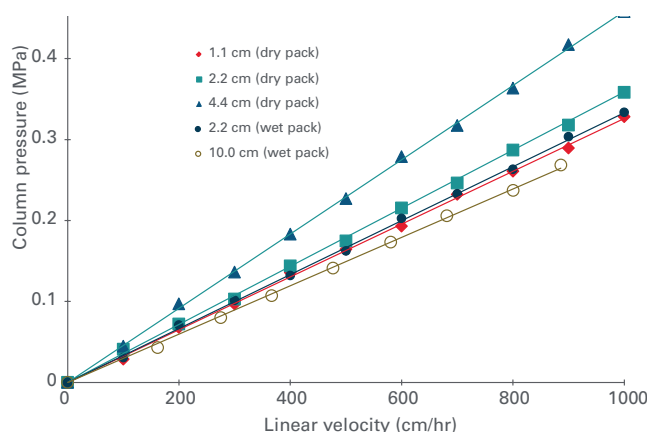


Figure 6

Ordering Information

Ca<sup>++</sup>Pure-HA - Process Media

Part-No	Description
0045045	Ca <sup>++</sup> Pure-HA , 50 g
0045039	Ca <sup>++</sup> Pure-HA , 100 g
0045040	Ca <sup>++</sup> Pure-HA , 250 g
0045041	Ca <sup>++</sup> Pure-HA , 500 g
0045042	Ca <sup>++</sup> Pure-HA , 1 kg
0045043	Ca <sup>++</sup> Pure-HA , 5 kg

Ca<sup>++</sup>Pure-HA - Process Development Tools

Part-No	Description
<b>MiniChrom</b>	
0045152	Minichrom Ca <sup>++</sup> Pure-HA, 5 mL
<b>RoboColumns</b>	
0045053	RoboColumn Ca <sup>++</sup> Pure-HA , 200 µL x 8
0045054	RoboColumn Ca <sup>++</sup> Pure-HA , 600 µL x 8
<b>ResinSeeker 96-well plates</b>	
0045512	ResinSeeker Ca <sup>++</sup> Pure-HA , 20 µL
0045513	ResinSeeker Ca <sup>++</sup> Pure-HA , 500 µL