TOSOH

Product Overview

Ca++Pure-HA™

Hydroxyapatite Media from Tosoh Bioscience

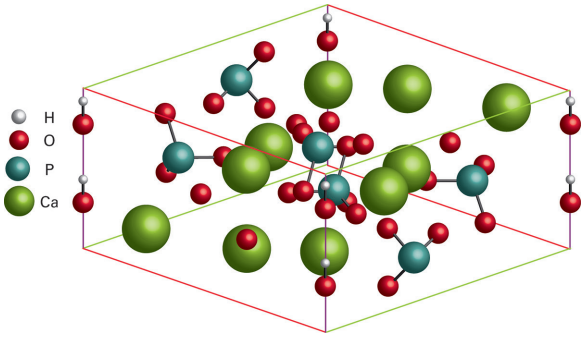
Introduction

Ca++Pure-HA is a hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) chromatography packing material composed of calcium and phosphate and is used in the separation of biomolecules. The formation of the Ca++Pure-HA particle, both the ligand and the base bead, is created simultaneously from the same source of materials. Ca++Pure-HA media offers unparalleled selectivity and resolution for process scale operations. Its highly selective nature often separates proteins otherwise shown to be homogeneous by electrophoresis and other chromatographic techniques.

While the selectivity and multiple modes of interaction of Ca++Pure-HA media point to a seemingly limitless field of applications for its use, it is best suited for applications associated with the separation of biomolecules. Ca++Pure-HA is specifically developed for the purification of monoclonal and polyclonal antibodies, the separation of antibody isoforms, isozymes, the purification of antibody fragments, and the isolation of single-stranded from double-stranded DNA.

Ca++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++Pure-HA allows for it to be used reproducibly for many cycles at high flow rates and in large columns.

Figure 1. Ca++Pure-HA crystalline structure

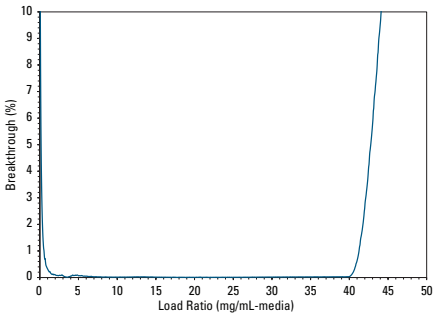


Product Attributes

Particle size (mean):	39 µm
Pressure rating:	10 MPa
Shipped as:	dry powder
pH stability:	6.5-14
Shelf life (estimated):	10 years

Ca++Pure-HA has a demonstrated dynamic binding capacity (DBC), at 5% breakthrough, of greater than 40 g/L human IgG at residence times as low as 4 minutes as shown in *Figure 2*.

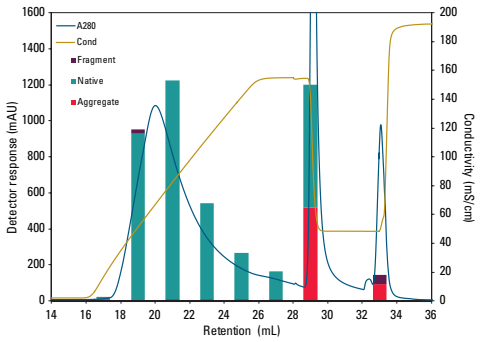
Figure 2. Ca++Pure-HA dynamic binding capacity



Media:	Ca++Pure-HA, lot CPBL122716A
Column:	5 mm × 5 cm (1.0 mL)
Equilibration:	20 mmol/L MES, 5 mmol/L KPO_4, pH 6.5
Elution/Strip:	500 mmol/L KPO_4, 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
Instrument:	ÄKTA® avant 25

Ca++Pure-HA is effective at removing aggregates and degradation products from mAbs with an elution buffer such as potassium chloride, as demonstrated in *Figure 3*. The separation profile shows high resolution between the monomer peak and the aggregate peaks.

Figure 3. Polished IgG sample (pooled fractions from post-protein A purification) using Ca++Pure-HA



SEC analysis of the peaks before and after purification on Ca++Pure-HA *(Figure 4)* show that high molecular weight aggregates and degradation products are reduced significantly. Analytical HPLC peak integration data summary is shown in *Table 1*.

Figure 4. SEC analysis of pooled monomer IgG eluted peak

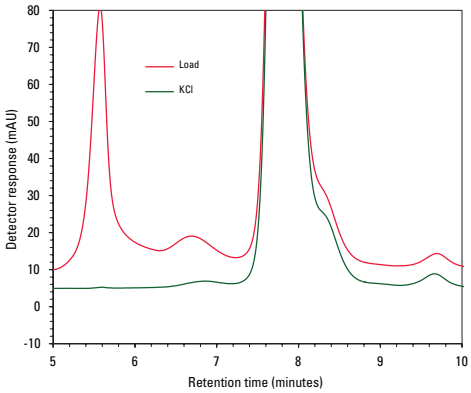
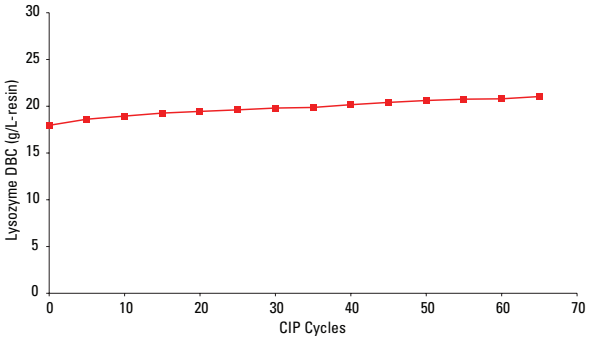


Table 1. Reduced IgG aggregates and fragments using Ca++Pure-HA media with KCl elution buffer

Condition	Media	Fraction (mL)	Aggregate (%)	Fragment (%)
Initial sample load			**18.8**	**1.3**
KCl Elution	Ca++Pure-HA	8.9	1.1	1.0

Ca++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)*. Dynamic binding capacity of lysozyme was measured after every 5th CIP cycle with 1.0 mol/L NaOH.

Figure 5. Caustic stability of Ca++Pure-HA



Ca++Pure-HA media offers 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++Pure-HA provides the flexibility to use this media at any stage in a process from capture to final polishing.

Ordering Information

Part#	Description	Resin Volume
0045045	**Ca++Pure-HA**	50 g
0045039	**Ca++Pure-HA**	100 g
0045040	**Ca++Pure-HA**	250 g
0045041	**Ca++Pure-HA**	500 g
0045042	**Ca++Pure-HA**	1 kg
0045043	**Ca++Pure-HA**	5 kg
0045225	**SkillPak Ca++Pure-HA 1 mL columns (qty. 5)**	5 × 1 mL
0045262	**SkillPak Ca++Pure-HA 5 mL column**	5 mL
CUS1004	**ToyoScreen Robocolumn, Ca++Pure-HA**	8 × 600 µL
CUS022318CT2	**ToyoScreen Robocolumn, Ca++Pure-HA**	8 × 200 µL
OC41MDCPHA	**Resin Seeker, Ca++Pure-HA, 96-well plate**	20 µL resin beds
OC41MDPHA-500	**Resin Seeker, Ca++Pure-HA, 96-well plate**	500 µL resin beds