Two Chromatographic Steps Purification of Monoclonal Antibody on TOYOPEARL AF-rProtein A-650F

Purification Step	HCP (ppm)	Virus reduction	DNA reduction	Aggregates (%)	Recovery (%)
	cum.	(LRV/step)	(LRV/step)	cum.	(step/cum.)
Harvest	228000	-	-	2.67	100/100
BTI clarification	116000	4 - 5	5	0.39	99/99
Protein A	< 1	3 - 5	3 - 4	0.22	95/97
Anion-exchange (flow-through)	FBLD*	3 - 4	3 - 4	0.05	99/96
HCP; host cell protein, by ELISA, Virus reduction; by infectivity, DNA reduction; by qPCR, Aggregates; by SEC					
Protein A; TOYOPEARL AF-rProtein A 650F					
Anion-exchange (flow-through); UNOsphere Q					
* FBLD; Far below the limit of detection					

Ref.; P. Gagnon et al., IBC Biopharmaceutical Development and Production Conference, Huntington Beach, (2012), modified

"TOYOPEARL AF-rProtein A 650F gave good mass transfer and sharper peak for removal of impurities, which might be due to larger pore size on the resin." (personal communication)

Purification of Bi-specific Monoclonal Antibody using TOYOPEARL AF-rProtein A 650F

Purification Step	HCP (ng/mg)	DNA (pg/mg)	AA, BB* (%)	AB** (%)	Aggregates (%)
Feed	45,855	2,596	37	35	> 5
Protein A eluate	42 - 220	4 - 6	37	NA	2.3 - 2.9
MCSGP CIEX eluate	14 - 38	3 - 8	< 0.5	NA	< 1.0
Anion-exchange (pH 6.0)	0 - 25	14 - 17	< 0.5	60 - 70%	< 1.0
Anion-exchange (pH 5.0)	26	28	< 0.5	60 - 70%	< 1.0
MM polish	11	2	< 0.5	60 - 70%	< 1.0

*Main isoform determined by CIEX analytics on TSKgel SP-STAT

**Determined by native mass spectrometry (MS)

Protein A; TOYOPEARL SuperQ-650M

Cation-exchange; Poros 50 HS

Anion-exchange (flow-through); TOYOPEARL SuperQ-650M

Ref.; T. Muller-Spath et al., BioProcess International, 11-5 (2013), May p36, modified

"We chose Tosoh rPA resin for our protein A capture step based on HCP clearance data."