

Fig 1

Met	Ala	Gln	Val	Leu	Arg	Gly	Thr	Val	Thr	Asp	Phe	Pro	Gly	Phe	Asp	
1				5					10					15		
Glu	Arg	Ala	Asp	Ala	Glu	Thr	Leu	Arg	Lys	Ala	Met	Lys	Gly	Leu	Gly	
			20					25					30			
Thr	Asp	Glu	Glu	Ser	Ile	Leu	Thr	Leu	Leu	Thr	Ser	Arg	Ser	Asn	Ala	
		35					40					45				
Gln	Arg	Gln	Glu	Ile	Ser	Ala	Ala	Phe	Lys	Thr	Leu	Phe	Gly	Arg	Asp	
	50					55					60					
Leu	Leu	Asp	Asp	Leu	Lys	Ser	Glu	Leu	Thr	Gly	Lys	Phe	Glu	Lys	Leu	
65					70					75					80	
Ile	Val	Ala	Leu	Met	Lys	Pro	Ser	Arg	Leu	Tyr	Asp	Ala	Tyr	Glu	Leu	
				85					90					95		
Lys	His	Ala	Leu	Lys	Gly	Ala	Gly	Thr	Asn	Glu	Lys	Val	Leu	Thr	Glu	
			100					105					110			
Ile	Ile	Ala	Ser	Arg	Thr	Pro	Glu	Glu	Leu	Arg	Ala	Ile	Lys	Gln	Val	
		115					120					125				
Tyr	Glu	Glu	Glu	Tyr	Gly	Ser	Ser	Leu	Glu	Asp	Asp	Val	Val	Gly	Asp	
	130					135					140					
Thr	Ser	Gly	Tyr	Tyr	Gln	Arg	Met	Leu	Val	Val	Leu	Leu	Gln	Ala	Asn	
145					150					155					160	
Arg	Asp	Pro	Asp	Ala	Gly	Ile	Asp	Glu	Ala	Gln	Val	Glu	Gln	Asp	Ala	
				165					170				175			
Gln	Ala	Leu	Phe	Gln	Ala	Gly	Glu	Leu	Lys	Trp	Gly	Thr	Asp	Glu	Glu	
			180					185					190			
Lys	Phe	Ile	Thr	Ile	Phe	Gly	Thr	Arg	Ser	Val	Ser	His	Leu	Arg	Lys	
		195					200					205				
Val	Phe	Asp	Lys	Tyr	Met	Thr	Ile	Ser	Gly	Phe	Gln	Ile	Glu	Glu	Thr	
	210						215				220					

Ile	Asp	Arg	Glu	Thr	Ser	Gly	Asn	Leu	Glu	Gln	Leu	Leu	Leu	Ala	Val
225								230						235	240
Val	Lys	Ser	Ile	Arg	Ser	Ile	Pro	Ala	Tyr	Leu	Ala	Glu	Thr	Leu	Tyr
								245						250	255
Tyr	Ala	Met	Lys	Gly	Ala	Gly	Thr	Asp	Asp	His	Thr	Leu	Ile	Arg	Val
								260						265	270
Met	Val	Ser	Arg	Ser	Glu	Ile	Asp	Leu	Phe	Asn	Ile	Arg	Lys	Glu	Phe
								275						280	285
Arg	Lys	Asn	Phe	Ala	Thr	Ser	Leu	Tyr	Ser	Met	Ile	Lys	Gly	Asp	Thr
								290						295	300
Ser	Gly	Asp	Tyr	Lys	Lys	Ala	Leu	Leu	Leu	Leu	Cys	Gly	Glu	Asp	Asp
305								310						315	320

Fig 2

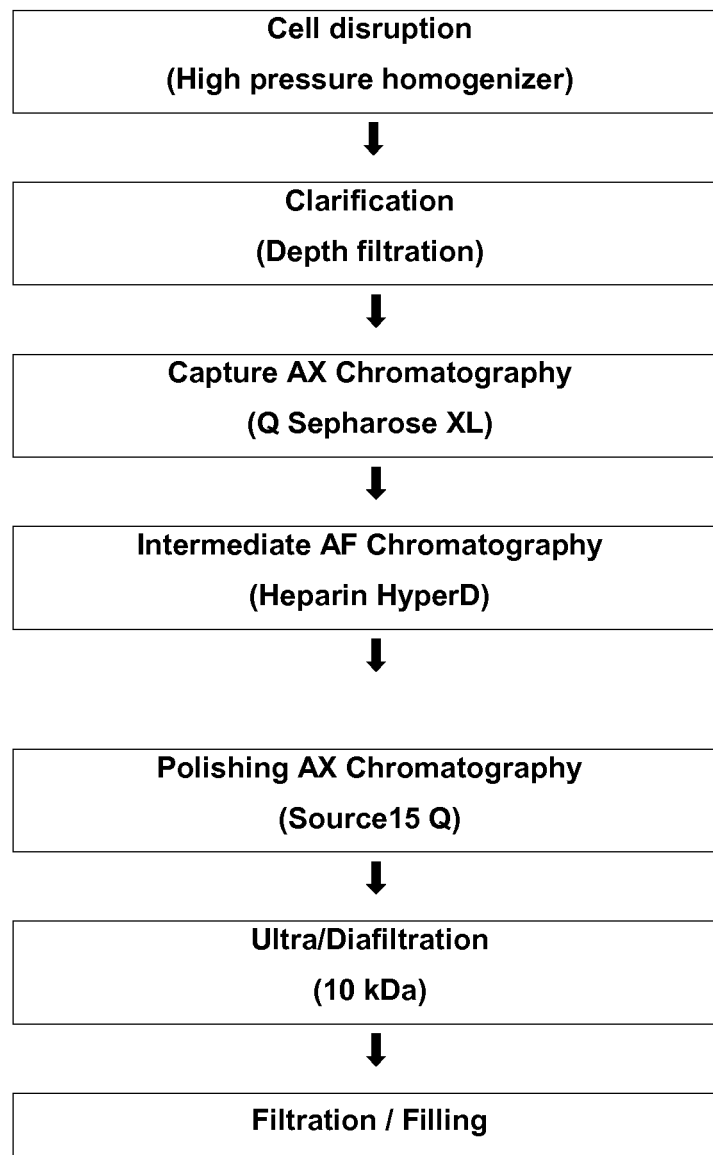


Fig 3

Process Step	Parameter	3 L process	100 L process
AX ▶	Resin:	Q Sepharose XL	Q Sepharose XL
	Column:	XK 50	BPG 300
	Column diameter (cm):	5	30
	Bed height (cm):	13.5	13.5
	Column volume (L):	0.265	9.5
	Flow rate:	200 cm/h	170 cm/h
	Buffer temperature:	Ambient temperature	Ambient temperature
▼	Pump:	with WATSON-MARLOW Tubing pump 323S	BioProcess system
EQ	Buffer A:	20 mM Tris pH 7.4, 0.1 % Tween80, 25 mM NaCl	20 mM Tris pH 7.4, 0.1 % Tween80, 25 mM NaCl
▼	Vol:	2 CV	3 CV
Load			Online dilution (1+1) with 1% (v/v) Tween 80, 4 mM EDTA, pH 8.0
▼			
Wash	Buffer:	20 mM Tris pH 7.4, 0.1 % Tween80, 25 mM NaCl	20 mM Tris pH 7.4, 0.1 % Tween80, 25 mM NaCl
▼	Vol:	10 CV	10 CV
Step Elution	100 % Buffer B:	20 mM Tris pH 7.4, 0.1 % Tween80, 300 mM NaCl	20 mM Tris pH 7.4, 0.1 % Tween80, 300 mM NaCl
▼	Vol:	9 CV	9 CV
Pooling	Criteria:	Main peak Pool (0.1 AU-0.2 AU)	Main peak Pool (target 0.2 AU-0.4 AU ; 5 mm light path)
▼			(within 3 weeks)
CIP	Step 1:	2 M NaCl	2 M NaCl
	Vol:	3 CV	3 CV
	Step 2:	1 M NaOH	1 M NaOH
	Vol:	3 CV	3 CV
▼	Incubation:	15 h	15 h
Storage	Storage:	20 mM NaOH	10 mM NaOH

Fig 4

Process Step	Parameter	3 L Process	100 L Process
AF ▶	Resin:	Heparin HyperD	Heparin HyperD
	Column:	XK 50	BPG300
	Column diameter (cm):	5	30
	Bed height (cm):	25	25
	Column volume (L):	0.491	17.7
	Flow rate:	60-100 cm/h	60-100 cm/h
	Buffer temperature:	Ambient temperature	Ambient temperature
▼ EQ ▼	Buffer A:	20 mM Tris pH 7.4; 0.1 % Tween80; 2 mM CaCl ₂ , 25 mM NaCl	20 mM Tris pH 7.4; 0.1 % Tween80; 2 mM CaCl ₂ , 25 mM NaCl
	Vol:	2 CV	3 CV
▼ Load ▼	Flow rate:	100 cm/h	60-100 cm/h; online 1:8 dilution of AX eluate with buffer A
Wash 1	Buffer:	20 mM Tris pH 7.4; 0.1 % Tween80; 2 mM CaCl ₂ , 25 mM NaCl	20 mM Tris pH 7.4; 0.1 % Tween80; 2 mM CaCl ₂ , 25 mM NaCl
	Vol:	15 CV	15 CV
	Flow rate:	100 cm/h	60-100 cm/h
▼ Wash 2 ▼	Buffer:	20 mM Tris pH 7.4; 0.1 % Tween80, 25 mM NaCl	20 mM Tris pH 7.4; 0.1 % Tween80, 25 mM NaCl
	Vol:	2 CV	2 CV
	Flow rate:	100 cm/h	60-100 cm/h
▼ Step Elution ▼	100 % Buffer B:	20 mM Tris pH 7.4; 0.1 % Tween80; 10 mM EDTA; 25 mM NaCl	20 mM Tris pH 7.4; 0.1 % Tween80; 10 mM EDTA; 25 mM NaCl
	Vol:	2-3 CV	2-3 CV
	Flow rate:	40-60 cm/h	40-60 cm/h
▼ Pooling ▼	Criteria:	Complete peak Pool (0.1 AU-0.1 AU)	Complete peak Pool (0.2 AU-0.2 AU ; 5 mm light path)
▼ CIP ▼	Step 1:	2 M NaCl	(within 3 weeks) 2 M NaCl
	Vol:	3 CV	3 CV
	Step 2:	0.1 M NaOH	0.1 M NaOH
	Vol:	3 CV	3 CV
▼ Storage	Incubation:	15 h	≥15 h (contact time, no pumping)
	Storage:	1 M NaCl, 25% EtOH	1 M NaCl, 25% EtOH

Fig 5

Process Step	Parameter	3 L process	100 L process
AX ▶	Resin:	Source15 Q	Source15 Q
	Column:	-	Fineline200
	Column diameter (cm):	-	20
	Bed height (cm):	15	15
	Column volume (L):	0.4	4.7
	Flow rate:	100 cm/h	100 cm/h
	Buffer temperature:	Ambient temperature	Ambient temperature
▼			
EQ	Buffer A:	20 mM Bis-Tris pH 7; 25 mM NaCl	20 mM Bis-Tris pH 7; 25 mM NaCl
▼	Vol:	2 CV	3 CV
Load	Flow rate:	100 cm/h	100 cm/h AF pool splitting (1/4-1/5)
▼			
Wash	Buffer:	20 mM Bis-Tris pH 7; 25 mM NaCl	20 mM Bis-Tris pH 7; 25 mM NaCl
	Vol:	3 CV	3 CV
	Flow rate:	100 cm/h	100 cm/h
▼			
Gradient Elution	Buffer B:	20 mM Bis-Tris pH 7; 180 mM NaCl 0-100% B in 33 CV	20 mM Bis-Tris pH 7; 180 mM NaCl 0-100% B in 33 CV
	Gradient:		
▼			
Pooling	Criteria:	major peak starting at 0.05 AU to the valley between peak 1 and peak 2	major peak starting at 0.1 AU to the valley between peak 1 and peak 2 (5 mm light path)
▼			
CIP	Step 1:	2 M NaCl	(within 3 weeks) 2 M NaCl
	Vol:	3 CV	3 CV
	Step 2:	1 M NaOH	1 M NaOH
	Vol:	3 CV	3 CV
	Incubation:	15 h	≥15 h (contact time, no pumping)
▼			
Storage	Storage:	25 mM NaOH	10 mM NaOH

Fig 6

Process Step	Parameter	3 L process	100 L process
Concentration	cassette: area: Target concentration: Processing time: Parameter: Concentration factor	Hydrosart 10 K 0.1 m ² 5 g/L ~ 2h TMP 0.9-1.1 Entrance pressure 1 bar Retentate back pressure 0.8 bar ~ 6	Hydrosart 10 K 2.4 m ² 5 g/L - TMP 0.9-1.1 Entrance pressure 1 bar Retentate back pressure 0.8 bar 6-8
▼			
Buffer change	► Dilution buffer: Diafiltration volumes: Concentration factor Target concentration: Dilution	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl ₂ pH 7 10 ~ 6 12 g/L Addition of cassette wash	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl ₂ pH 7 10 ~ 6 12 g/L Addition of cassette wash
▼			
Addition of Tween80	► Buffer: Target concentration Tween80: Target concentration bulk:	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl ₂ , 10 % Tween80 pH 7 0.05 % 10 g/L	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl ₂ , 10 % Tween80 pH 7 0.05 % 10 g/L
▼			
Sterile filtration	► Device: Cut off: Target concentration bulk:	Sartopore2 0.45-0.2 µm 10 g/L	Sartopore2 0.45-0.2 µm 10 g/L
▼			
Storage of bulk	► Temperature:	2-8°C	2-8°C

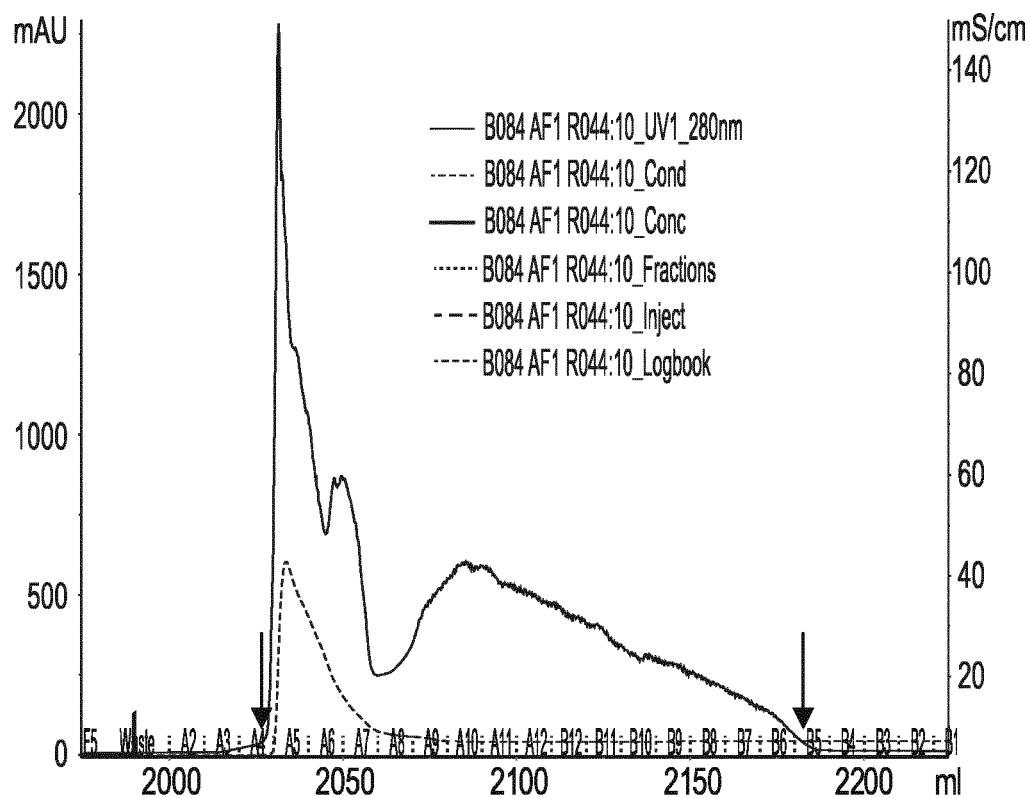


FIG. 7A

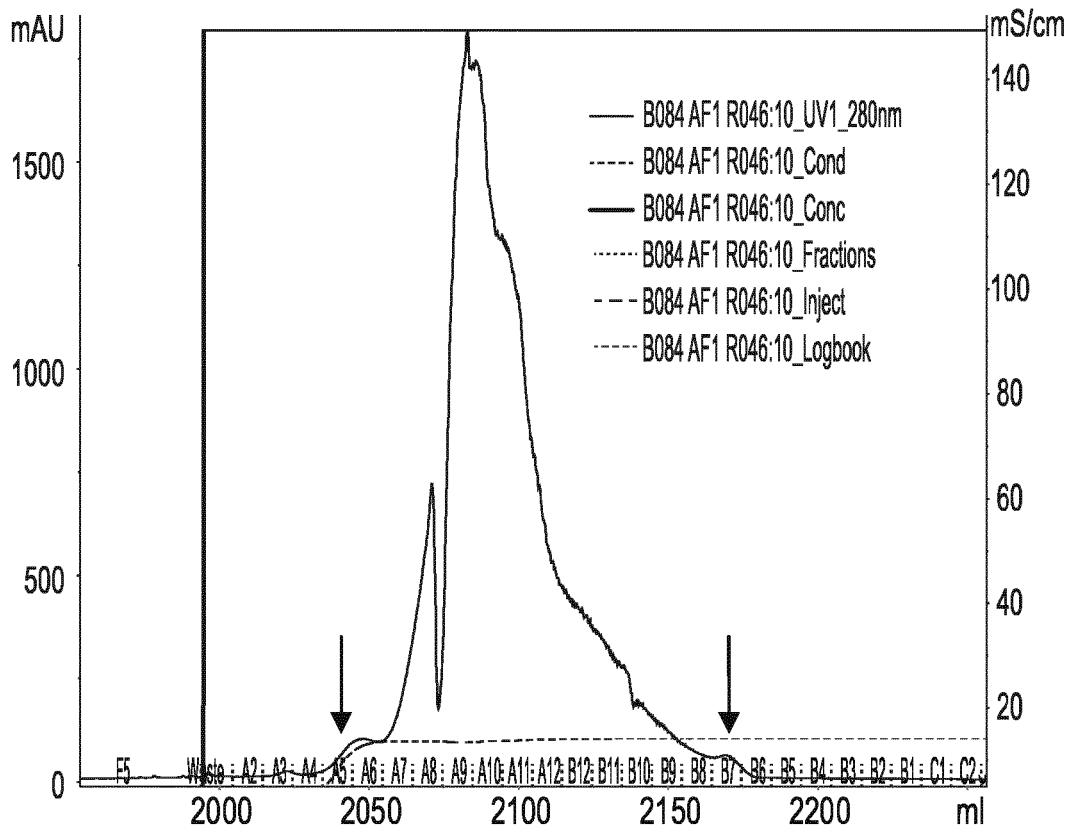


FIG. 7B