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
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```
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

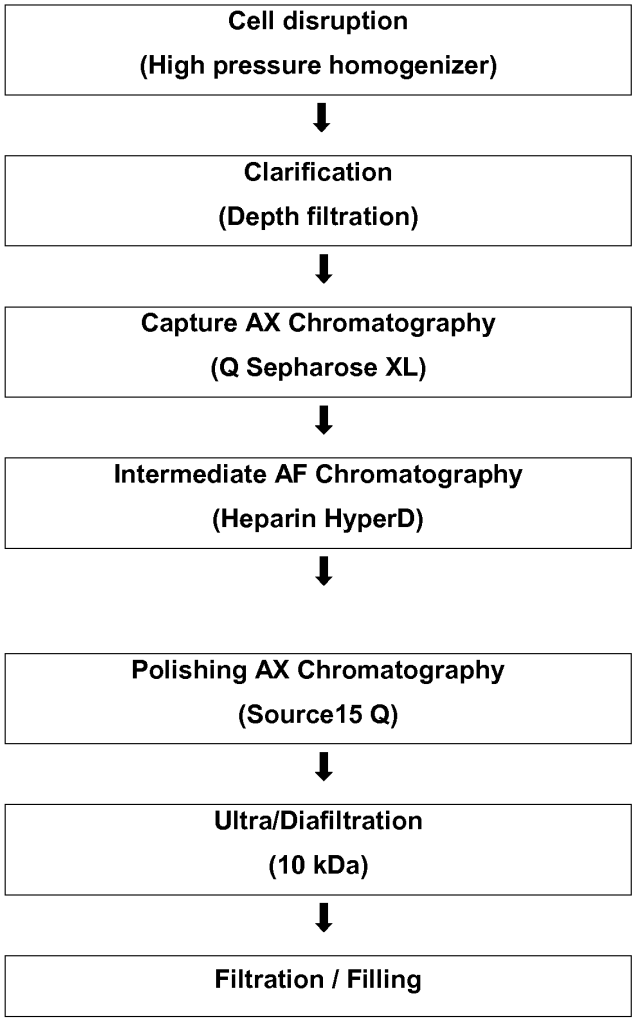

Cell disruption
(High pressure homogenizer)
Clarification
(Depth filtration)
Capture AX Chromatography
(Q Sepharose XL)
Intermediate AF Chromatography
(Heparin HyperD)
Polishing AX Chromatography
(Source15 Q)
Ultra/Diafiltration
(10 kDa)
Filtration / Filling

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

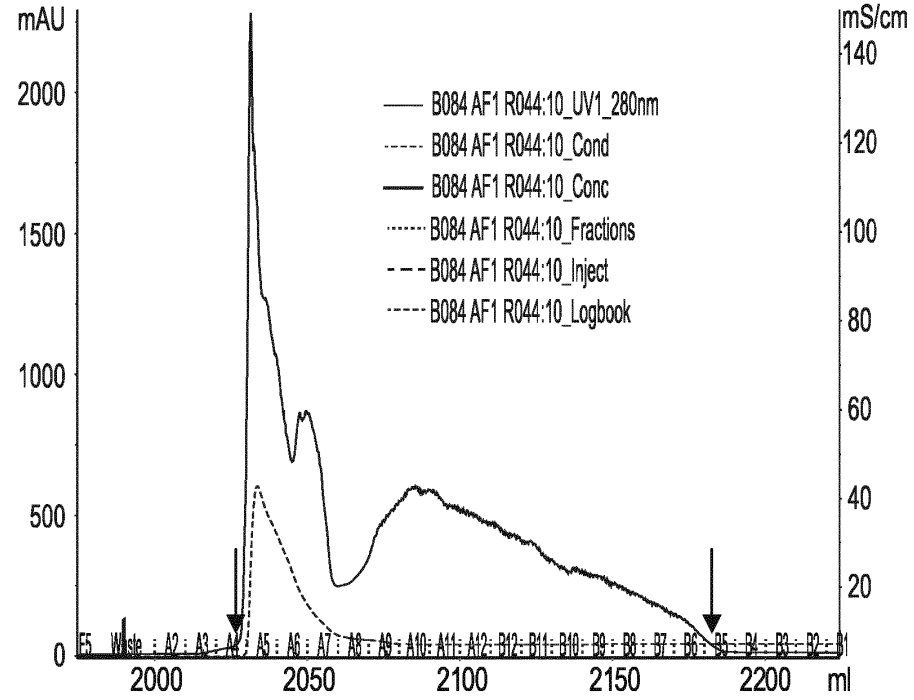
Process Step	Parameter	3 L Process	100 L Process
AF ▶	Resin:	Heparin HyperD	Heparin HyperD
	Column:	XK 50	BPG300
	Column diameter (cm):	5	30
	Bed height (cm):	25	25
	Column volume (L):	0.491	17.7
	Flow rate:	60-100 cm/h	60-100 cm/h
	Buffer temperature:	Ambient temperature	Ambient temperature
▼ EQ ▼	Buffer 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			(within 3 weeks)
	Step 1:	2 M NaCl	2 M NaCl
	Vol:	3 CV	3 CV
	Step 2:	0.1 M NaOH	0.1 M NaOH
	Vol:	3 CV	3 CV
	Incubation:	15 h	≥15 h (contact time, no pumping)
▼ Storage	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			(within 3 weeks)
	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 (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:	Hydrosart 10 K 0.1 m²	Hydrosart 10 K 2.4 m²
	Target concentration:	5 g/L	5 g/L
	Processing time:	~ 2h	-
	Parameter:	TMP 0.9-1.1 Entrance pressure 1 bar Retentate back pressure 0.8 bar	TMP 0.9-1.1 Entrance pressure 1 bar Retentate back pressure 0.8 bar
	Concentration factor	~ 6	6-8
▼			
Buffer change ►	Dilution buffer:	20 mM Bis-Tris, 150 mM NaCl, 1mM $CaCl_2$ pH 7	20 mM Bis-Tris, 150 mM NaCl, 1mM $CaCl_2$ pH 7
	Diafiltration volumes:	10	10
	Concentration factor	~ 6	~ 6
	Target concentration:	12 g/L	12 g/L
	Dilution	Addition of cassette wash	Addition of cassette wash
▼			
Addition of Tween80 ►	Buffer:	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl2, 10 % Tween80 pH 7	20 mM Bis-Tris, 150 mM NaCl, 1mM CaCl2, 10 % Tween80 pH 7
	Target concentration Tween80:	0.05 %	0.05 %
	Target concentration bulk:	10 g/L	10 g/L
▼			
Sterile filtration ►	Device:	Sartopore2	Sartopore2
	Cut off:	0.45-0.2 µm	0.45-0.2 µm
	Target concentration bulk:	10 g/L	10 g/L
▼			
Storage of bulk ►	Temperature:	2-8°C	2-8°C


mAU
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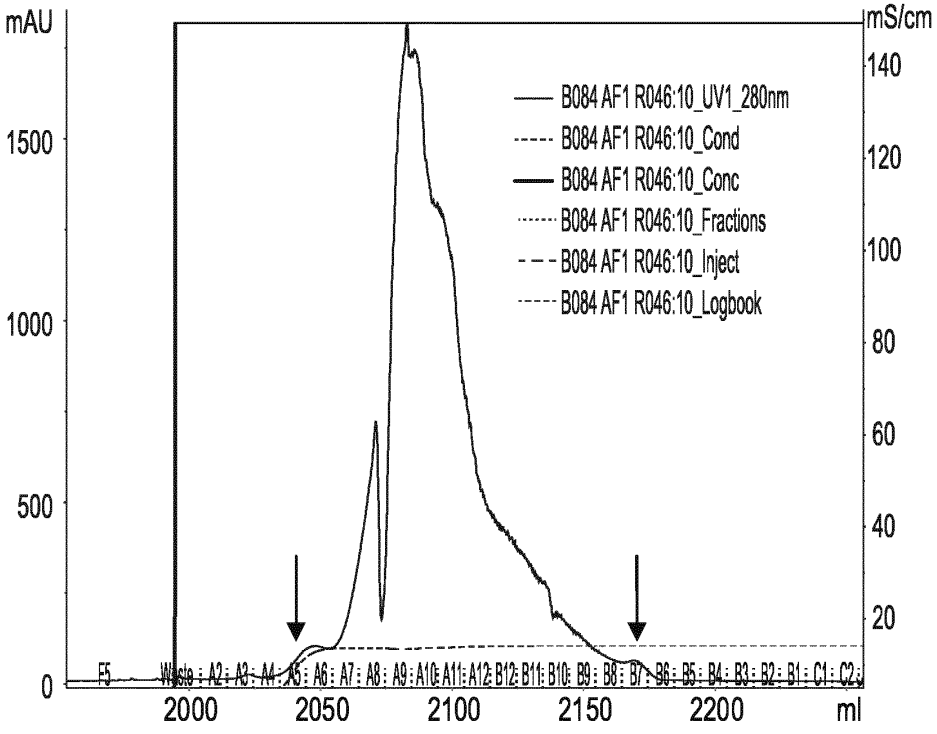
1500
1000
500
0
mS/cm
140
120
100
80
60
40
20
B084 AF1 R044:10_UV1_280nm
B084 AF1 R044:10_Cond
B084 AF1 R044:10_Conc
B084 AF1 R044:10_Fractions
B084 AF1 R044:10_Inject
B084 AF1 R044:10_Logbook
2000
2050
2100
2150
2200
ml

FIG. 7A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1500
1000
500
0
mS/cm
140
120
100
80
60
40
20
B084 AF1 R046:10_UV1_280nm
B084 AF1 R046:10_Cond
B084 AF1 R046:10_Conc
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2000
2050
2100
2150
2200
ml

FIG. 7B