Figure 1

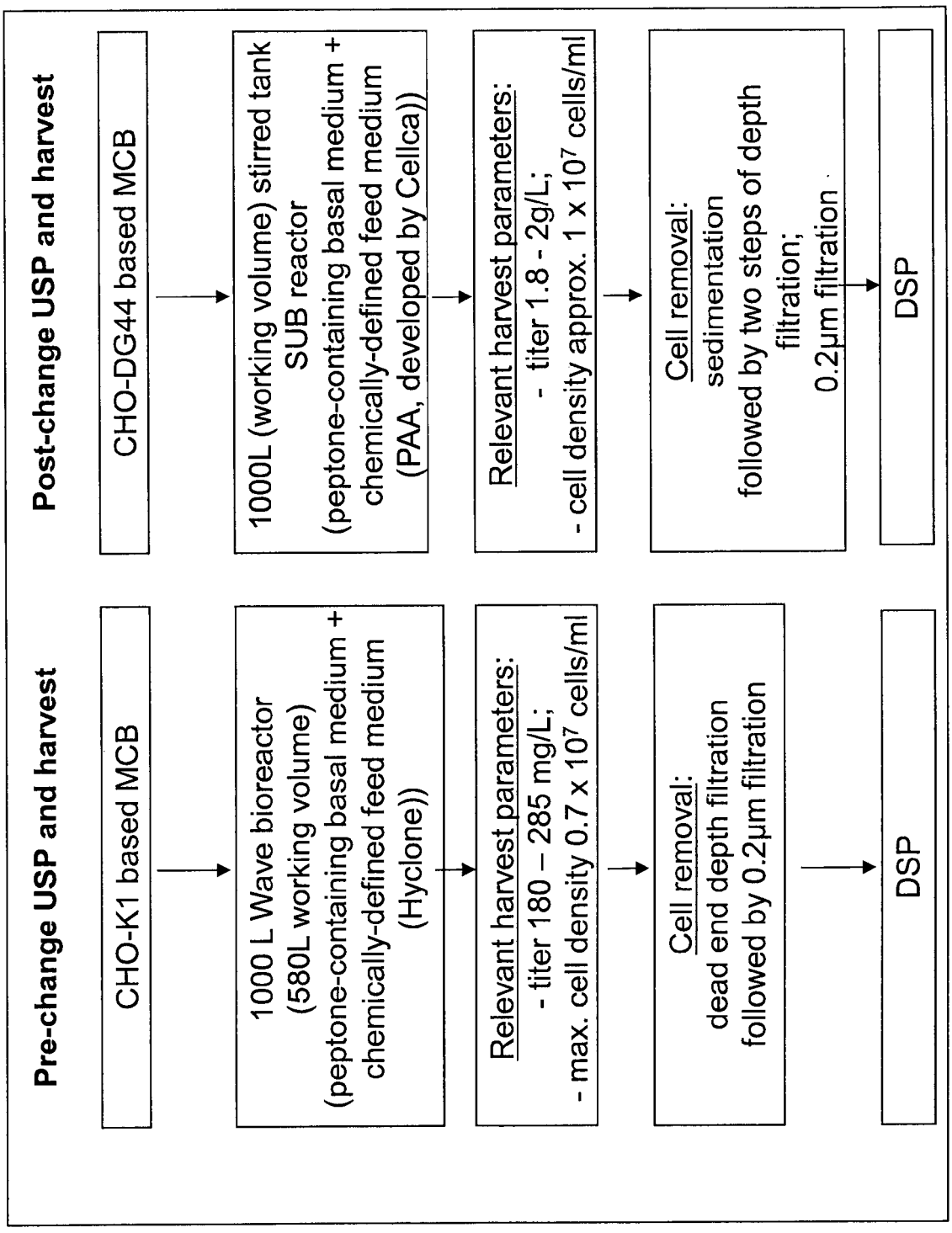

Pre-change USP and harvest
CHO-K1 based MCB
1000 L Wave bioreactor (580L working volume) (peptone-containing basal medium + chemically-defined feed medium (Hyclone))
Relevant harvest parameters: - titer 180 – 285 mg/L; - max. cell density 0.7 x 10^7 cells/ml
Cell removal: dead end depth filtration followed by 0.2µm filtration
DSP
Post-change USP and harvest
CHO-DG44 based MCB
1000L (working volume) stirred tank SUB reactor (peptone-containing basal medium + chemically-defined feed medium (PAA, developed by Cellca))
Relevant harvest parameters: - titer 1.8 - 2g/L; - cell density approx. 1 x 10^7 cells/ml
Cell removal: sedimentation followed by two steps of depth filtration; 0.2µm filtration
DSP

Figure 2

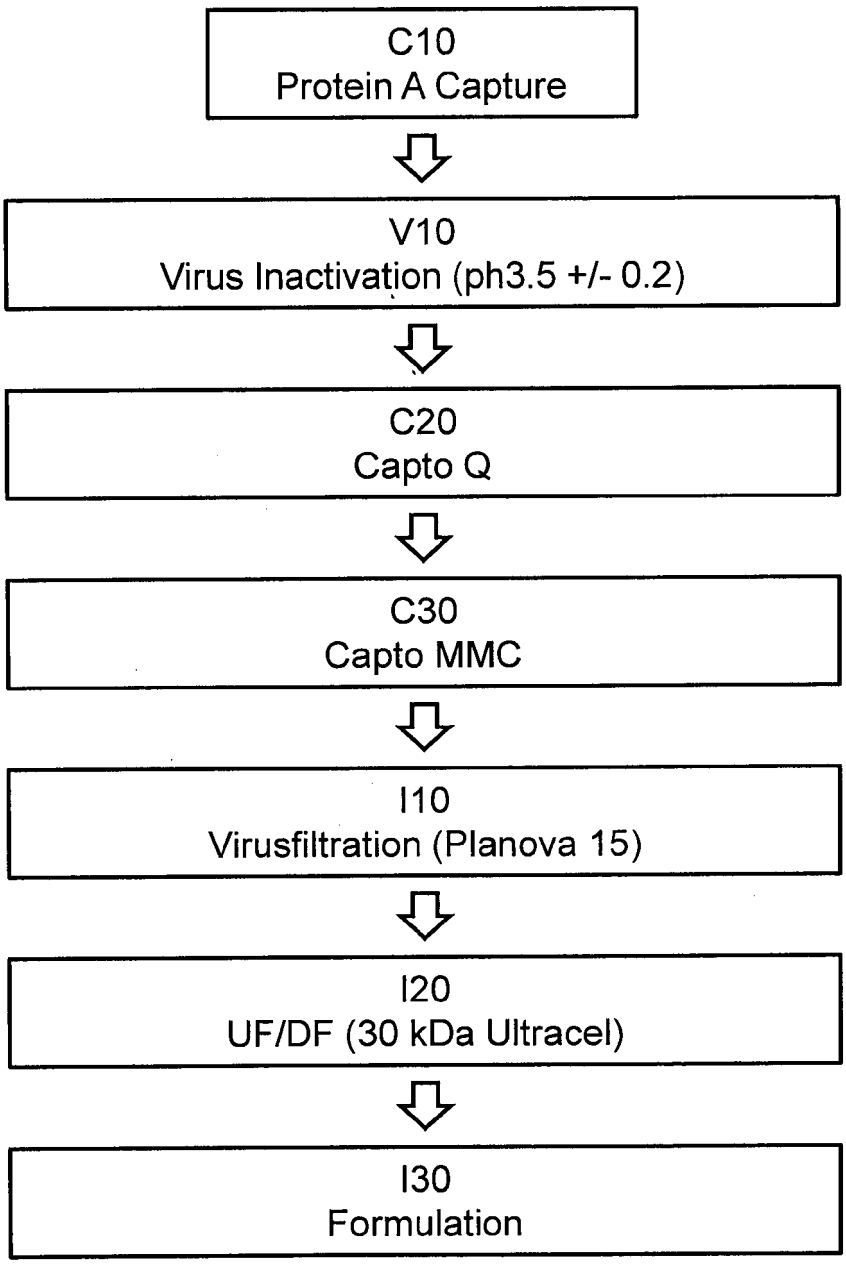

C10
Protein A Capture
V10
Virus Inactivation (ph3.5 +/- 0.2)
C20
Capto Q
C30
Capto MMC
I10
Virusfiltration (Planova 15)
I20
UF/DF (30 kDa Ultracel)
I30
Formulation

Figure 3

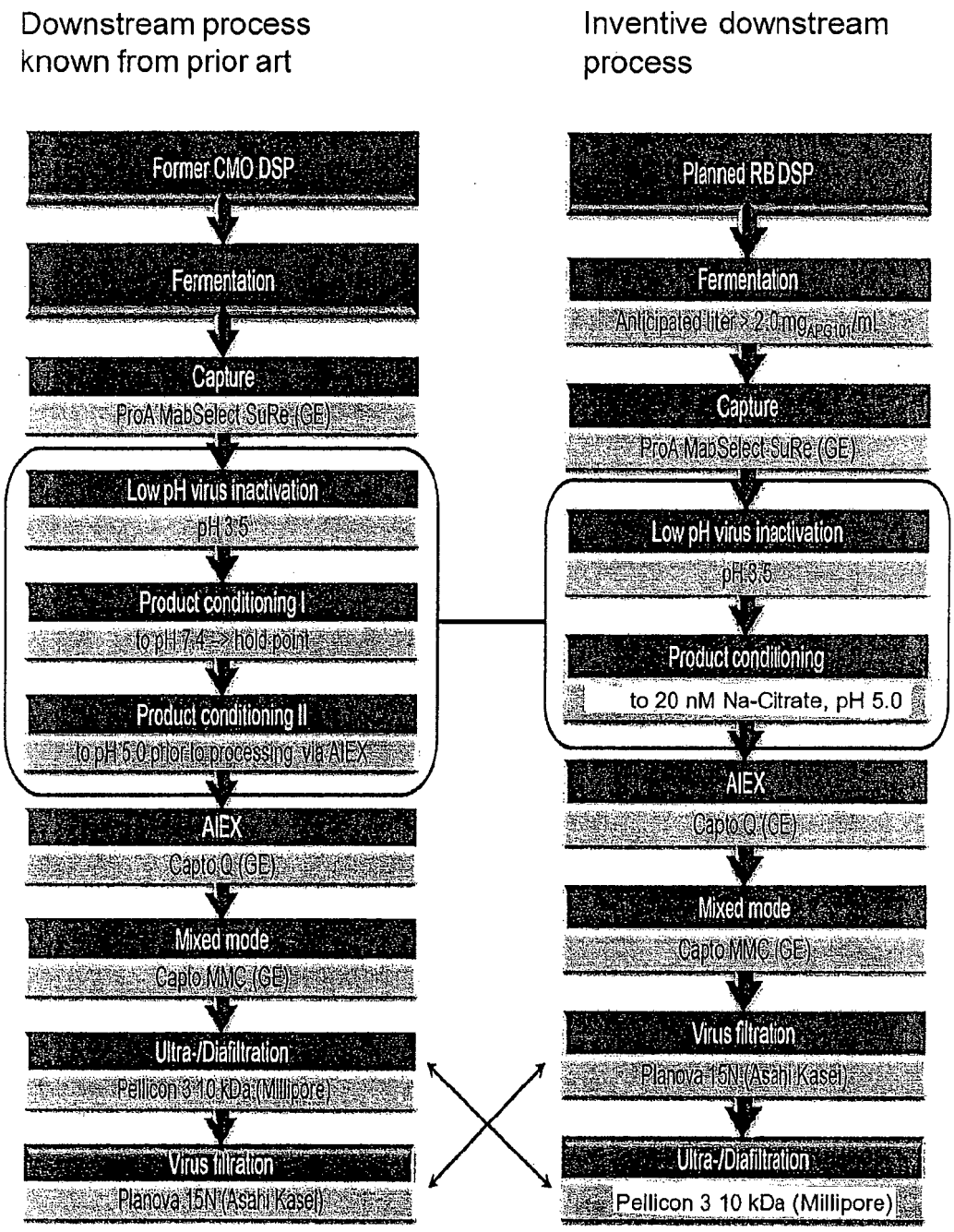

Downstream process known from prior art
Former CMO DSP
Fermentation
Capture
ProA MabSelect SuRe (GE)
Low pH virus inactivation
pH 3.5
Product conditioning I
to pH 7.4 -> hold point
Product conditioning II
to pH 5.0 prior to processing via AIEX
AIEX
Capto Q (GE)
Mixed mode
Capto MMC (GE)
Ultra-/Diafiltration
Pellicon 3 10 kDa (Millipore)
Virus filtration
Planova 15N (Asahi Kasei)
Inventive downstream process
Planned RB DSP
Fermentation
Anticipated titer > 2.0 mg$_{APG101}$/mL
Capture
ProA MabSelect SuRe (GE)
Low pH virus inactivation
pH 3.5
Product conditioning
to 20 nM Na-Citrate, pH 5.0
AIEX
Capto Q (GE)
Mixed mode
Capto MMC (GE)
Virus filtration
Planova 15N (Asahi Kasei)
Ultra-/Diafiltration
Pellicon 3 10 kDa (Millipore)

Figure 4a

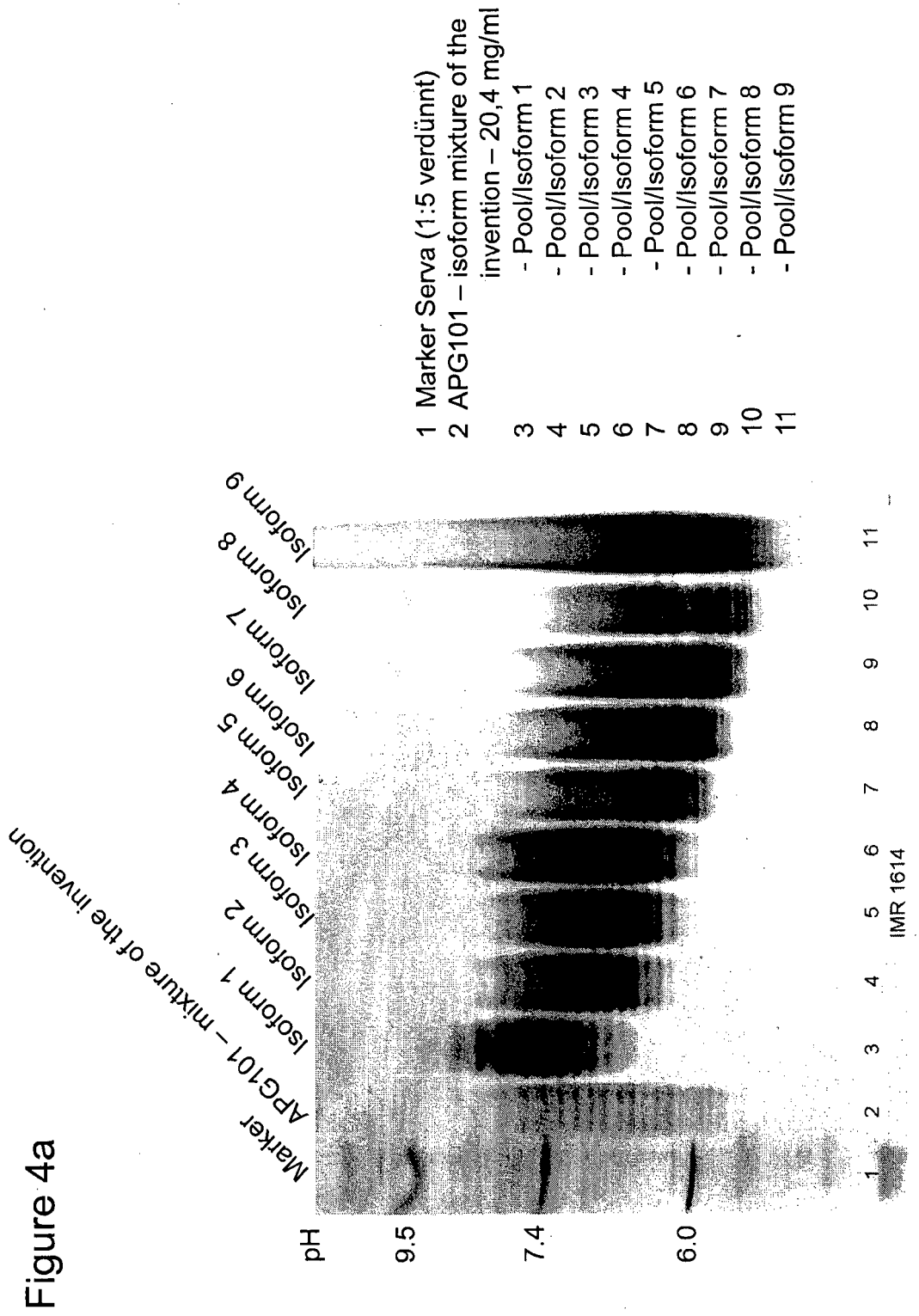

1 Marker Serva (1:5 verdünnt)
2 APG101 – isoform mixture of the invention – 20,4 mg/ml
3 - Pool/Isoform 1
4 - Pool/Isoform 2
5 - Pool/Isoform 3
6 - Pool/Isoform 4
7 - Pool/Isoform 5
8 - Pool/Isoform 6
9 - Pool/Isoform 7
10 - Pool/Isoform 8
11 - Pool/Isoform 9
pH
9.5
7.4
6.0
1 2 3 4 5 6 7 8 9 10 11
IMR 1614
Marker
APG101 – mixture of the invention
isoform 1
isoform 2
isoform 3
isoform 4
isoform 5
isoform 6
isoform 7
isoform 8
isoform 9

Figure 4b

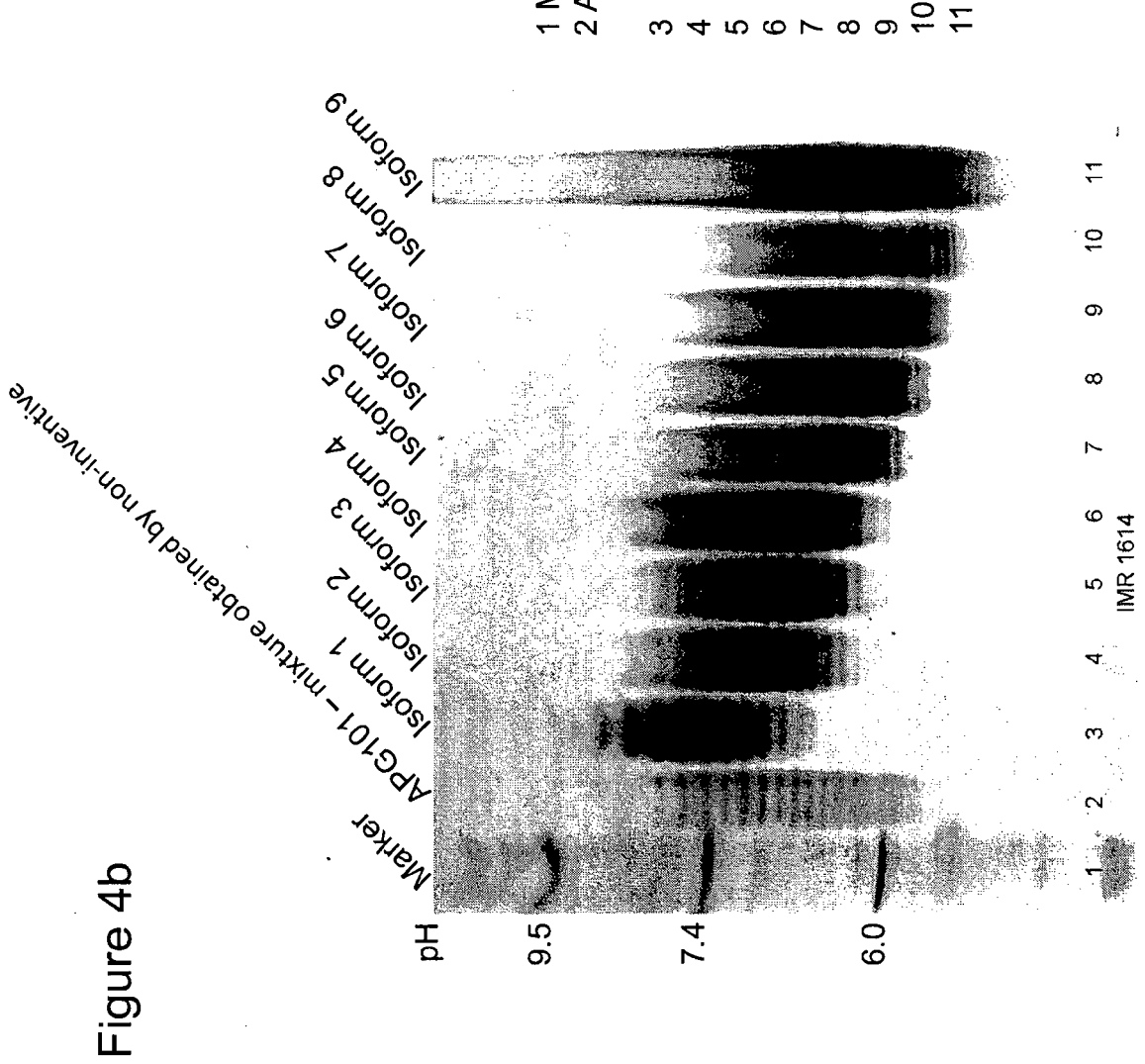
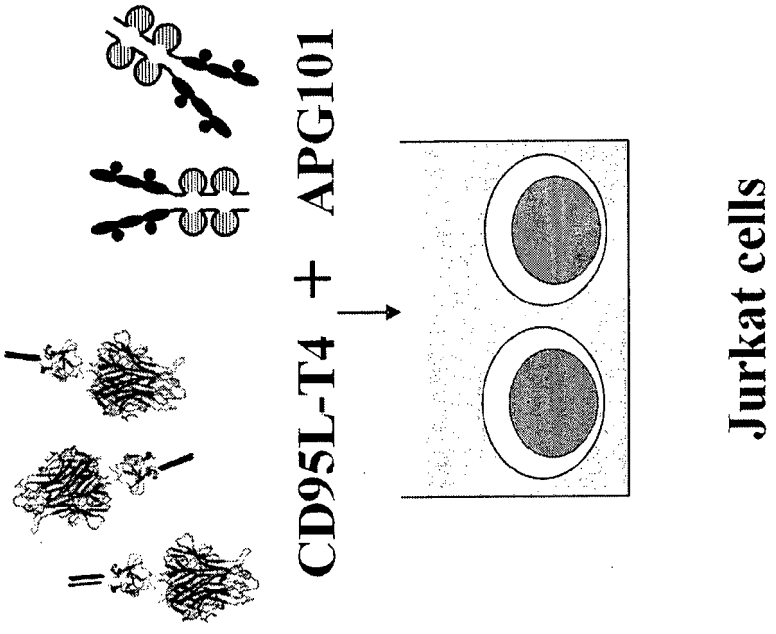

1 Marker Serva (1:5 verdünnt)
2 APG101 – mixture obtained by non-inventive method
3 – Pool / Isoform 1
4 – Pool / Isoform 2
5 – Pool / Isoform 3
6 – Pool / Isoform 4
7 – Pool / Isoform 5
8 – Pool / Isoform 6
9 – Pool / Isoform 7
10 – Pool / Isoform 8
11 – Pool / Isoform 9
pH
9.5
7.4
6.0
1 2 3 4 5 6 7 8 9 10 11
IMR 1614
Marker
APG101 – mixture obtained by non-inventive
Isoform 1
Isoform 2
Isoform 3
Isoform 4
Isoform 5
Isoform 6
Isoform 7
Isoform 8
Isoform 9

Figure 5



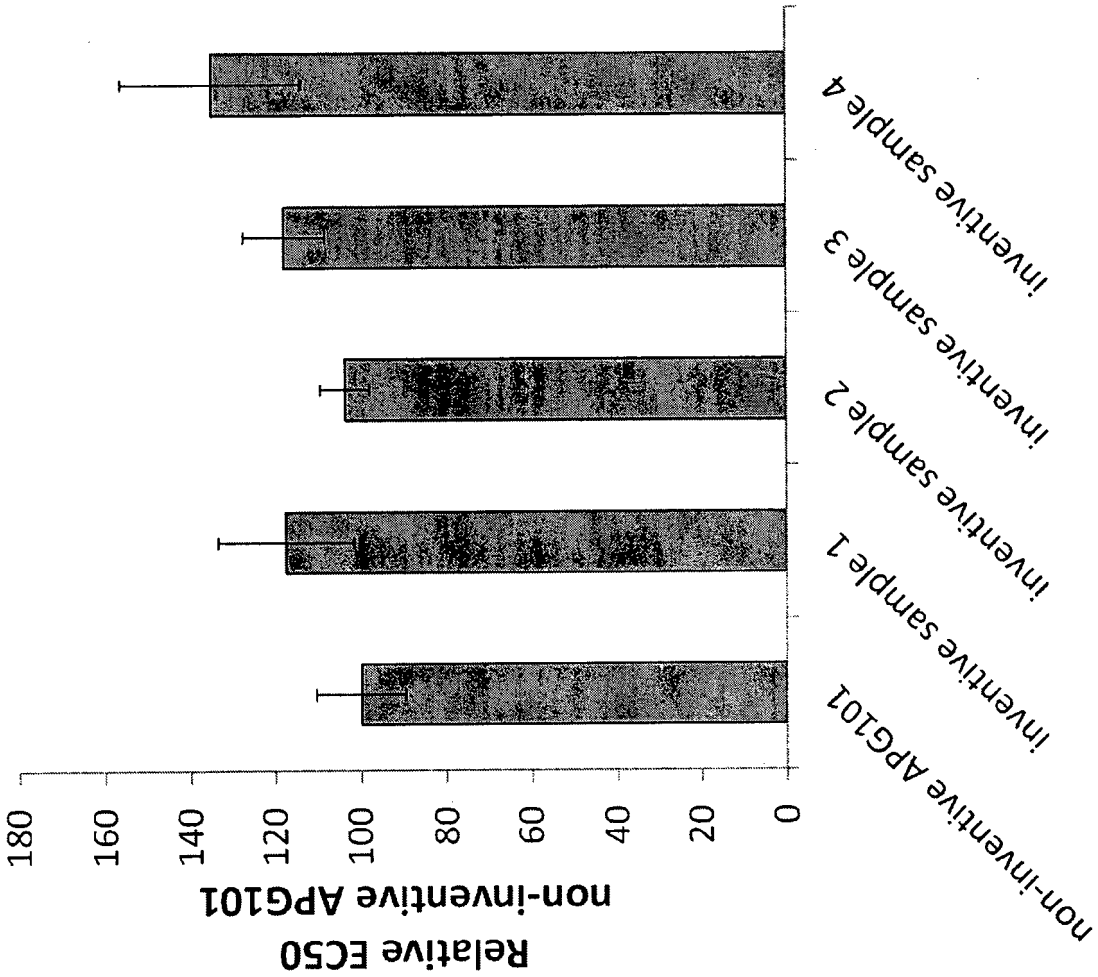

Relative EC50
non-inventive APG101
180
160
140
120
100
80
60
40
20
0
non-inventive APG101
inventive sample 1
inventive sample 2
inventive sample 3
inventive sample 4

Figure 6

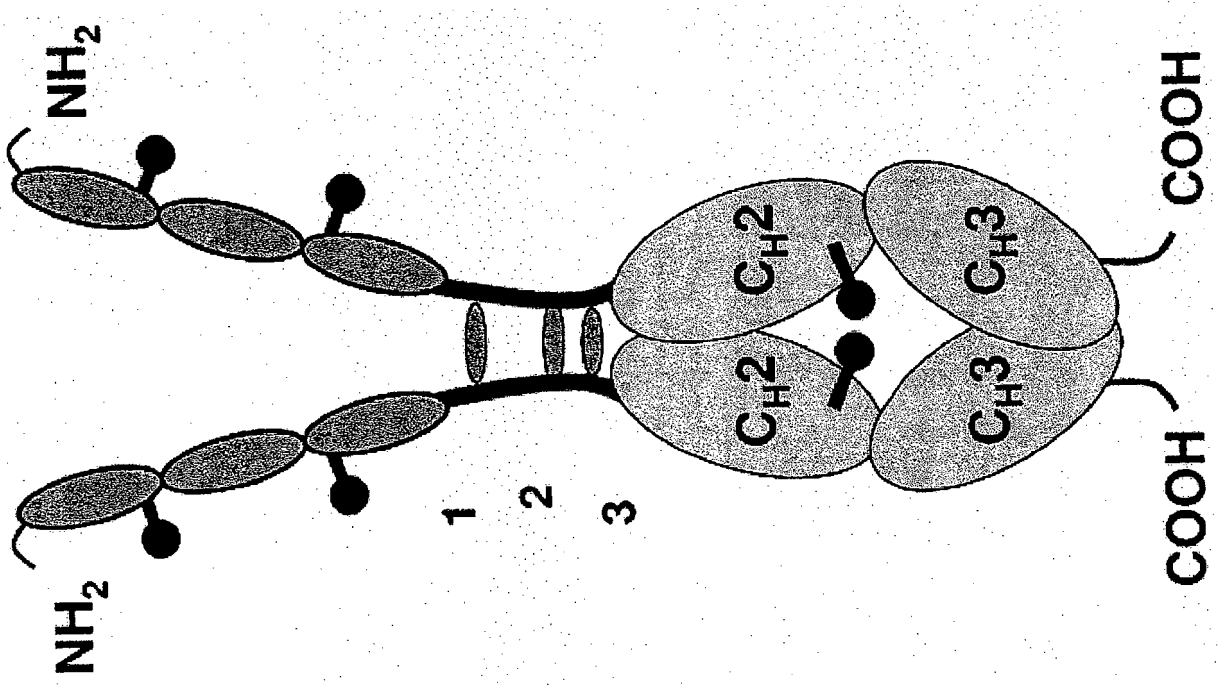


Figure 7

Figure 8

Buffer		pH 7.5	pH 7.0	pH 6.5	pH 6.0	PBS
Histidine / HCl	50 mM	67.3	67.0	66.5	N/A	67.1
	30 mM	66.9	66.9	66.2		
	10 mM	66.9	66.8	66.4		
	5 mM	N/A	66.9	66.5		
50 mM Histidine	25 mM NaCl	67.1	67.1	66.2	N/A	
	50 mM NaCl	67.5	67.0	66.2		
	100 mM NaCl	67.7	67.0	66.3		
L-Histidine / Phosphate	50 mM	67.3	67.0	66.5	N/A	
	30 mM	66.9	66.9	66.2		
	10 mM	66.9	66.8	66.4		
	5 mM	N/A	66.9	66.5		
Na-Acetate	150 mM	67.3	67.5	67.6	N/A	67.3
	50 mM	67.3	67.4	67.5		
	30 mM	67.2	67.4	67.3		
	10 mM	66.9	67.1	67.2		
	5 mM	67.1	67.1	66.9		
Na-Phosphate	50 mM	67.3	67.5	67.5	67.0	67.1
	30 mM	67.4	67.5	67.3	67.0	
	10 mM	67.4	67.3	67.2	66.9	
	5 mM	67.3	67.3	67.1	66.9	
Na- Succinate	50 mM	67.5	67.5	67.4	N/A	67.3
	30 mM	67.5	67.5	67.5		
	10 mM	67.4	67.4	67.3		
	5 mM	67.5	67.5	67.3		
Arginine / Phosphate	50 mM	67.3	67.3	67.2	N/A	
	30 mM	67.3	67.4	67.4		
	10 mM	67.4	67.3	67.4		
	5 mM	67.4	67.5	67.3		
Na-Citrate	50 mM	N/A				67.3
	30 mM	67.0	67.3	66.8	66.1	
	10 mM	67.3	67.5	67.2	66.9	
	5 mM	67.2	67.1	67.1	66.9	

Figure 9

Excipients		Saccharose		Sorbitole		Glycine		Gly-Gly		PEG 6000	
concentration	0.0 %	2.5 %	5.0 %	2.5 %	5.0 %	125 mM	250 mM	5.0 %	10.0 %	2.5 %	5.0 %
150 mM Na-Acetate, pH 6.5	66.6	68.1	N/A	68.1	N/A	67.7	N/A	66.4	N/A	67.3	N/A
50 mM Histidine / 100 mM NaCl, pH 7.5	67.5	68.1	68.3	68.1	68.7	67.9	68.1	66.7	66.4	67.3	66.7
30 mM Na-Succinate, pH 7.0	67.6	68.0	68.5	68.1	68.3	67.8	68.3	66.3	66.1	67.2	66.8
30 mM Na-Phosphate, pH 6.5	67.3	67.8	68.3	67.7	68.3	67.6	68.0	66.7	66.1	67.5	67.2
50 mM Na-Phosphate, pH 6.5	66.7	67.8	68.3	67.9	68.5	67.9	68.1	67.1	66.7	67.5	67.3

Figure 10

	Buffer	SE-HPLC (%)			MFI	SDS-PAGE. red.	IEF
		Aggregates	Homodimer	Fragments	Particles (#/mL)	Colloidal blue	
1	PBS (unstressed control)	4.98	95.02	n.d.	15011		
2	50 mM Histidine, 100 mM NaCl, 5.0 % Sorbitole, pH 7.5	4.13	94.48	1.39	13432	-	-
3	50 mM Histidine, 100 mM NaCl, 5.0 % Sorbitole, 20 µM EDTA, pH 7.5	4.17	94.55	1.28	7051	-	-
4	30 mM Na-Phosphate, 5 % Saccharose, pH 6.5	6.18	92.85	0.96	5386	-	+
5	30 mM Na-Phosphate, 5 % Sorbitole, pH 6.5	5.95	93.22	0.83	9769	+	+
6	50 mM Na-Phosphate, 5 % Sorbitole, pH 6.5	5.72	93.43	0.85	7056	+	+
7	50 mM Na-Phosphate, 250 mM Glycine, pH 6.5	5.08	94.24	0.68	8629	+	-

SUBSTITUTE SHEET (RULE 26)

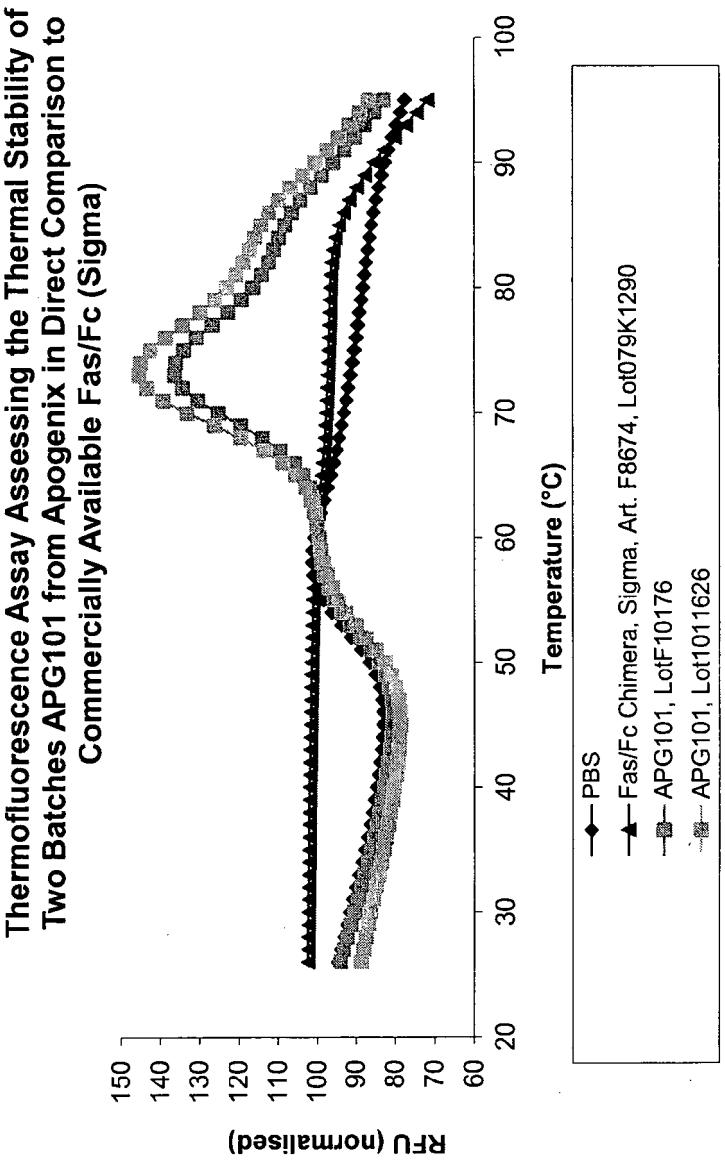

Thermofluorescence Assay Assessing the Thermal Stability of Two Batches APG101 from Apogenix in Direct Comparison to Commercially Available Fas/Fc (Sigma)
RFU (normalised)
150
140
130
120
110
100
90
80
70
60
20
30
40
50
60
70
80
90
100
Temperature (°C)
PBS
Fas/Fc Chimera, Sigma, Art. F8674, Lot079K1290
APG101, LotF10176
APG101, Lot1011626

Figure 11