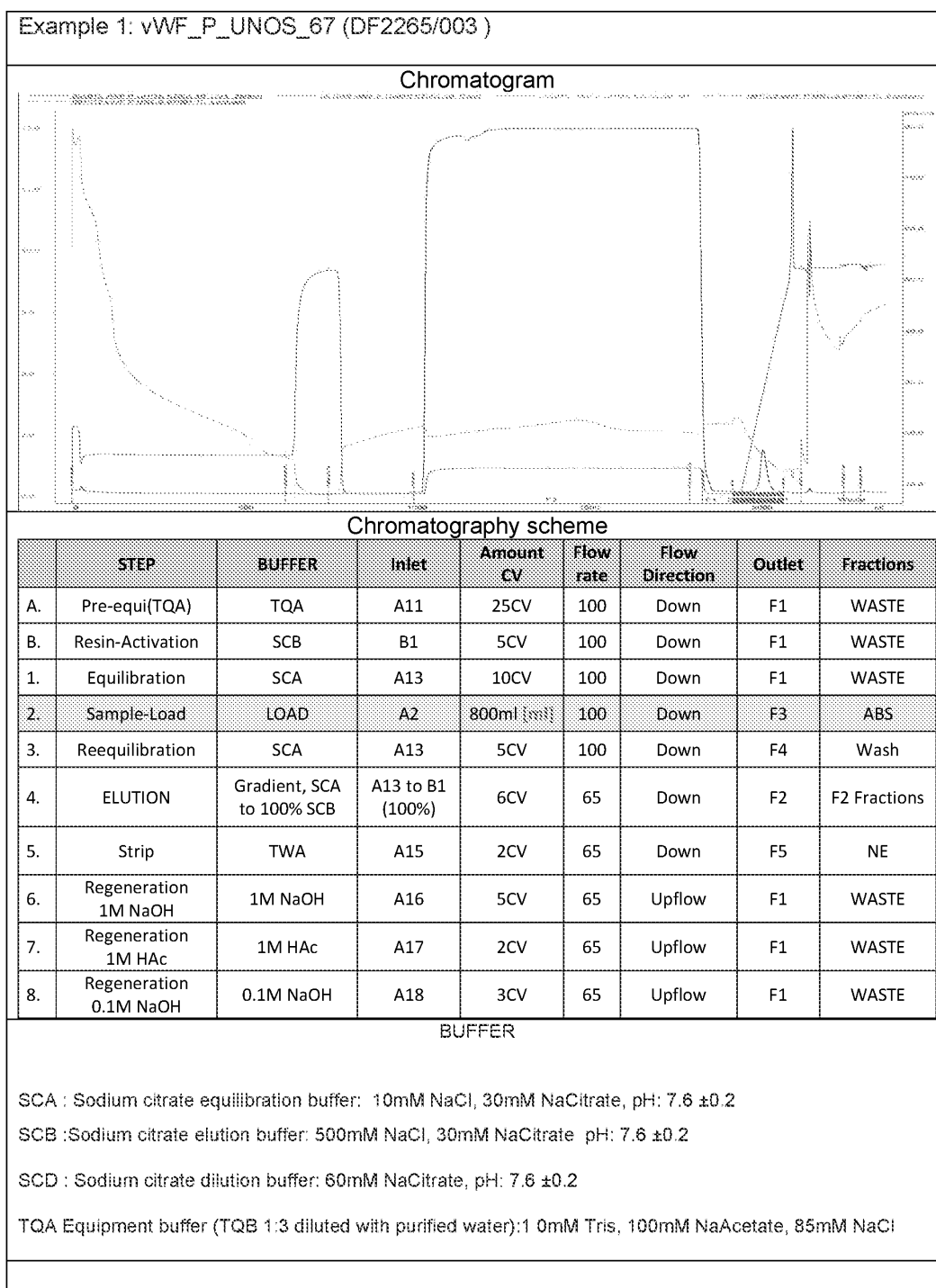


FIG. 1



YIELD

		vWF AG ELISA SOP: OR-13-00127				vWF-Riisto SOP: OR13-00427 (HN22 Standard)			
Sample Code	Amount [g]	Conc. [µg/mL]	Total [µg]	Total [U]	Yield LOAD [%]	Riisto [U/mL]	Total [U]	spec. Akt. (U Riisto/Ag)	Yield LOAD [%]
LOAD	817.10	77	62 916.70	6 291.67	100.0%	2.55	2053.61	0.33	100%
ELUATE Pool	58.17	211.5	12 302.96	1 230.30	10.6%	15.6	907.45	0.74	43.6%

		CHO AG ELISA BOP GR-13-00467				VWF-prope and AG ELISA BOP GR-13-00468			
Sample Code	Amount [g]	CHO-HCP [µg/ml]	Total [µg]	Purity [µg/10000 Ratio]	Yield [%]	Conc [µg/mL]	Total [µg]	Yield [%]	
LOAD	817.10	1.70	1391.223	667.7	100%	12.098	9.885.28	100%	
ELUATE Pool	58.17	0.38	22.317	24.6	1.60%	0.071	4.13	0.04%	

FIG. 3

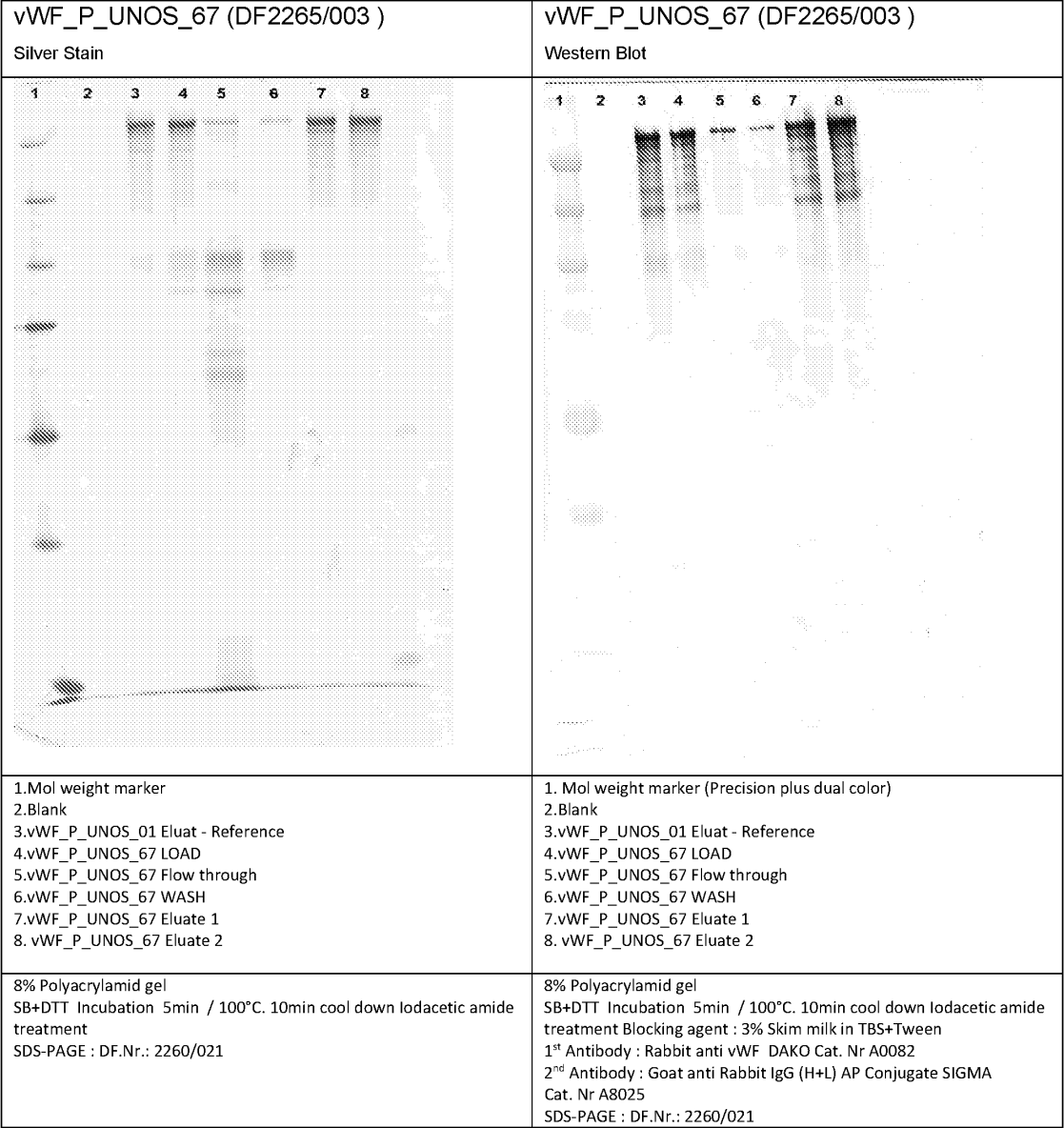


FIG. 4

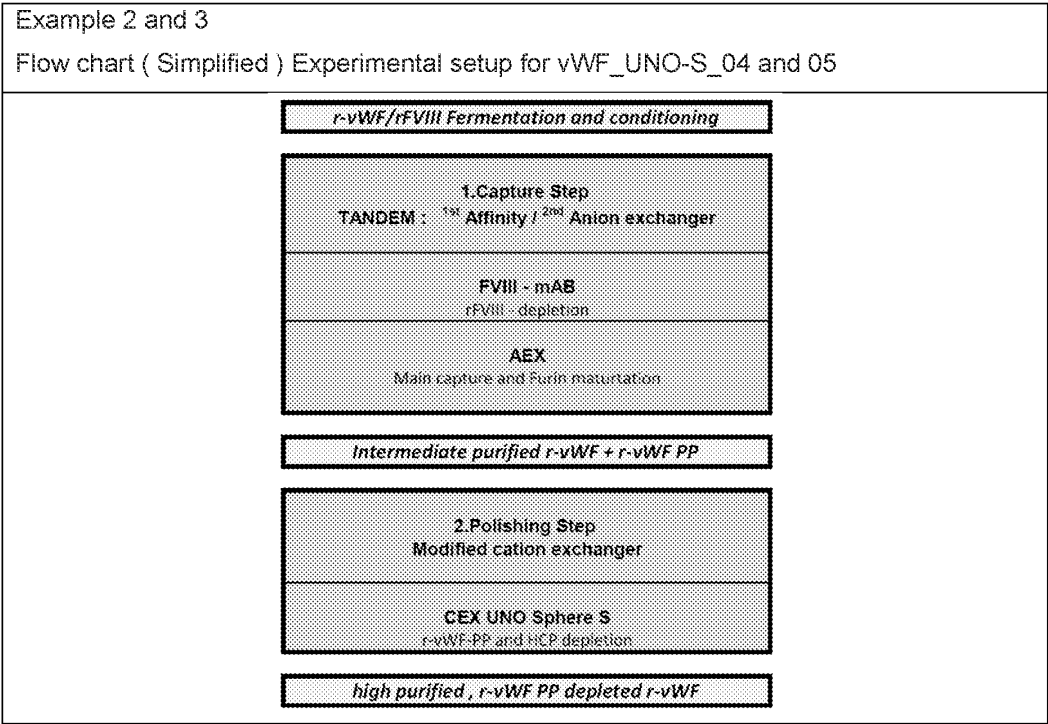


FIG. 5

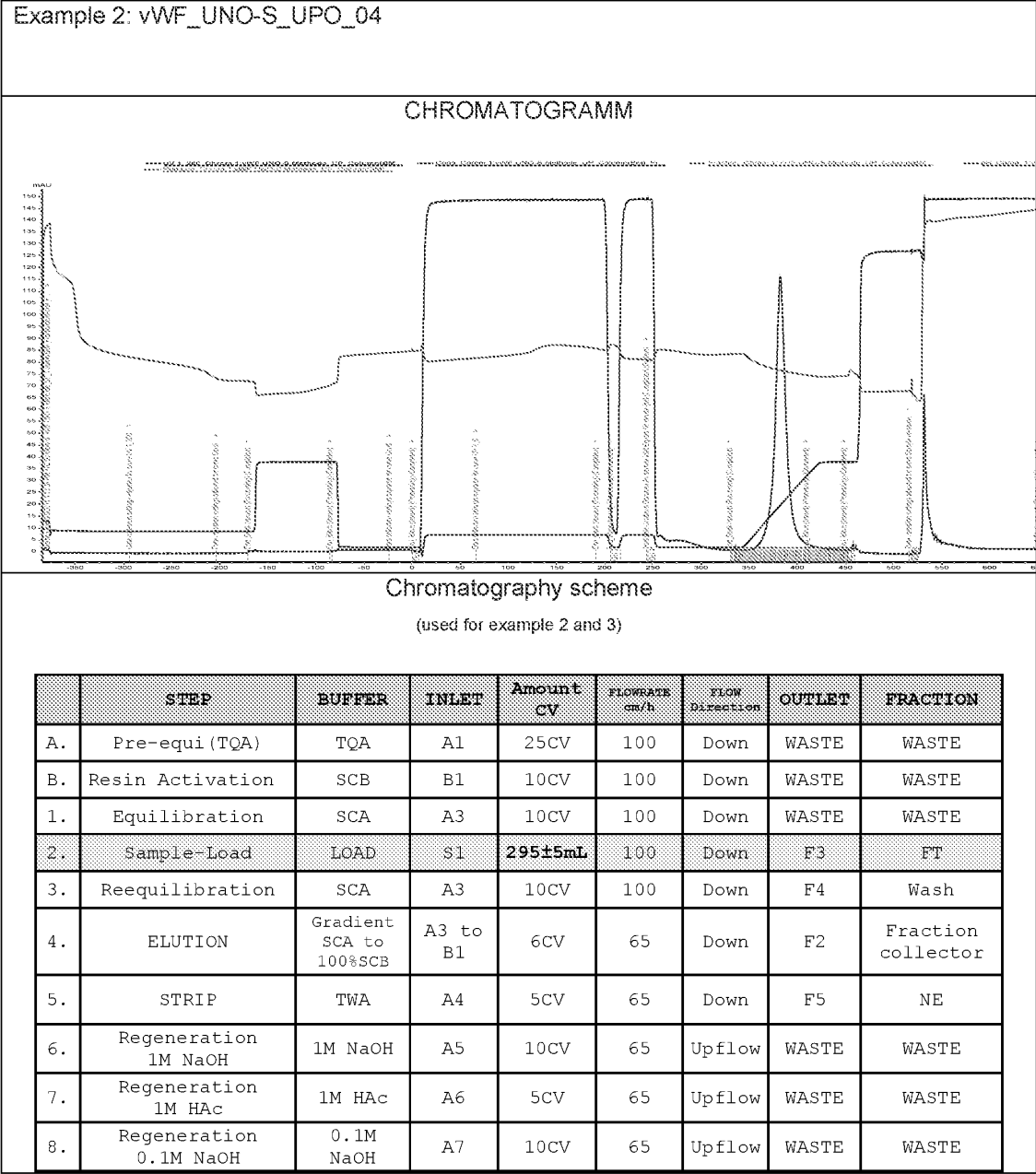


FIG. 6

BUFFER

<u>Na Citrate Dilution buffer</u> 10mM Na Citrate pH: 7.574 at 22.7°C C: 2.56 mS/cm at 22.6°C Lot.: Reag_RCF_14/047, 08.07.2014 Exp date: 08.07.2015	<u>TWA Puffer</u> 2M NaCl pH: 6.452 at 21.9°C C: 158.6 mS/cm at 21.8°C Lot.: Reag_RCF_14/052, 08.07.2014 Exp date: 08.07.2015
<u>Na Citrate Equilibrationbuffer</u> 10mM NaCl, 30mM Na Citrate, 2mM Citric acid pH: 7.559 at 22.9°C C: 7.71 mS/cm at 22.9°C Lot.: Reag_RCF_14/046, 08.07.2014 Exp date: 08.07.2015	<u>1M NaOH</u> Lot.: ORMPSHD14504 Exp date: 31.08.2014
<u>Na Citrate Elution buffer</u> 500mM NaCl, 30mM Na Citrate pH: 7.593 at 21.9 °C C: 51.0 mS/cm at 21.8°C Lot.: Reag_RCF_14/048, 08.07.2014 Exp date: 08.07.2015	<u>1M HAc</u> Lot.: ORMPEB14501 Exp date: 20.07.2014
<u>TQA Buffer (TQB 1:3 diluted with purified water):</u> 10mM Tris, 100mM Na Acetate, 85mM NaCl pH: 6.547 at 22.5°C C: 15.84 mS/cm at 22.4°C Lot.: Reag_RCF_14/051,09.07.2014 Exp date: 08.07.2015	<u>0.1M NaOH</u> Lot.: Reag_RCF_14/049 Exp date: 09.07.2015

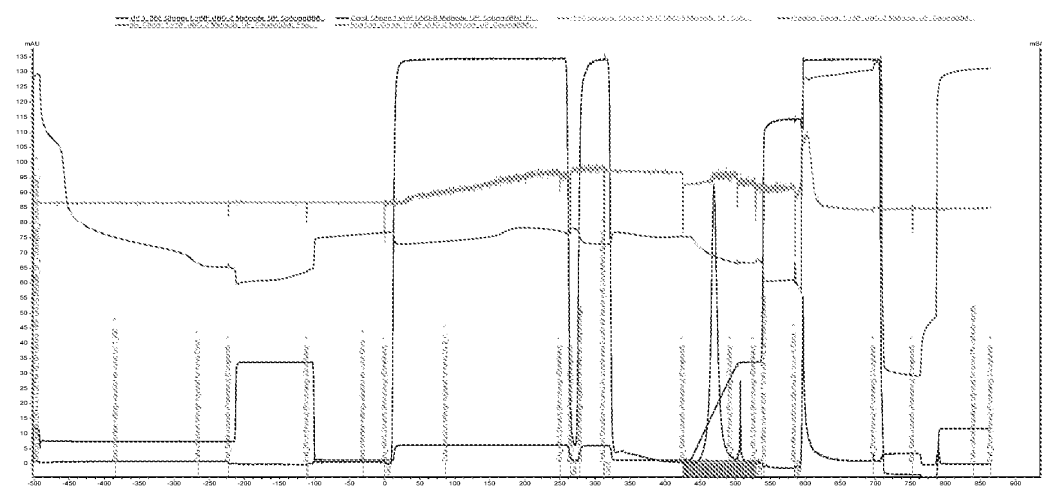
YIELD

vWF AG ELISA SOP: OR-13-00127						vWF-Risto SOP: OR13-00407		
Sample Code	Amount [g]	Conc [g/mL]	Total [g]	Total [g]	Yield LOAD [%]	Risto [U/mL]	Total [g]	spec. Akt. [U Risto/U Ag]
LOAD	296.36	51	14.956,18	1.456,62	100,0%			
ELUAT (Pool)	16.00	514,0	8.224,00	822,40	55,0%	23,7	378.58	0,46

CHO AG ELISA SOP: OR-13-00437						vWF-propeptid AG ELISA SOP: OR-13-00405		
Sample Code	Amount [g]	CHO-HCP [µg/mL]	Total [µg]	Purity [µg/1000U Risto]	Yield [%]	Conc. [µg/mL]	Total [µg]	Yield [%]
LOAD	296,36	5,28	1563,406		100%	13,039	3.864,12	100%
ELUAT (Pool)	16,00	1,33	21,200	56,003	1,36%	0,564	9,02	0,23%

FIG. 7

Chromatogram



YIELD

		vWF AG ELISA SOP: CR-13-00127				vWF-Risto SOP: CR13-00407		
Sample Code	Amount [g]	Conc [µg/mL]	Total [µg]	Total [U]	Ausbeute LOAD [%]	Risto [U/mL]	Total [U]	spez. Act. [U Risto/U AG]
LOAD	302.31	39	11.760,09	1.175,01	100,0%			
ELUAT 2	16,00	443,0	7.088,00	708,80	60,1%	19,7	314,72	0,44

Sample Code	Amount [g]	CHO AG ELISA SOP: OR-13-00497				VWF-propeptid AG ELISA SOP: OR-13-00405			
		CHO [µg/ml]	Total [µg]	Purity [µg/1000U Risto]	Ausbeute [%]	Conc [µg/mL]	Total [µg]	Yield [%]	
LOAD	302,31	2.76	834,339		100%	8,801	2.660,69	100%	
ELUAT 2	16.00	0.75	12,075	38,367	1,45%	0,416	6.65	0,25%	

FIG. 8

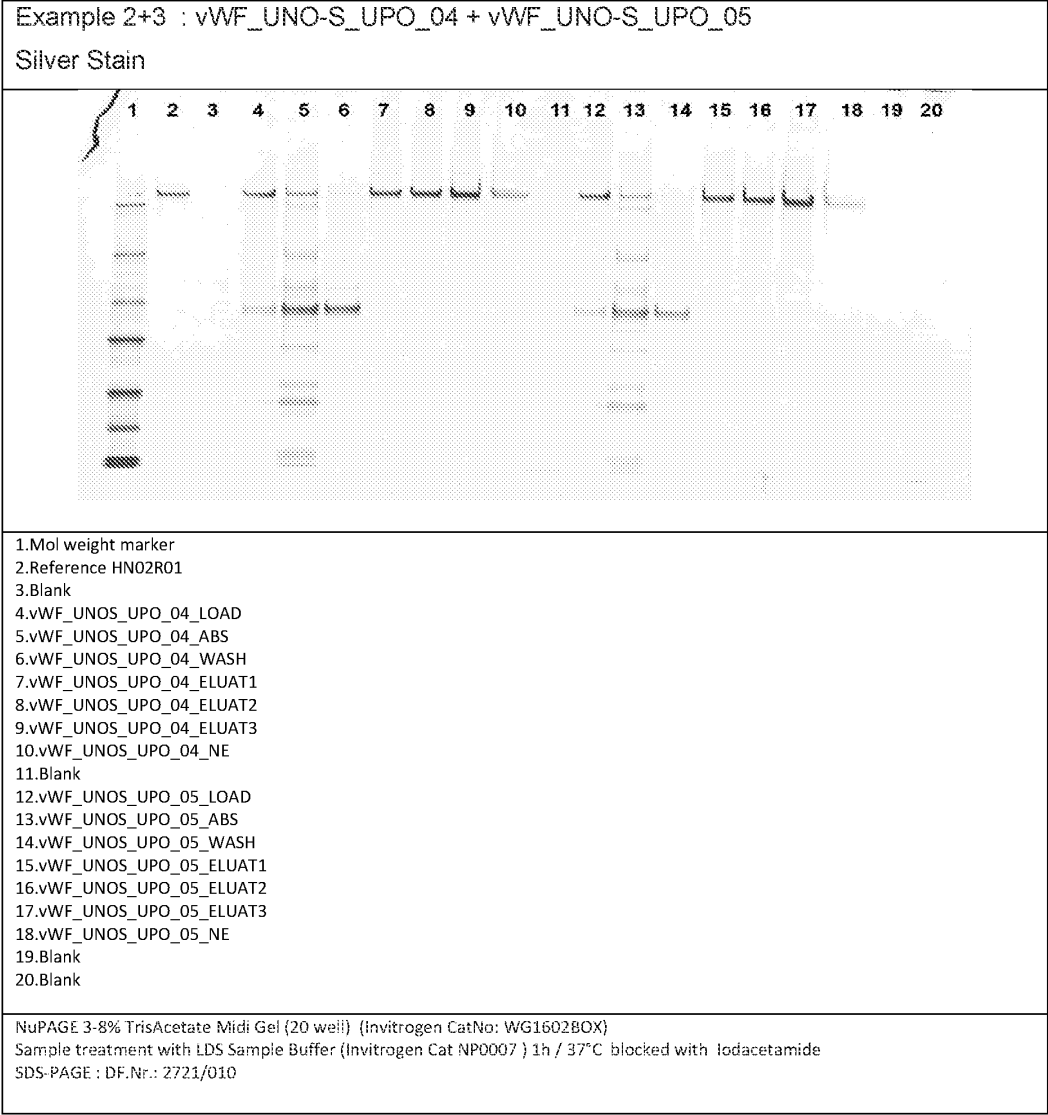


FIG. 9

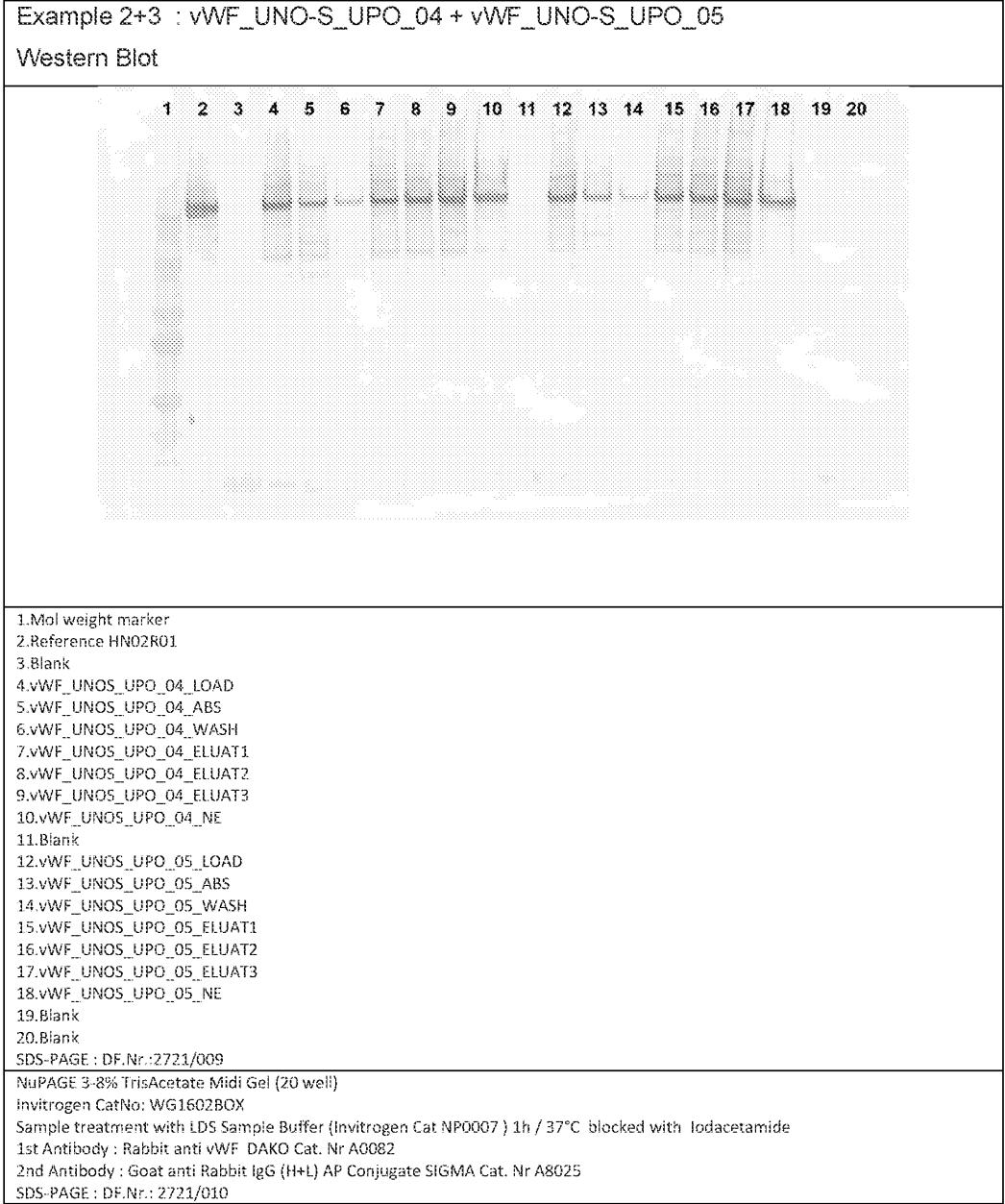


FIG. 10

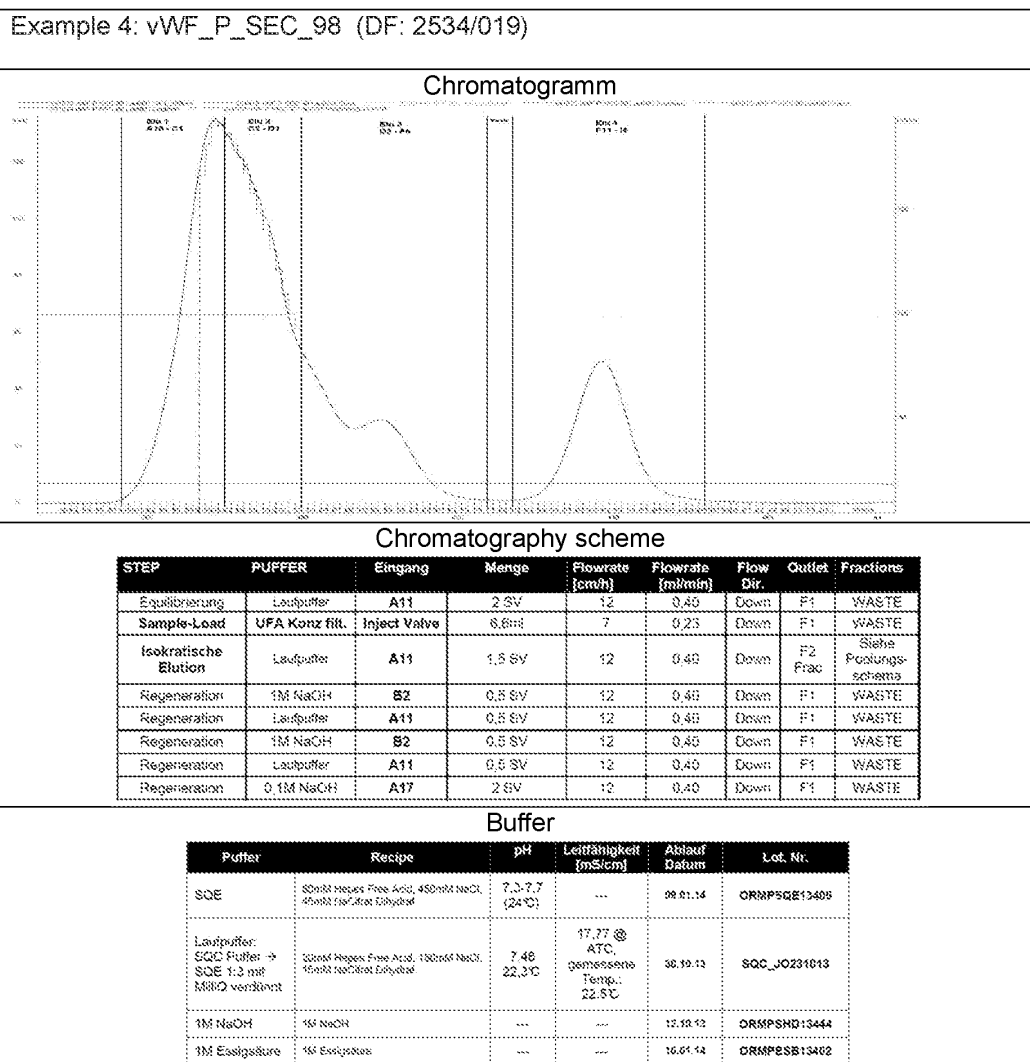


FIG. 11

Example 4 : vWF_P_SEC_98									
YIELD									
Sample Code	Amount [g]	vWF:Ag SOP: OR-13-00127				vWF-Risto (SHP Standard) SOP: OR-13-00407			
		Antigen [µg/mL]	Total [µg]	Total [U]	Yield [%]	RiCoF [U/mL]	Total [U]	spec. Act. [U Risto/U Ag]	Yield [%]
ORUFA12016RF	6,60	2416,6667	15950,00	1595		136,499	901	0,56	
LOAD	6,60	2350	15510	1551	100,0%	140,097	925	0,60	100,0%
ELU1 A10-C1	12,94	290	3752,6	375,26	24,2%	37,239	482	1,28	52,1%
ELU2 C2-D1	9,51	605	5753,55	575,355	37,1%	31,639	301	0,52	32,5%
ELU3 D2-F6	23,77	185	4397,45	439,745	28,4%	1,677	40	0,09	4,3%
ELU4 F11-I4	24,73	2,78	68,7494	6,87494	0,4%	<0,50	<12,365	<1,80	<1,3%

Sample Code	Amount [g]	CHO AG ELISA SOP: OR-13-B0487				vWF propeptide ELISA SOP: OR-13-00405			
		CHO [ng/mL]	Total [ng]	Yield [%]	Purity [µg/1000U Risto]	propeptide [µg/mL]	Total [µg]	Yield [%]	PP/vWF [µg/mg vWF] µg/1000U Risto
ORUFA12016RF	6,60								
LOAD	6,60	12753,46	84172,836	100%	91,03	288,380	1903,308	100%	122,715 2058,431
ELU1 A10-C1	12,94	40,301	521,495	0,62%	1,08	<0,03125	<0,404	<0,02%	<0,108 <0,839
ELU2 C2-D1	9,51	167,03	1588,4553	2%	5,28	0,04320	0,411	0,02%	0,07 1,365
ELU3 D2-F6	23,77	539,72	12829,1444	15%	321,84	0,04320	1,026864	0,05%	0,234 25,760
ELU4 F11-I4	24,73	229,608	5676,20584	7%	459,22	56,3060	1392,44738	73,16%	20253,957 112612,000

FIG. 12

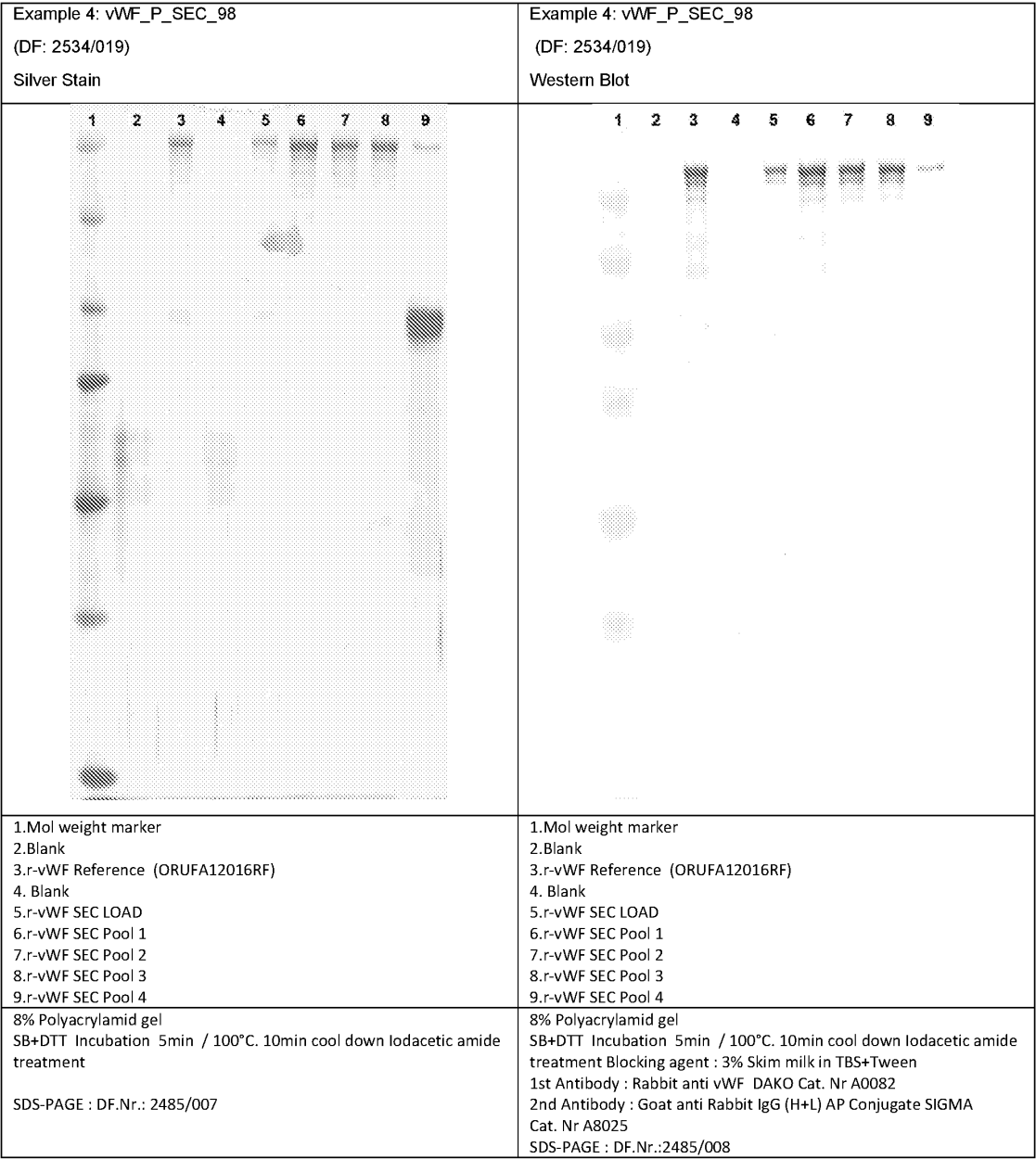


FIG. 13

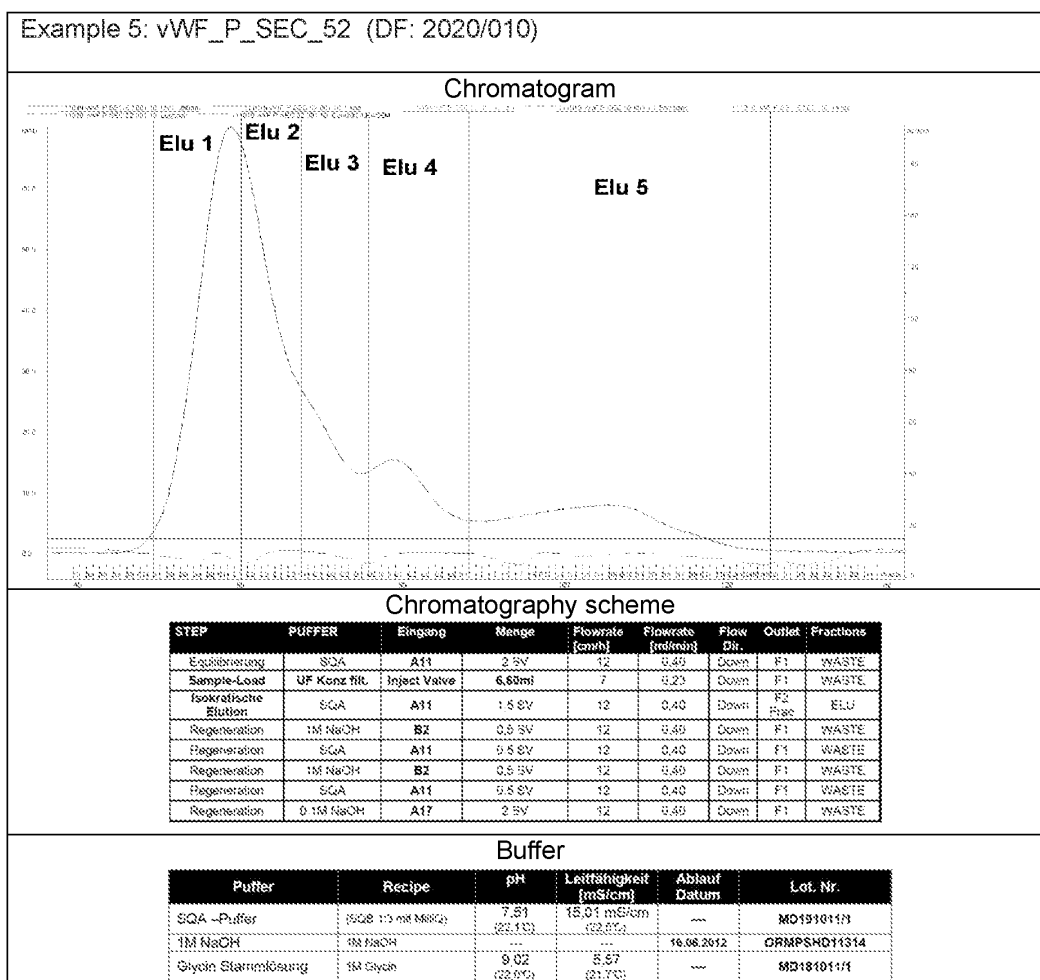


FIG. 14

YIELD												
Proben Code	Menge [g]	vWF:Ag SOP: OR-13-00127				vWF-Risto SOP: OR-13-00487				CHO Ag ELISA SOP: OR-13-00487		
		Aktivität [µg/mL]	Gesamt [µg]	Gesamt [U]	Ausbeute [%]	Risto [U/mL]	Gesamt [U]	spez. Akt. [U Risto/U Ag]	Ausbeute [%]	CHO [ng/mL]	Gesamt [ng]	Ausbeute [%]
LOAD_11002	6.60	1800	9600	560	100.0%	63.2	361	0.35	100.0%	1411.4	5315.24	100%
ELU1 A12-C1	10.93	186	2054.84	205.484	20.6%	15.96	174	0.85	49.6%	<31.250	<341.663	<3.7%
ELU2 C2-C10	5.74	440	2365.6	236.56	30.0%	11.70	79	0.27	22.6%	92.932	528.36168	7%
ELU3 C11-DB	7.86	184	1445.24	144.524	14.6%	1.38	11	0.03	3.1%	150.005	1450.4	16%
ELU4 D5-E12	12.60	87	1098.2	109.82	11.1%	0.25	3	0.03	0.9%	285.360	3658.01	39%
ELU5 F1-F8	34.77	1.95	67.8015	6.78015	0.7%	<0.19	<7	<1.03	<2.0%	84.305	2931.31562	31%
Wiederfindung					77.2%				78.1%			97%

Proben Code	Menge [g]	vWF propeptide ELISA SOP: OR-13-00400				F8 Ag ELISA SOP: OR-13-00408			
		propeptide [µg/mL]	Gesamt [µg]	Ausbeute [%]	µg/mg vWF	µg/1000U Risto	F8 [ng/mL]	Gesamt [ng]	FVIII Ag Gehalt µg/1000U Risto µg/mg vWF Ag
LOAD_11002	6.60	187.264	1235.9424	100%	124.843	3520.000			
ELU1 A12-C1	10.93	0.108	1.51	0.12%	0.73	5.679	160.60	1973.958	11.36
ELU2 C2-C10	5.74	2.768	19.66	1.51%	5.29	236.561	290.50	1957.970	24.83
ELU3 C11-DB	7.86	4.908	38.57688	0.12%	26.674	3688.824			
ELU4 D5-E12	12.60	10.628	133.5128	10.83%	122.161	42512.060			
ELU5 F1-F8	34.77	15.351	533.75427	43.13%	7672.366	80784.737			
Wiederfindung				58%					

Yellow marked: product containing fraction / No SDS-PAGE Available

FIG. 15

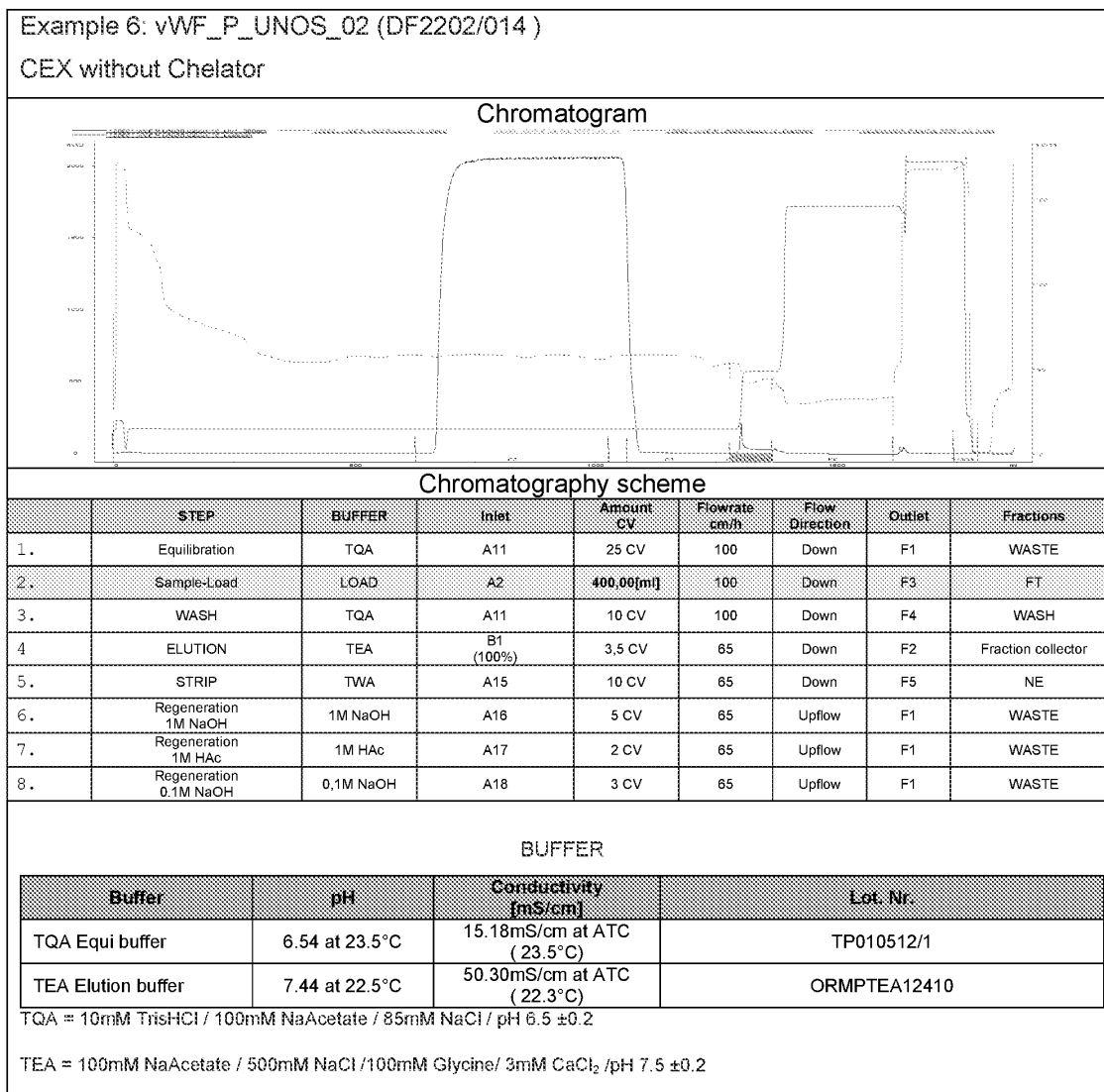


FIG. 16

Example 6: vWF_P_UNOS_02 (DF2202/014)
CEX without Chelator

YIELD
(vWF-Antigen / vWF Risto)

	vWF AG ELISA				vWF Risto			
	Konz. [µg/mL]	Gesamt [µg]	Ausbeute [%]	Yield [µg/µg]	Konz. [µg/mL]	Gesamt [µg]	Ausbeute [%]	Yield [µg/µg]
23. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
24. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
25. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
26. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
27. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
28. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
29. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
30. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
31. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
32. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
33. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
34. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00

(CHO AG ELISA/ Protein BCA / vWF-propeptid AG ELISA)

	CHO AG ELISA				Protein BCA				vWF-propeptid AG ELISA			
	Konz. [µg/mL]	Gesamt [µg]	Ausbeute [%]	Yield [µg/µg]	Konz. [µg/mL]	Gesamt [µg]	Ausbeute [%]	Yield [µg/µg]	Konz. [µg/mL]	Gesamt [µg]	Ausbeute [%]	Yield [µg/µg]
24. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
25. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
26. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
27. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
28. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
29. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
30. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
31. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
32. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
33. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00
34. Purkay Cone	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00	262.39	262.39	100.00%	1.00

FIG. 17

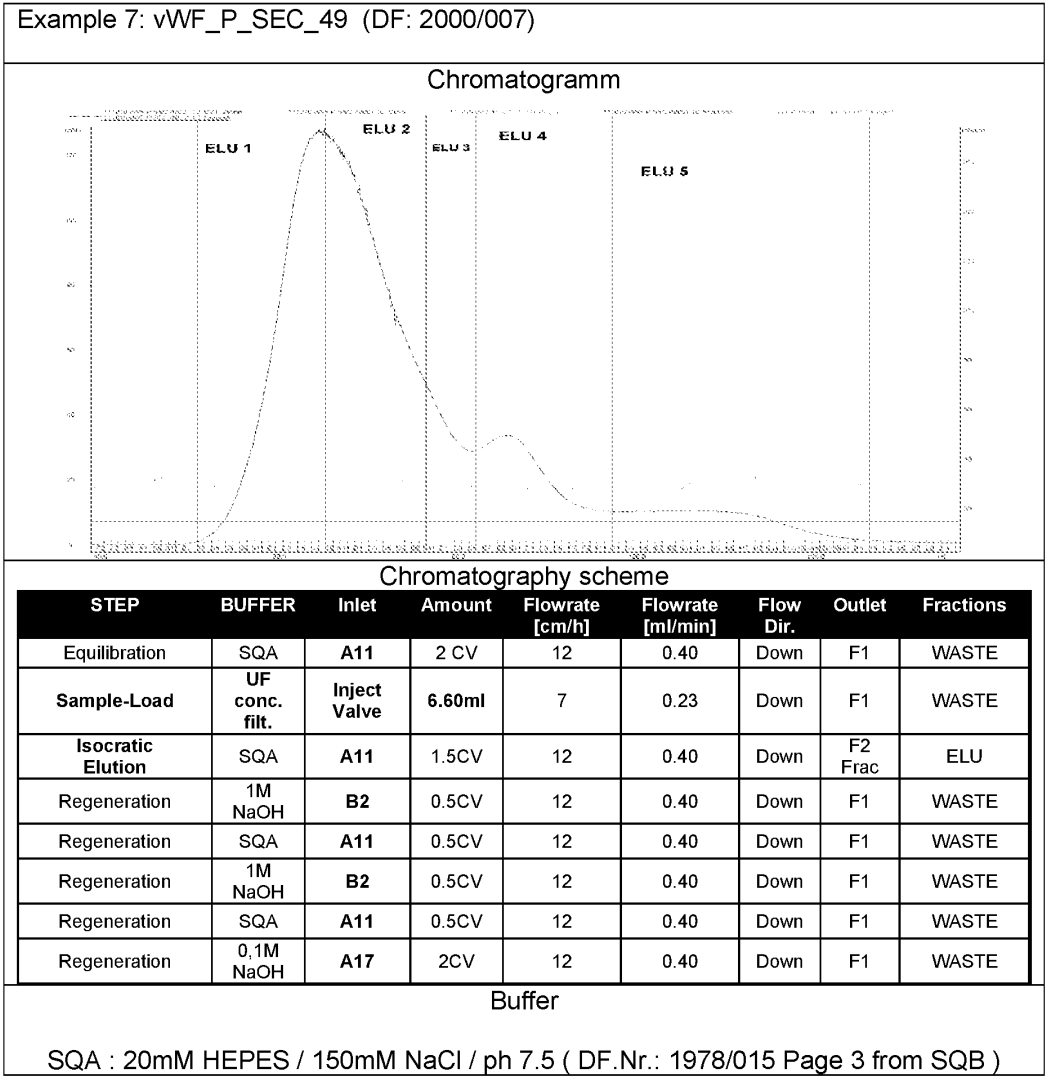


FIG. 18

Example 7: vWF_P_SEC_49 (DF: 2000/007)

YIELD

Proben Code	Menge [g]	vWF:Ag SOP: OR-13-00127				vWF-Risto SOP: OR-13-00407				CHO Ag ELISA SOP: OR-13-00387			
		Aktivität [µg/mL]	Gesamt [µg]	Ausbeute [%]	Risto [µmL]	Gesamt [µg]	spez. Akt. [µg/1000U Risto]	Ausbeute [%]	CHO [µg/mL]	Gesamt [µg]	Ausbeute [%]	FVIII-Ag Gehalt [µg/1000U Risto]	
LOAD_110006	6.00	2640	18744	100.0%	133.2	813	0.43	100.0%	11916.5	78648.9	100%	96.72	
ELU1 B3-C6	12.62	274.00	3512.68	18.7%	24.70	317	0.30	39.0%	32.298	414.000	0.005	<1.308	
ELU2 C9-D10	9.35	675.00	6341.25	33.7%	21.80	202	0.32	24.8%	329.188	3077.6891	4%	15.24	
ELU3 D11-E5	5.09	346.00	1688.44	8.6%	2.17	11	0.07	1.4%	765.285	4048.05155	5%	366.50	
ELU4 E6-F12	13.74	158.00	2170.92	11.6%	0.90	8	0.04	1.0%	847.336	11642.39964	15%	1613.10	
ELU5 G1-I12	26.24	4.14	108.6336	0.6%	0.22	8	0.55	0.7%	349.454	8382.47266	41%	1452.06	
Wiederfindung				73.2%				66.9%			36%		

Proben Code	Menge [g]	vWF propeptide ELISA SOP: OR-13-00405				F8 Ag ELISA SOP: OR-13-00408			
		propeptide [µg/mL]	Gesamt [µg]	Ausbeute [%]	F8 [µg/mL]	Gesamt [µg]	FVIII-Ag Gehalt [µg/1000U Risto]	F8 [µg/mL]	FVIII-Ag Gehalt [µg/1000U Risto]
LOAD_110006	6.00	354.793	2341.6338	100%	2879.813				
ELU1 B3-C6	12.62	0.457	5.66	0.25%	18.502	6584.276	20.72	511.60	20.72
ELU2 C9-D10	9.35	19.062	178.23	7.61%	882.500	8831.920	42.74	923.20	42.74
ELU3 D11-E5	5.09	21.875	111.34375	4.75%	10080.645				
ELU4 E6-F12	13.74	26.718	367.10592	15.68%	47710.714				
ELU5 G1-I12	26.24	16.504	513.35936	21.92%	85927.273				
Wiederfindung				50%					

Yellow marked: product containing fraction / No SDS-PAGE Available
The product containing fraction of example 7 results in low yield and vWF propeptide containing material

FIG. 19

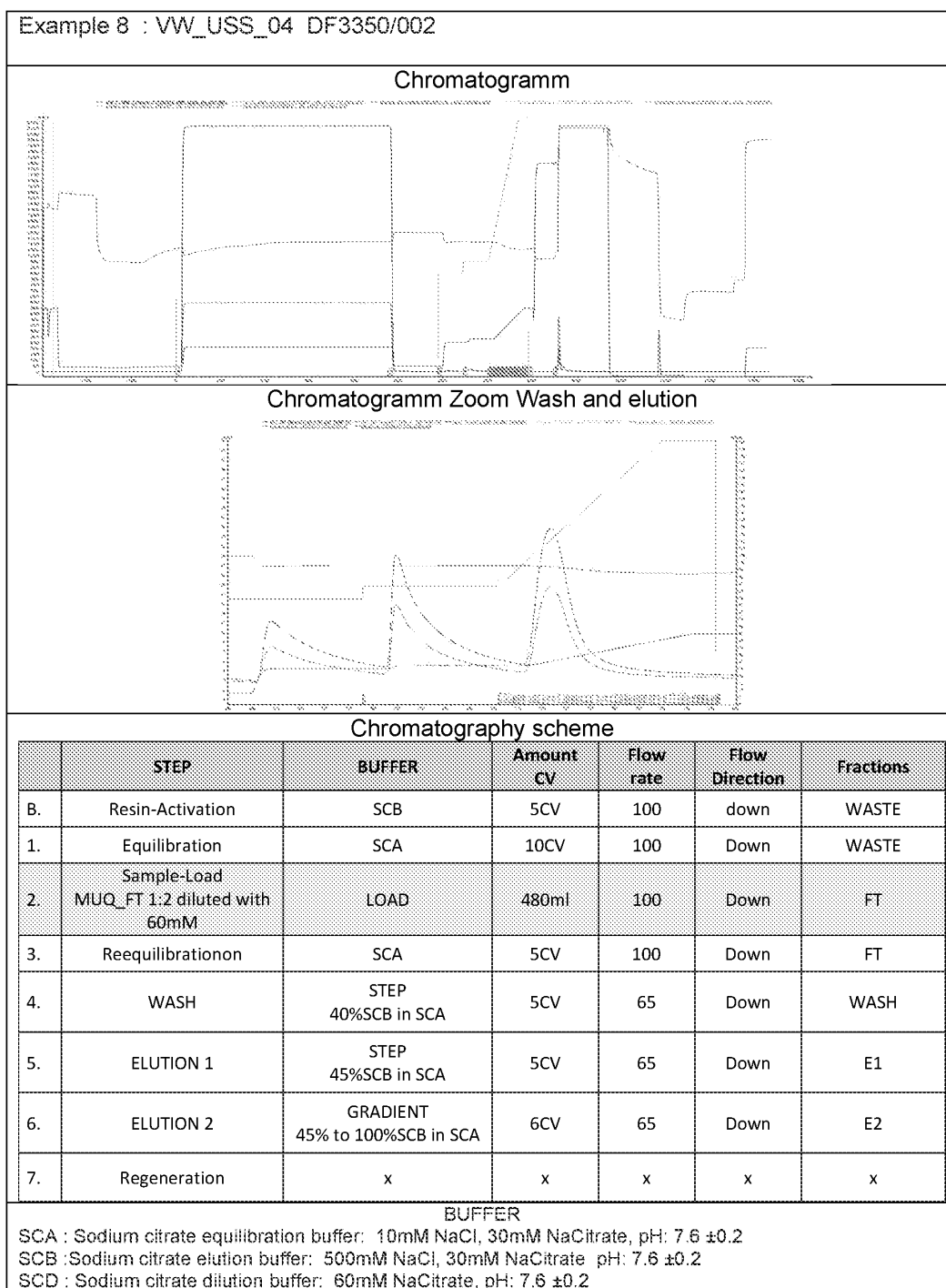


FIG. 20

YIELD

VW_USS_04 DF3350/002

Code	Volumen [l]	VWF Ausgussgewicht CDE 13-0078 [kg / m ³]			Bestimmung der Rückhaltung CDE-Abdruck bei VWF CDE 13-0077 [g/cm ³]			Spezifische Aktivität UR-Rückhaltung VWF/AS
		[kg / m ³]	[kg]	[m ³]	[g]	[g]	[m ³]	
L.prior SDVI	240.22	100.00	24422.00	100.00%	8.347	2005.12	100.00%	83.47
FT	555.53	23.50	13397.31	55.3%	< 0.5	—	—	x
WASH	55.75	27.20	1543.60	6.4%	1.535	92.84	4.8%	60.1
E1	55.87	39.50	2245.37	9.4%	5.673	322.62	16.1%	145.6
E2	27.59	70.60	1947.85	8.1%	12.431	342.97	17.1%	175.1

VW_USS_04 DF3350/002

Code	Volumen [l]	PVA-Querschnitt CDE 13-002278 [g]		Rückhalt VWF/AS CDE 13-0078 [g]		Rückhalt VWF/AS CDE 13-0077 [g]		Rückhalt VWF/AS CDE 13-0077 [g]		Spezifische Aktivität UR-Rückhaltung VWF/AS
		[g]	[m ³]	[g]	[m ³]	[g]	[m ³]	[g]	[m ³]	
L.prior SDVI	240.22	0.38	91.26	100.00%	1.88	451.81	100.00%	2887.10	689.215.16	100.00%
FT	555.53	< 0.2	—	—	0.86	512.24	113.4%	15320.80	91255.25.10	132.6%
WASH	55.75	< 0.2	—	—	< 0.14	—	—	658.10	37247.15	0.5%
E1	55.87	< 0.2	—	—	< 0.14	—	—	30.62	1744.26	0.0%
E2	27.59	< 0.2	—	—	< 0.14	—	—	23.77	855.81	0.0%

FIG. 21

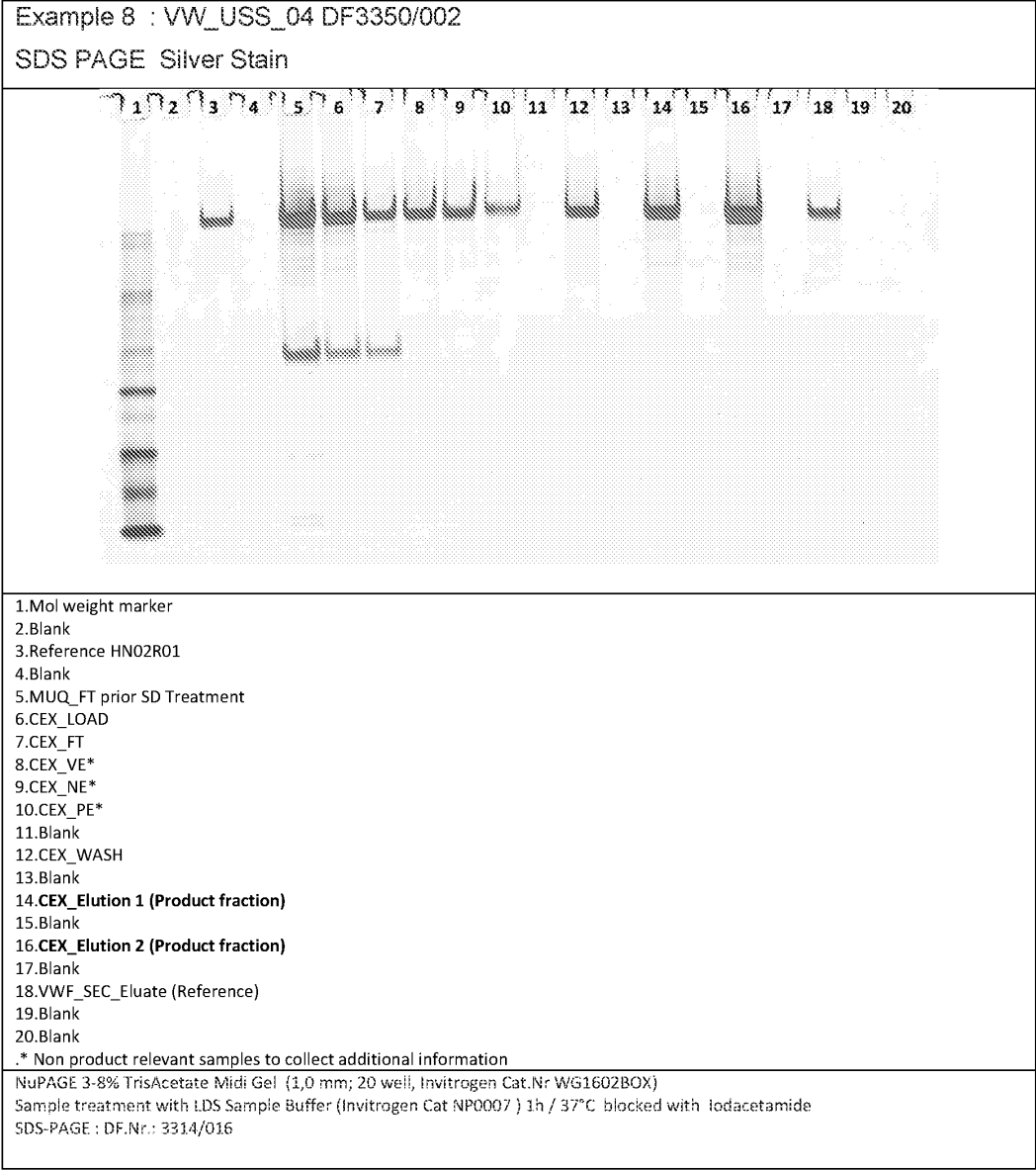


FIG. 22

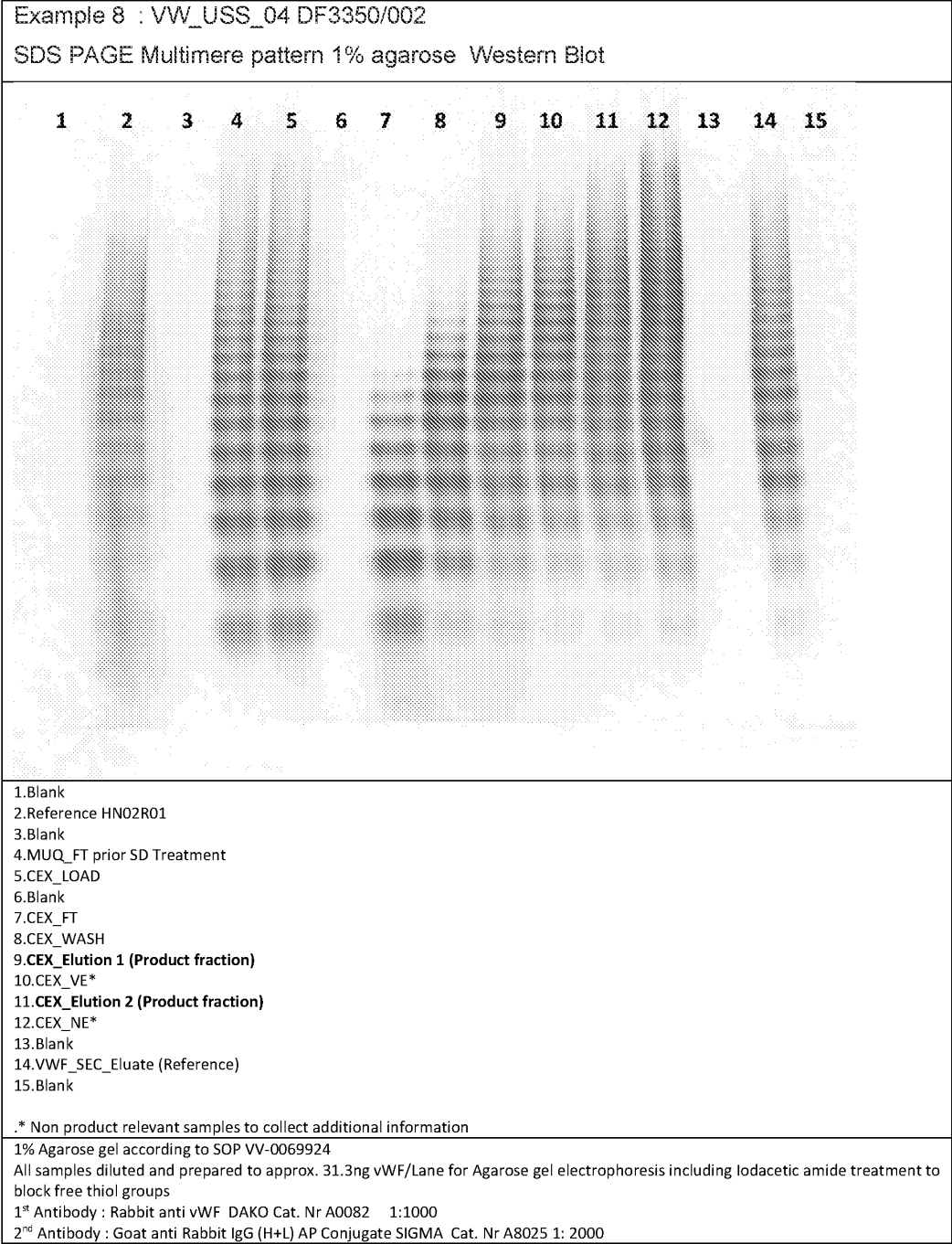


FIG. 23

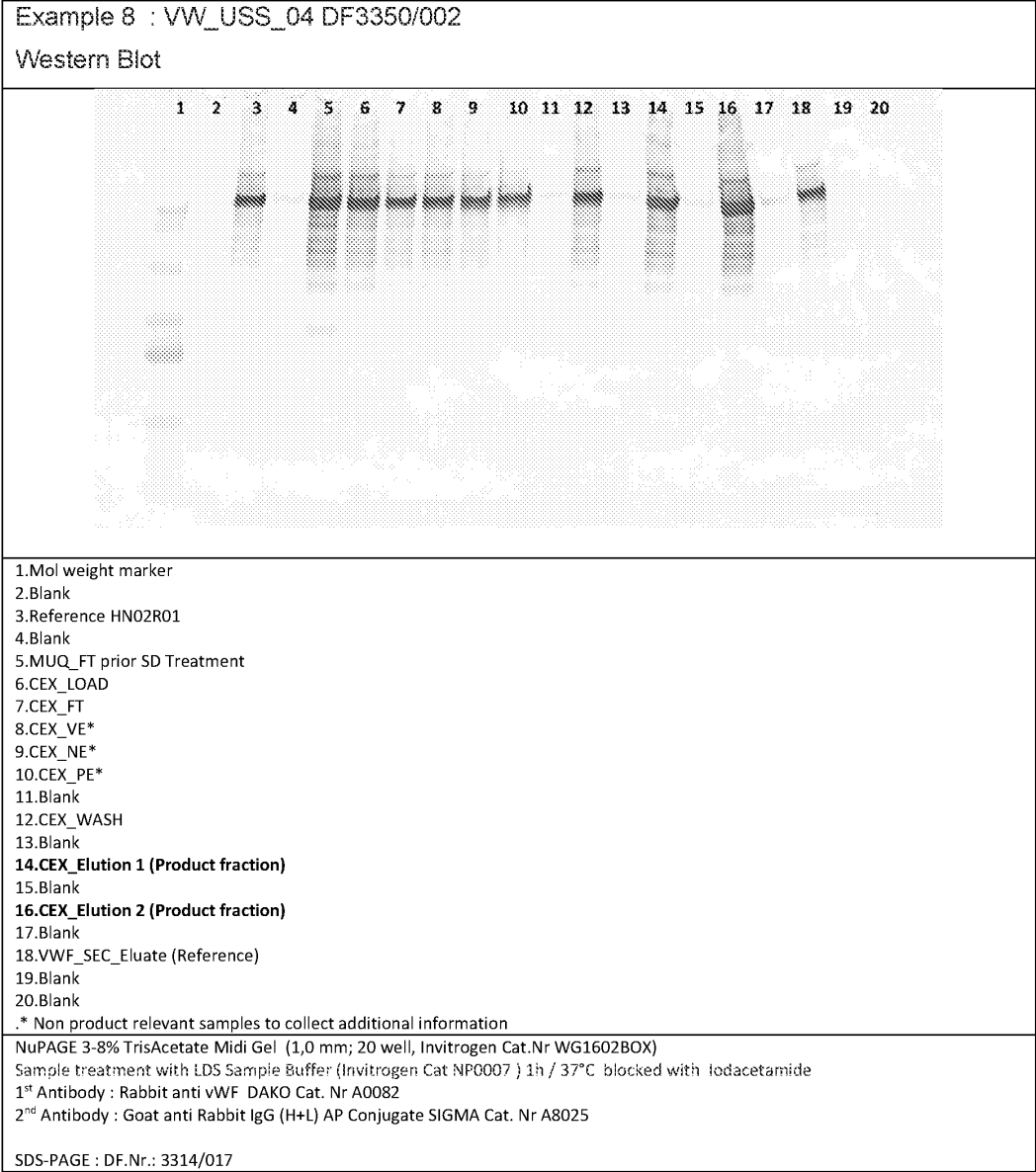


FIG. 24

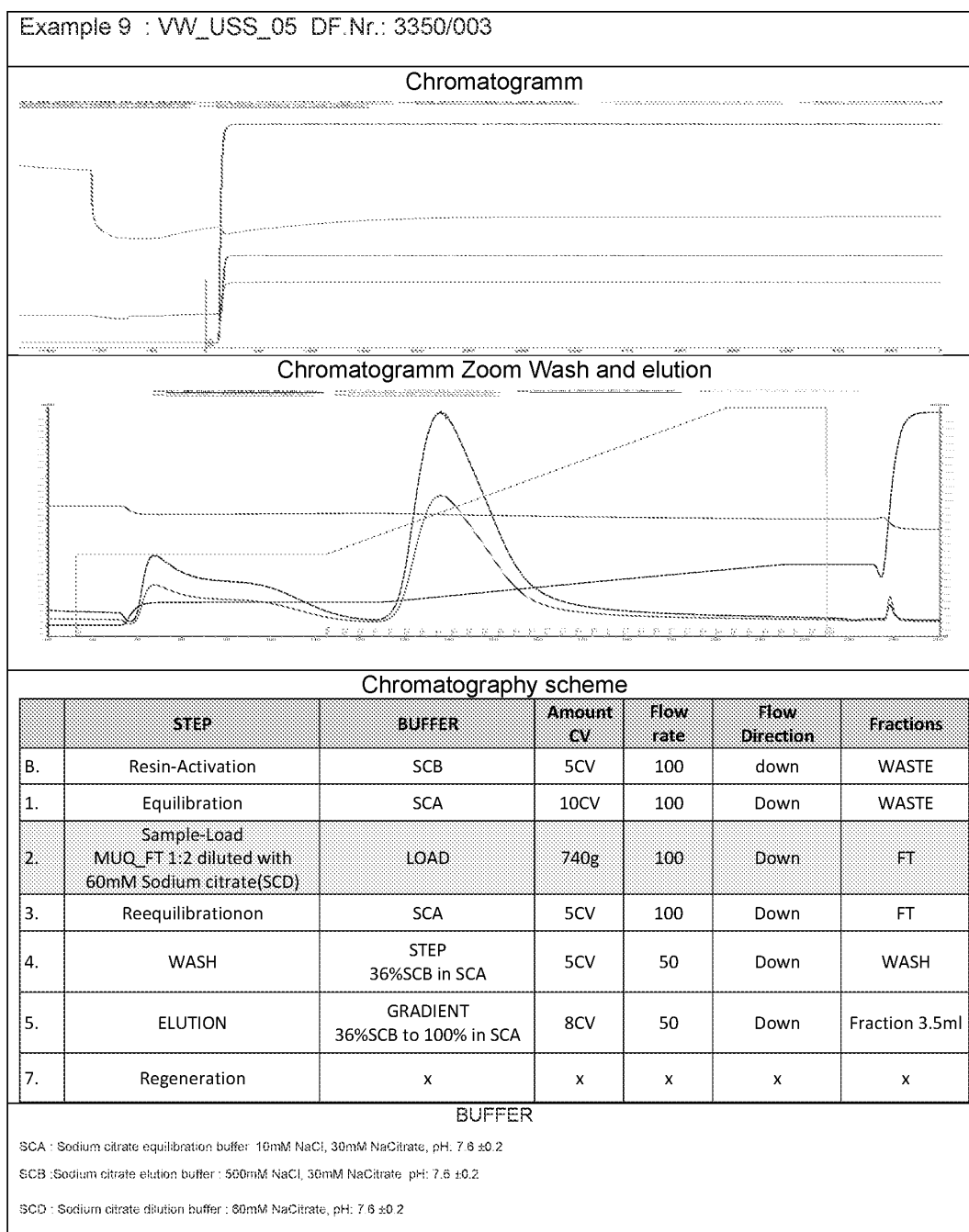


FIG. 25

YIELD

Example 9 : VW_USS_05 DF.Nr.: 3350/003

Code	Amount [g]	vWF Antigen (OR-13-0072)			Hyaluron-Catalin-Activity (WF OR-13-0087)			Specific Activity		
		[µg / ml]	[g]	[%]	[IC50 ml]	[g]	[%]	[U RC50/mg vWF:Ag]		
L prior SD	364.03	121.00	44047.63	100.00%	8.243	3000.70	100.00%	68.12		
FT	798.98	25.60	20453.89	46.4%	< 0.5	—	—	x		
E	34.69	231.50	8030.74	18.2%	19.696	683.25	22.8%	65.1		

Example 9 : VW_USS_05 DF.Nr.: 3350/003

Code	Amount [g]	FVIII Chromogen (E % 13-48777B)			C1C100F Elisa (E % 335616A)			CAG Protein Elisa (E % 335616A)			Properdin Elisa	
		[mU / ml]	[g]	[%]	[µg / ml]	[g]	[%]	[µg / ml]	[g]	[ng / ml]	[g]	[%]
L prior SD	364.03	0.66	203.86	100.00%	1.22	444.12	100.00%	2.39	36201.25	13178341.04	100.00%	
FT	798.98	< 0.2	—	—	0.59	471.40	106.1%	—	14512.80	11595436.94	88.0%	
E	34.69	0.83	28.79	14.1%	0.008	0.28	0.1%	<0.0625	142.60	4946.79	0.0%	

FIG. 26

Analysis	Value
vWF: Antigen	231,5 μ g /ml
RiCoF activity	19,7U/ml
Specific activity RiCoF / Antigen	85,1U/mg
Factor VIII activity (Chromogen)	0,83U/ml
CHO-HCP	0,008 μ g/ml
CHO-HCP / 1000U RiCoF activity	0,41 μ g/1000U
r-vWF-Propeptide	142ng/ml
Depletion factor CHO – HCP Load to Eluate	1575
Depletion factor vWF-Propeptide Load to Eluate	2664
Depletion factor FVIII- Chromogen activity Load to Eluate	7,1

FIG. 27

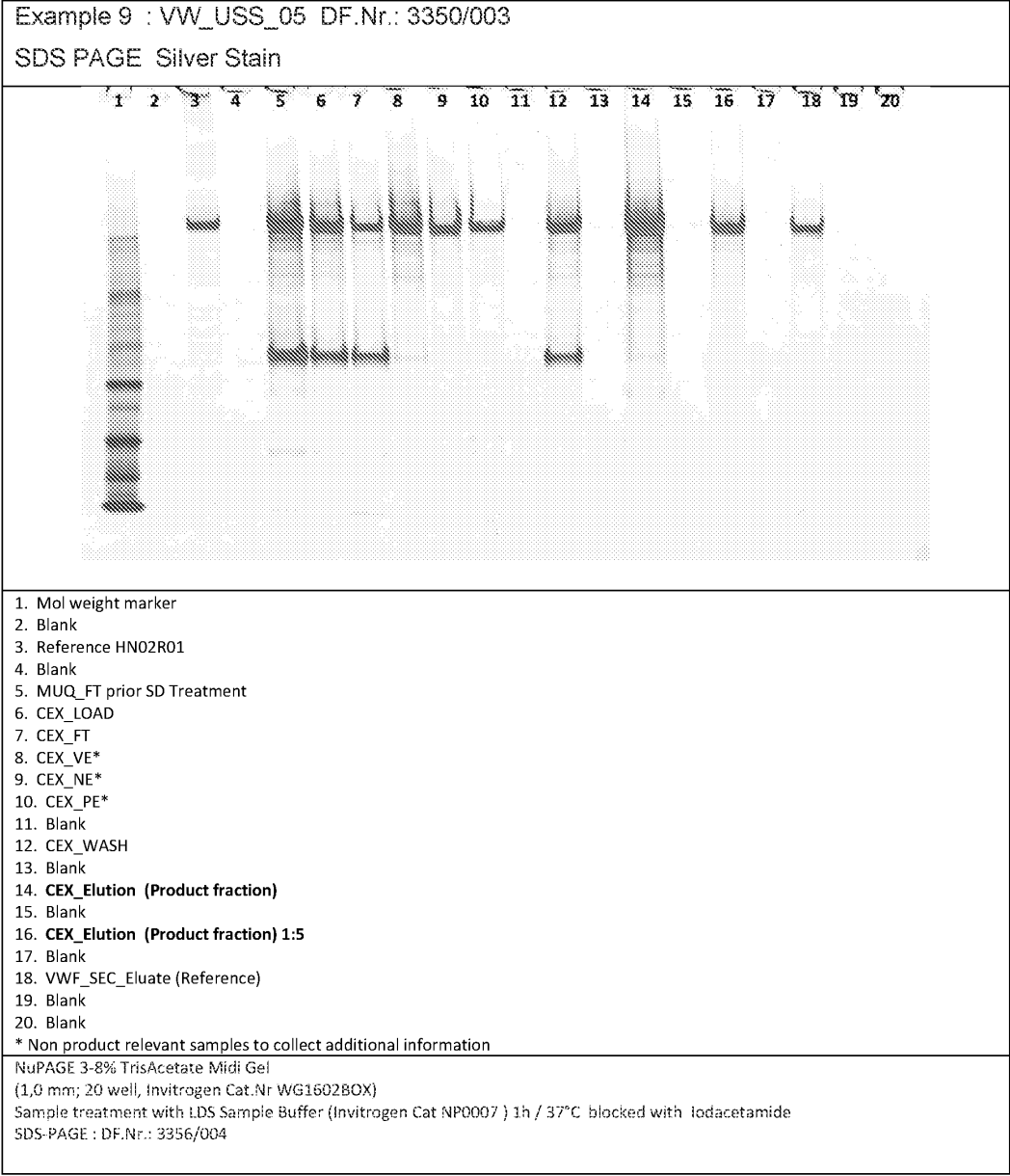


FIG. 28

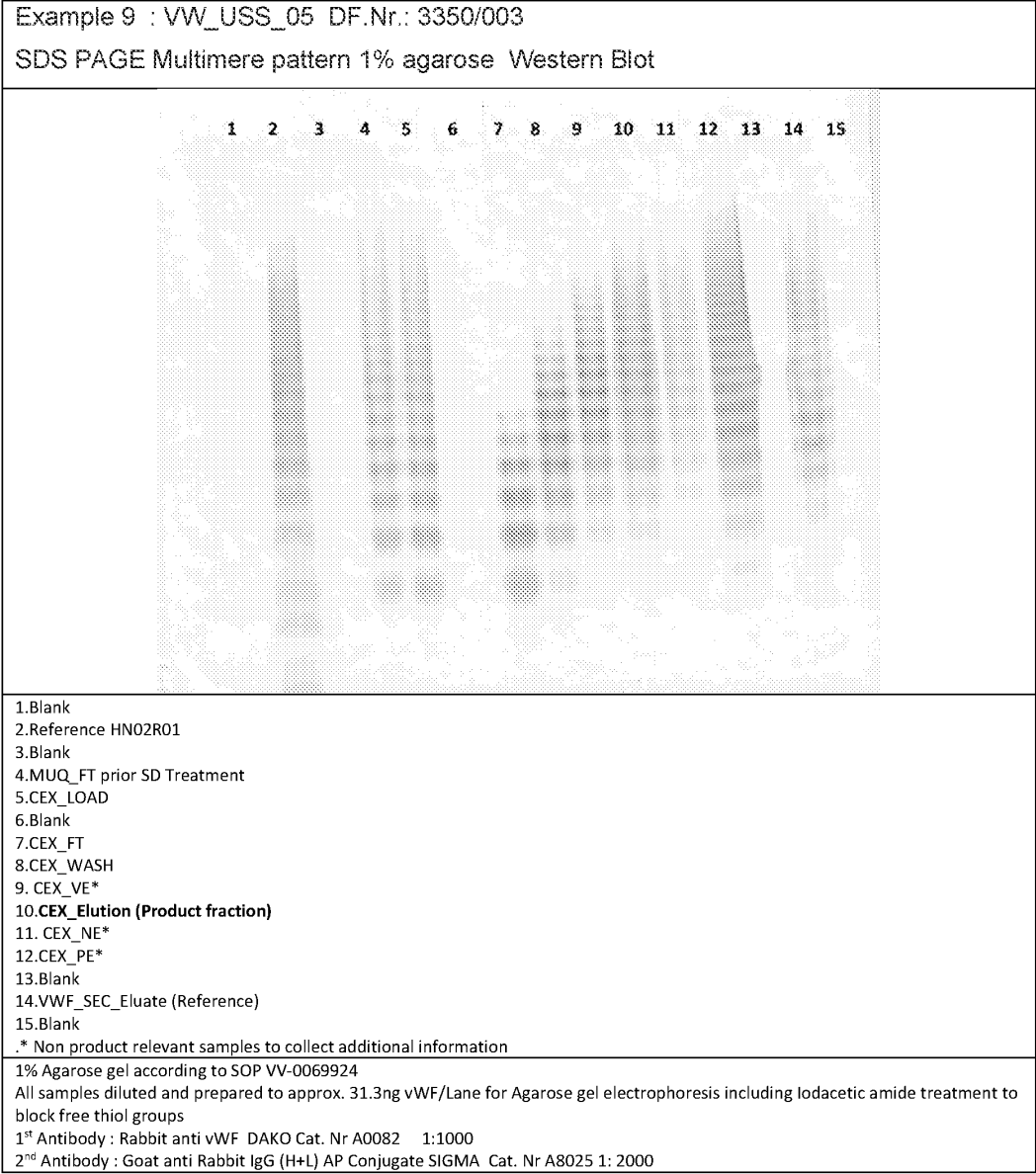


FIG. 29

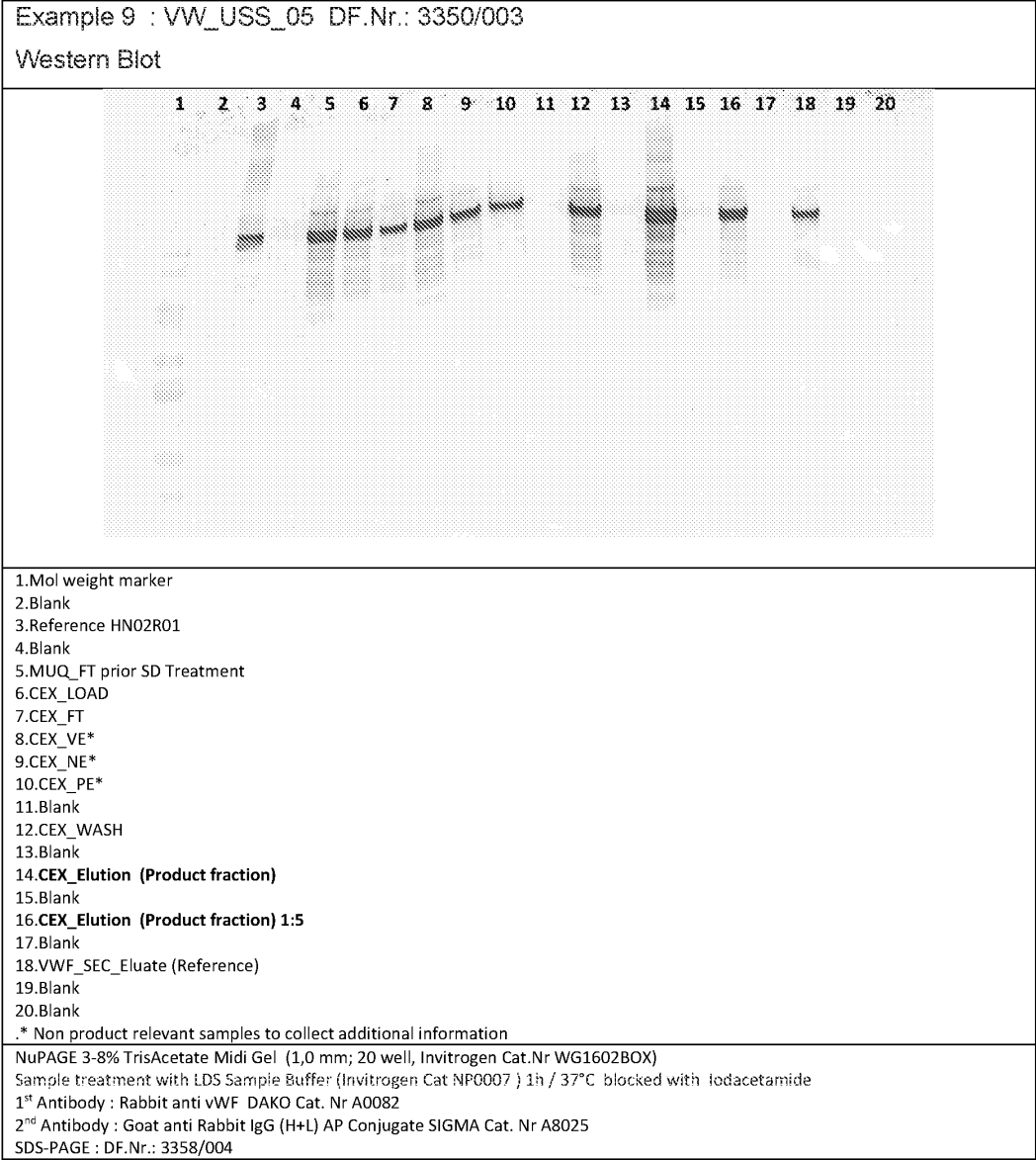


FIG. 30

ID	Method	vWF-PP/vWF ng/ μ g Antigen/Antigen	vWF-PP/RiCoF μ g/1000Units Antigen/Activity	CHO-HCP/vWF ng/ μ g Antigen/Antigen
Example 1	Citrate buffer Version 1	0.34	4.55	1.814
Example 2	Citrate buffer Version 2	1.10	23.83	2.578
Example 3	Citrate buffer Version 2	0.94	21.14	1.704
Example 6	Without chelating agent	163.3	3599	2.073
Example 8	Optimized Citrate buffer	E1 0.78	E1 5.4	E1 <5.15
		E2 0.34	E2 1.9	E2 <1.98
Example 9	Optimized Citrate buffer protocol for manufacturing	E 0.62	E 7.2	E 0.035

FIG. 31**DEPLETION FACTOR of product related impurities**

ID	Method	Depletion factor vWF-Propeptide Load/Eluate	Depletion factor CHO-HCP Load/Eluate
Example 1	Citrate buffer Version 1	2393	62.3
Example 2	Citrate buffer Version 2	428	73.7
Example 3	Citrate buffer Version 2	400	69.1
Example 6	Without chelating agent	3.5	12.2
Example 8**	Optimized Citrate buffer	E1 3957 E2 10508	E1 >56.7 E2 >116.9
Example 9	Optimized Citrate buffer Protocol for manufacturing	E 2664	E 1574

** two eluates E1 and E2 represent one final product after pooling

In Example 8 the CHO-HCP value was under the detection limit of 140ng/ml

FIG. 32**Enhanced Size Exclusion Chromatography (SEC)**

ID	Method	vWF-PP/vWF ng/μg Antigen/Antigen	vWF-PP/RiCoF μg/1000Units Antigen/Activity	CHO-HCP/vWF ng/μg Antigen/Antigen
Example 4	Chelator Citrate	<0.11	<0.84	0.14
Example 5	Elevated pH	0.73	8.68	<0.17

FIG. 33**DEPLETION FACTOR of product related impurities**

ID	Method	Depletion factor vWF-Propeptide Load/Eluate	Depletion factor CHO-HCP Load/Eluate
Example 4	Chelator Citrate	>4710	84.3
Example 5	Elevated pH	819	>13.3

FIG. 34**Material**

SETUP	Detail	Remark
COLUMN	TMAE 4ml	ID 10mm / h = approx. 5cm
Buffer A	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	-
Buffer B	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5 +1000mM NaCl	-
Buffer C	50mM TrisHCl + 2mM EDTA pH 8.5 + 16.6g/kg SD Reagent [10.54g Triton X100, 3.15g Polysorbate 80, 2.91g Tri-n- butyl phosphate]	Buffer for SD_VI Treatment "ON-COLUMN"

FIG. 35**Conditioning of the LOAD**

Original	Conditioning	Remark
ORVW16102MUQ-E	r-vWF MUQ – FT diluted 1:4 in BUFFER A	Incubation > 120min
25ml	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	

FIG. 36**Chromatography**

Chromatography	Buffer	Buffer Details	CV	Flow rate	Fraction
1. Activation	2M NaCl		5	0.6ml/min	Waste
2. Equilibration	Buffer A	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	8	0.6ml/min	Waste
3. Loading	r-vWF MUQ – FT diluted 1:4 in Buffer A	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	x	0.6ml/min	FT
4. Re- Equilibration	Buffer A	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	5	0.6ml/min	FT
5. On Column SD_VI	Buffer C	50mM TrisHCl + 2mM EDTA pH 8.5 + SD Reagent 16.6g/kg	10	0.6ml/min	REG_W
6. Re- Equilibration II	Buffer A	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	20	0.6ml/min	REG_W
7. Elution	Gradient 0% Buffer A to 50% Buffer B In 20CV	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5 + 1000mM NaCl in 50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5	20	0.6ml/min	Fraction - 1.5ml
8. Post Elution	50% Buffer B	50mM TrisHCl + 2mM EDTA + 0.1% Tween80 /pH 8.5 + 1000mM NaCl	8	0.6ml/min	Fraction - 1.5ml

CONDITIONS / PARAMETER

LOAD: rvWF_MUQ_FT – Furin processed r-vWF

Buffer + Split conditions: TrisHCl + elevated pH 8.5 + EDTA

Gradient counter: Chloride Cl⁻

SD_VI Treatment _ On Column post Wash 1 (Duration ca. 60min)

FIG. 37

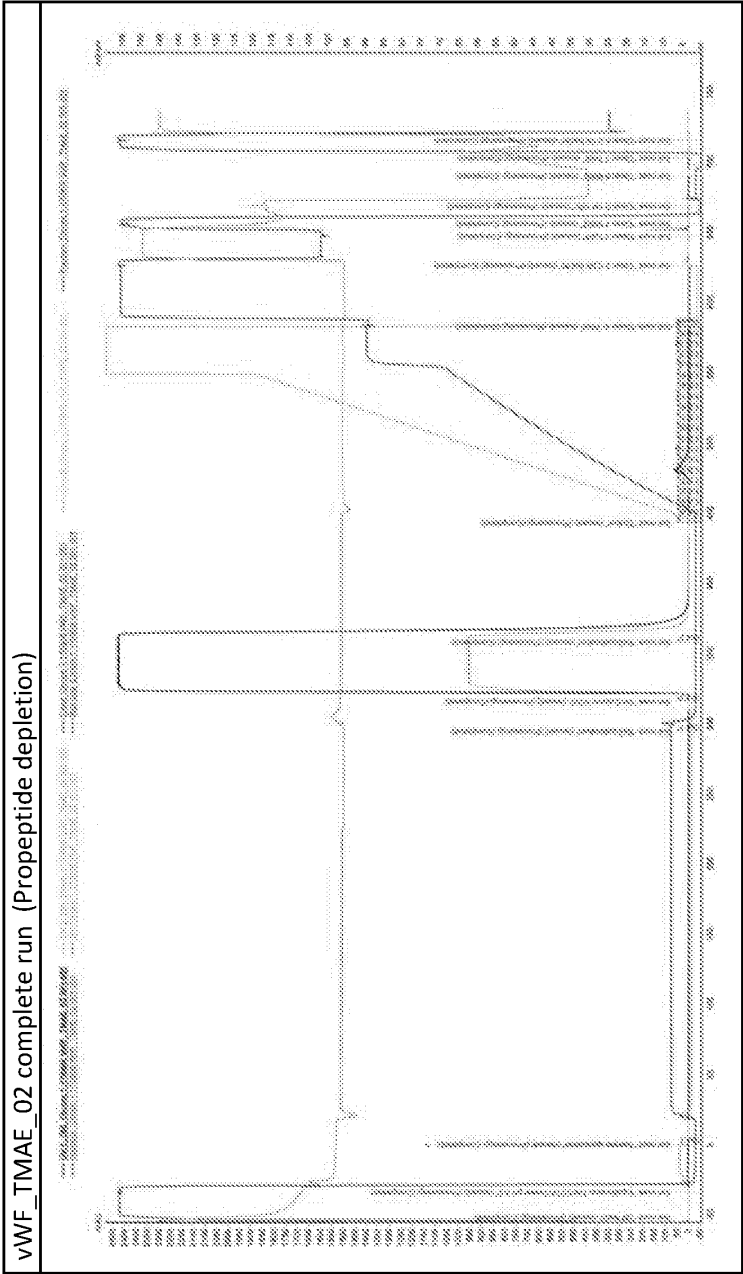


FIG. 38

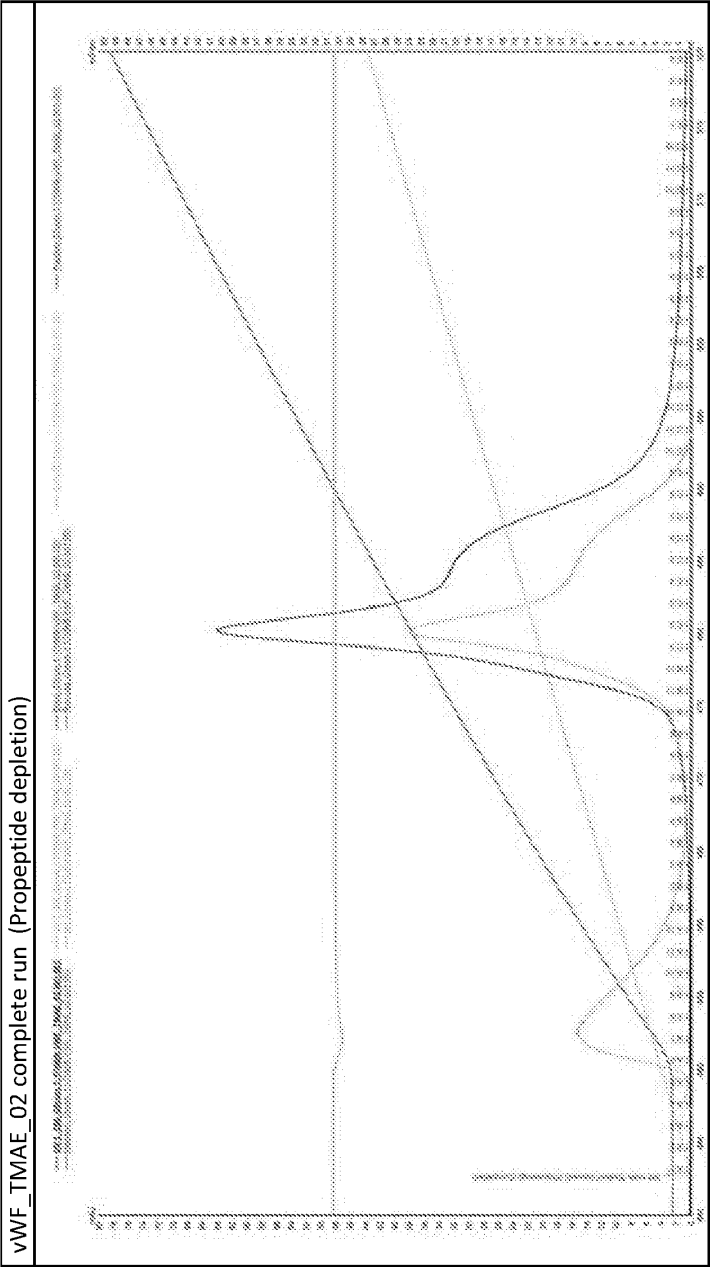


FIG. 39A

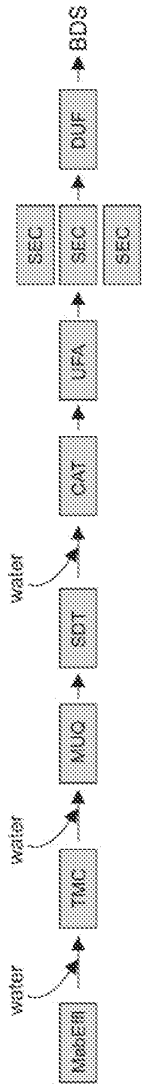


FIG. 39B

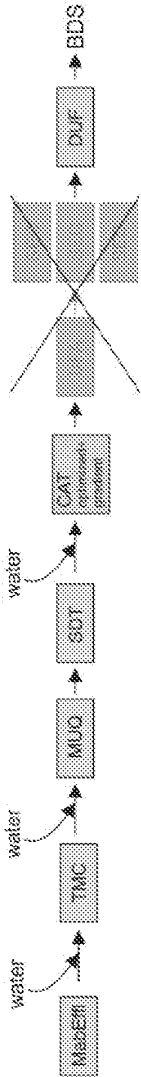


FIG. 40

CAT Feature	CAT 1.0	CAT 2.0	Comment
Concentration of product	yes	yes	No change
Removal of host cell protein impurities	Yes, reduction factor of about 40 to be determined	Yes, reduction factor of > 1000 to be determined	Significant improvement
Host cell DNA removal	no	Yes, reduction of > 2000	Significant improvement
VWF pro-peptide removal	reduction factor of < 10	Yes, replacing SEC	No improvement
Residual FVIII removal	yes		Significant improvement
Separation and pooling of VWF multimers			

FIG. 41

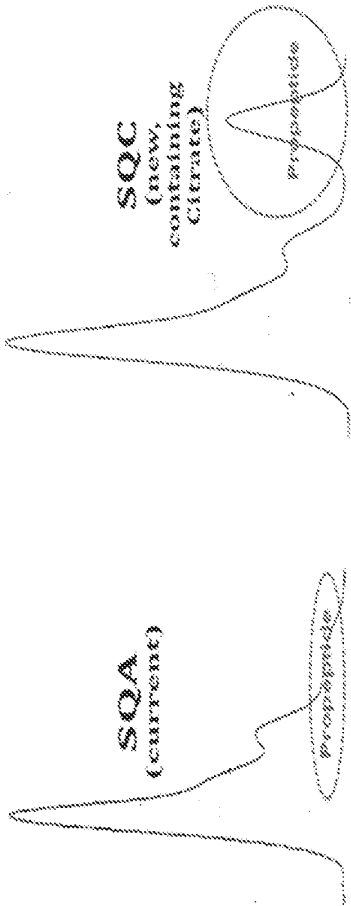


FIG. 42

SEC Feature	SEC (SQA buffer)	Improved SEC (SQC buffer)	Comment
Removal of host cell protein impurities	Yes, reduction factor of approx. 100	Yes, reduction factor of > approx. 100	No improvement
VWF pro-peptide removal	Yes, but not robust (impurity levels ranging from 20 – 250 µg/1000 Units)	Yes, very robust (impurity levels < 2 µg/1000 Units)	Significant improvement in terms of level and robustness
Residual FVIII removal	reduction factor of < 10	reduction factor of < 10	No improvement
Separation and pooling of VWF multimers	yes	yes	No improvement

FIG. 43A

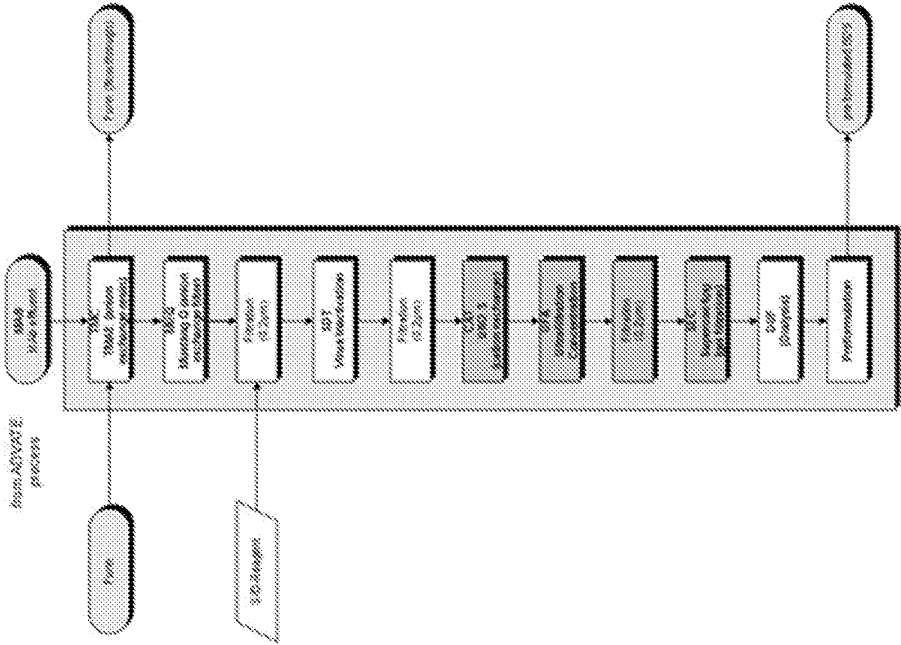


FIG. 43B

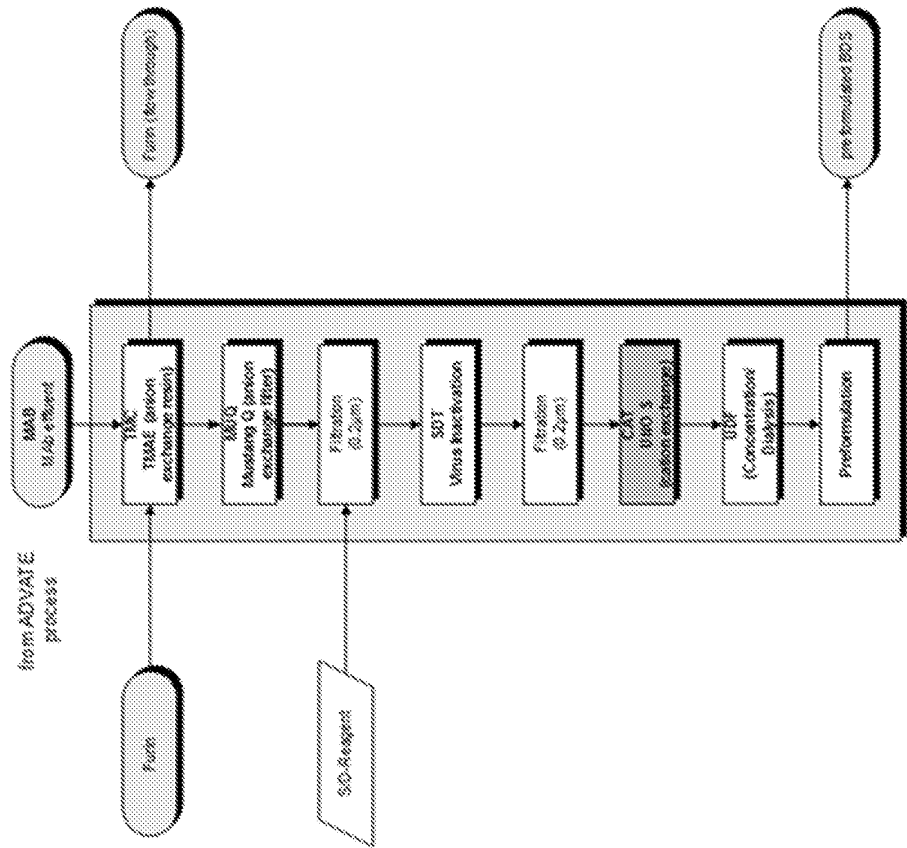


FIG. 44

Column parameter	1 st generation MFG NE	2 nd generation small scale	Difference/ Rationale
Cation Exchange media	UNO_Sphere S (BioRad)		No
Mean Pore size	80°µm		
Column	Pall Resolution	KronLab 10/250	Yes / scale dependent
Bed height	14 cm (15 – 18°cm)	14.3 cm	No
Bed diameter	120 cm	1 cm	
Column volume	Approx. 170 – 204°L	11.23°mL	Yes / scale dependent
Frit material	Stainless steel 20 µm	10 µm Polypropylene	
Plates/meter	≥ 2500	1565	
Asymmetry	0.8 – 1.8	1.93	
filter	Online filtration	Off line filtration in course of S/D treatment prior to CAT loading	
	Sartoguard membrane or Pall Supor EAV	Pall Kleenpack; Supor membrane EA	
	7.2 m² (3 x 30” cartridge)	260 cm²	
	0.2 µm	0.2 µm	No
System parameter			
Chromatography system	Millipore	GE-Healthcare AKTA Pure 25	Yes / scale dependent

FIG. 45

Run I.D.	Wash	Elution	2M NaCl
1 st Gen process (MFG)	Wash buffer 100 cm/h; 10 - 11 CV	Elution buffer 65 cm/h; 3.3 - 3.6 CV	65 cm/h; 10 CV
VW_USS_01	100% A 100 cm/h; 10 CV	0%B - 100%B 65 cm/h; 12 CV	65 cm/h; 5 CV
VW_USS_02	55% B; 65 cm/h; 10 CV	55%B - 100%B 65 cm/h; 6 CV	65 cm/h; 5 CV
VW_USS_03 and VW_USS_04	40% B; 65 cm/h; 5 CV	45%B - 100%B 65 cm/h; 6 CV	65 cm/h; 5 CV
VW_USS_05	36% B; 50 cm/h; 5 CV	36%B - 100%B 50 cm/h; 6 CV	65 cm/h; 5 CV

FIG. 46

Step	Buffer	1 st generation MFG NE		2 nd generation small scale setting		Difference / Rationale
		Amount [CV]	Flow rate [cm/h]	Amount [CV]	Flow rate [cm/h]	
1	Equilibration	25	100	≥ 32	100	Yes; improved impurity removal
2	Product load	16 – 19	100	38 - 66	100	Yes; improved capacity and impurity removal
		in-line filtration 0.2 µm; Supor EAV, 70 L/h/m ²		-		
3	Wash	Load: 60 – 140 IU/ml resin		Load 90 – 270 IU /ml resin		Yes; improved impurity removal
4.1. 4.2.	Elution	10 – 11	100	5 - 20	100	Yes; replacement of SEC
		3.3 – 3.6	65	8 -18	65	
	Pool collection	when the UV signal rises ≥ 0.1 O.D. above baseline; Vol. collected: 3.3 – 3.6 CV		to be explored		
5	Wash 3	10 – 11	100	5	65	No

All chromatographic steps were performed at room temperature at lab scale the specification for MFG is +18°C - +25°C.

FIG. 48

Buffer ID		1 st generation MFG NE		2 nd generation small scale		Difference / Rationale
Steps	Buffer	Composition	Conductivity [mS/cm] at +25°C	Composition	Conductivity [mS/cm] at +25°C	
S/D Dilution buffer	WFI	Inactivated MUQ-E is 1:2 diluted with WFI to reach a pH of 7.0 ± 0.5	After dilution a conductivity of 13 - 17 mS/cm is reached	Inactivated MUQ-E is diluted 1:2 with 60mM Na-citrate buffer that will sets the CAT feed to a preferred pH of 7.5 – 8.0 (pH testing range 6.0 – 9.0)	After dilution a preferred conductivity of 10 – 30 mS/cm is reached (testing range 5 – 40 mS/cm)	Yes / New buffer conditions to optimize the removal of product and process related impurities
CAT wash	TQA	100 mM NaOAc, 10 mM Tris, 85 mM NaCl, pH= 6.5 ± 0.2 (RT)	13 – 17	30 mM Na-Citrate, 2 mM Citric Acid, 10 mM NaCl; pH testing range 6.0 – 9.0	testing range 5 – 40 mS/cm	
CAT step Elution	CAT-Elu	100 mM NaOAc, 100 mM Glycine, 500 mM NaCl, 3 mM CaCl ₂ , pH= 7.5 ± 0.2 (RT)	47 - 53	-	-	
CAT Gradient Elution	CAT-Elu A	-	-	30 mM Na-Citrate, 2 mM Citric Acid, 10 mM NaCl; pH testing range 6.0 – 9.0	testing range 5 – 40 mS/cm	
	CAT-Elu B	-	-	30 mM Na-Citrate, 500 mM NaCl; pH testing range 6.0 – 9.0	testing range 5 – 40 mS/cm	

FIG. 49A

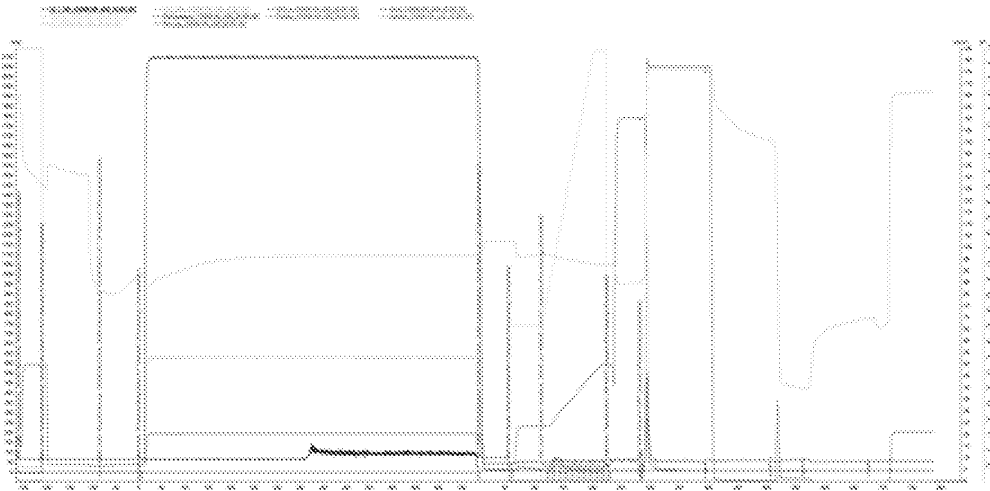


FIG. 49B

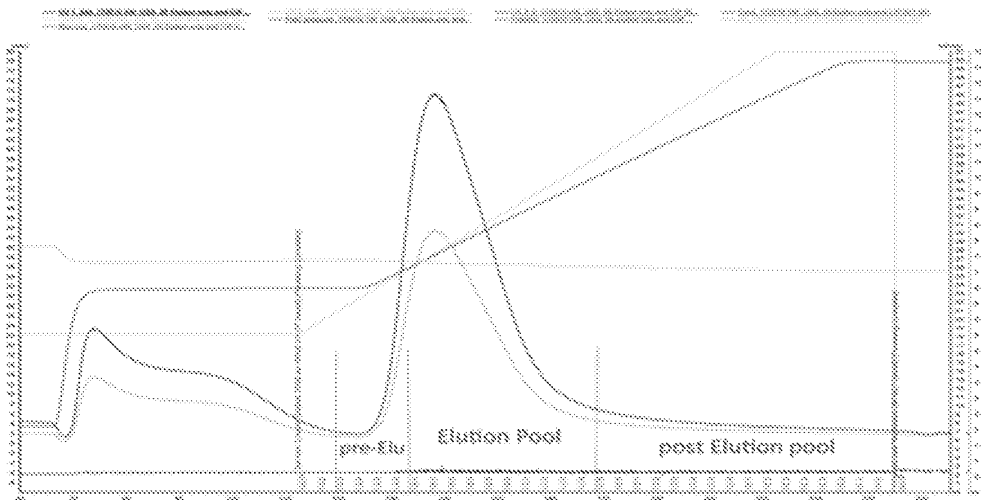


FIG. 50

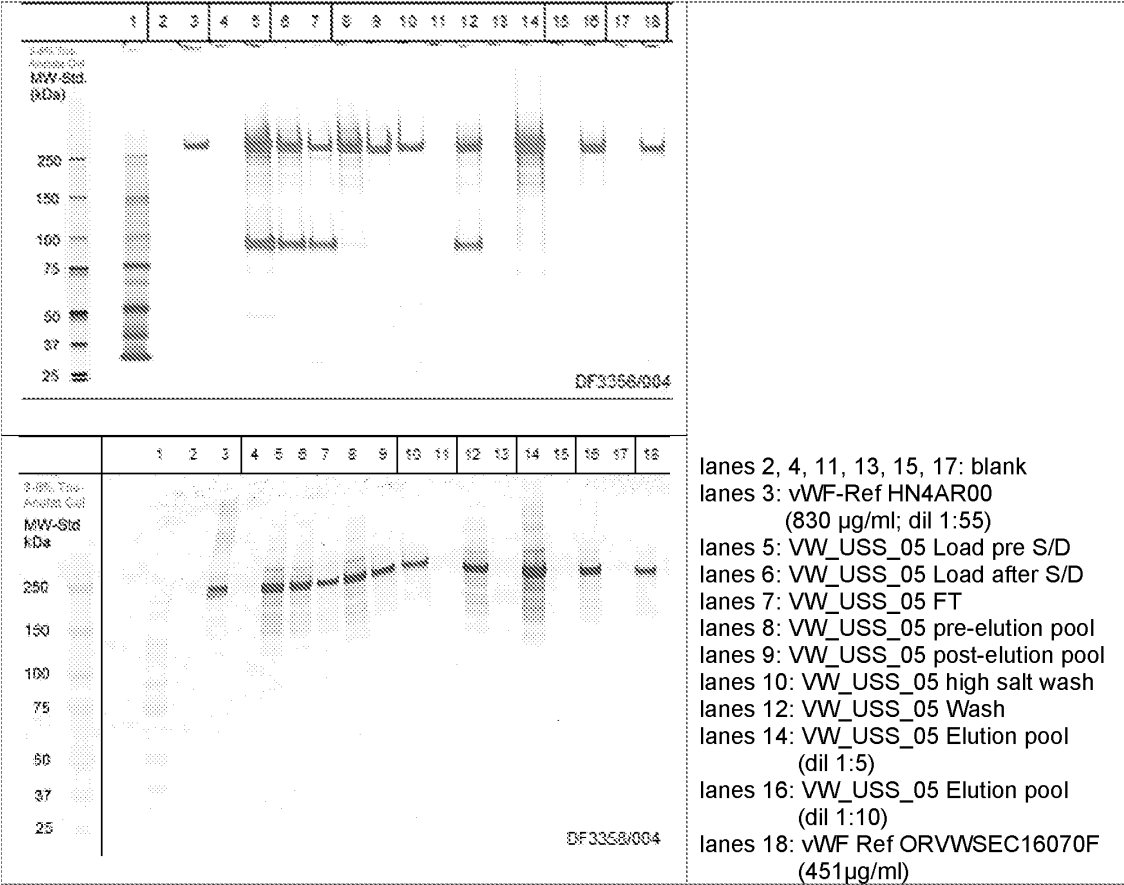


FIG. 51

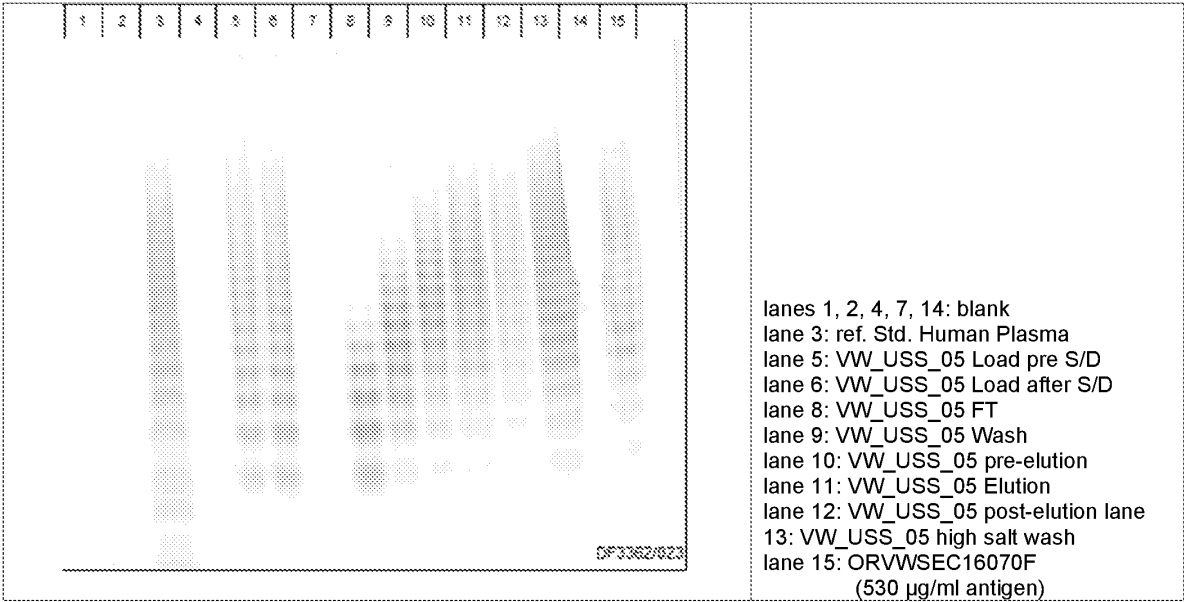


FIG. 52

Run I.D.	rVWF:Ag Yield %								comment
	FT	Wash(es)		pre-elu	elu	post-elu	High Salt	Total Rec	
VW_USS_01	2.30	0.20		0.02	41.5	0.70	0.10	44.8	---
VW_USS_02	38.3	24.5		n.d.	0.10	n.d.	0.05	63.0	wash conductivity to high
VW_USS_03	46.8	15.2	11.2	0.70	7.20	0.50	0.30	82.0	
VW_USS_04	58.3	6.43	9.35	0.50	8.10	0.60	0.10	83.4	---
VW_USS_05	46.4	7.20		2.50	18.2	1.60	0.30	76.2	---

FIG. 53

Run I.D.	rVWF Risto Co Activity %								comment
	FT	Wash(es)		pre-elu	elu	post-elu	High Salt	Total Rec	
VW_USS_01	n.d.	n.d.		n.d.	27.1	n.d.	n.d.	27.1	---
VW_USS_02	n.d.	52.5		n.d.	n.d.	n.d.	n.d.	52.5	wash conductivity to high
VW_USS_03	n.d.	10.4	18.4	1.10	12.9	n.d.	n.d.	43.2	
VW_USS_04	n.d.	4.63	16.1	n.d.	17.1	1.30	6.00	45.1	---
VW_USS_05	n.d.	5.00		2.40	22.8	1.60	n.d.	31.7	---

FIG. 54

Run I.D.	pro-peptide [µg/mg rVWF:Ag]							
	Load	FT	Wash(es)		pre-elu	elu	post-elu	High Salt
VW_USS_01	n.d.	n.d.	n.d.		n.d.	0.10	n.d.	n.d.
VW_USS_02	272	747	62.5		n.d.	4.51	n.d.	< 14.2
VW_USS_03	388	722	184	2.35	0.95	0.60	2.67	< 2.58
VW_USS_04	287	652	24.2	0.78	0.41	0.34	7.12	24.5
VW_USS_05	299	567	465		4.29	0.62	2.46	19.3

FIG. 55

Run I.D.	pro-peptide [µg PP /1000 U Risto]							
	Load	FT	Wash(es)		pre-elu	elu	post-elu	High Salt
VW_USS_01	n.d.	n.d.	n.d.		n.d.	2.90	n.d.	n.d.
VW_USS_02	3997	29150	429		n.d.	14.8	n.d.	< 6.25
VW_USS_03	4794	30486	3306	17.3	7.89	4.14	14.6	< 6.25
VW_USS_04	3437	30642	40.0	5.40	9.02	1.91	35.98	7.15
VW_USS_05	4392	29026	9927		66.0	7.24	35.43	79.0

FIG. 56

Run I.D.	Eluate Pools					
	VWF: Ag	VWF Risto Act.	Spec. act	CHO Ag / rVWF act.	PP / rVWF act.	FVIII act. / rVWF:Rco
	yield [%]	yield [%]	[RIU/IU:Ag]	[μ g/1000U Risto]	[μ g PP /1000U Risto]	[IU/ IU]
VW_USS_01	41.5	27.1	0.44	66	2.90	< 0.054
VW_USS_02	0.10	n.d.	n.d.	n.d.	14.8	n.d.
VW_USS_03	7.20	12.9	1.45	< 7.7	4.14	< 0.021
VW_USS_04	8.10	17.1	1.76	< 11.3	1.91	< 0.016
VW_USS_05	18.2	22.8	0.85	< 0.41	7.24	0.042

FIG. 57

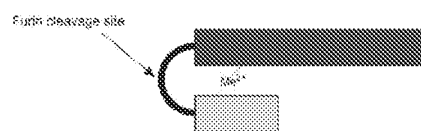
Test	Acceptance criteria	Result run VW_USS_05
Specific activity: ratio RCo Activity rVWF / rVWF:Ag (ELISA) /	0.7 – 1.4 IU/IU	0.85
CHO impurity ratio: CHO-HCP (ELISA) / RCo Activity rVWF	$\leq 2 \mu\text{g} / 1000 \text{ IU}$	< 0.41
FVIII impurity ratio: rFVIII:Ag (ELISA) / RCo Activity rVWF	$\leq 25 \mu\text{g} / 1000 \text{ IU}$	n.d.
pro-rVWF impurity ratio: pro-rVWF:Ag (ELISA) / RCo Activity rVWF	$\leq 50 \mu\text{g} / 1000 \text{ IU}$	n.d.
rVWF pro-peptide impurity ratio: rVWF pro-peptide:Ag (ELISA) / RCo Activity rVWF	$\leq 2 \mu\text{g} / 1000 \text{ IU}$	7.24 (1.91 in run VW_USS_04)
N-Glycane Profile	Monosialo: 20 – 35% Area Disialo 35 – 50% Area N-glycan Index 150 - 175	n.d.
Ratio Sialic Acid / Total Protein (BCA)	60 – 150 nmol/mg	n.d.
Ratio FVIII Activity / rVWF:RCo	$\leq 0.04 \text{ IU} / \text{IU}$	0.042 (< 0.016 in run VW_USS_04)
rVWF Multimer Analysis	≥ 15 multimer bands $\leq 40\%$ multimers larger than band 15	≥ 15 multimer bands $\leq 40\%$ multimers larger than band 15
Ratio CHO-DNA / rVWF:RCo Activity	$\leq 0.5^\circ\text{pg} / \text{IU}$	n.d.

FIG. 58

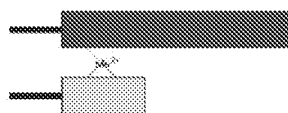
Unit operation	Mode of unit operation	Load	Wash	Elution	comment
AEX	Binding mode	no	no	Yes, Gradient • high pH • Chelat + neutral • both	
	Flow through mode	Yes • Low pH+chelate	no	no	
CEX	Binding mode	Yes • high pH • Chelate + neutral • both	Yes • high pH • Chelate + neutral • both	Yes, Gradient • high pH • Chelate + neutral • both	
	Flow through mode	no	no	no	
SEC		• high pH • neutral+chelate • both			

No/yes: is physical separation of matVWF and VWF-PP possible

FIG. 59



pro-VWF



matVWF/ VWF-PP complex



matVWF



VWF-PP

FIG. 60

SEQ ID NO: 1

agctcacagc	tattgtggtg	ggaaggag	ggtggttgg	ggatgtcaca	gcttgggctt	60
tatctccccc	agcagtgggg	actccacagc	ccctgggcta	cataacagca	agacagtccg	120
gagctgtagc	agacctgatt	gagcctttgc	agcagctgag	agcatggcct	aggggtggcg	180
gcaccattgt	ccagcagctg	agtttcccag	ggaccttgg	gatagccgca	gccctcattt	240
gcagggggaag	atgattccctg	ccagattttc	cgggtgtgctg	cttgtctctg	ccctcatttt	300
gccagggacc	ctttgtgcag	aaggaactcg	cggcaggtca	tcacaggccc	gatgcagcct	360
tttcggaagt	gacttcgtca	acacctttga	tgggagcatg	tacagctttg	cgggatactg	420
cagttacctc	ctggcagggg	gctgccagaa	acgctccttc	tcgattattg	gggacttcca	480
gaatggcaag	agagtgaacc	tctccgtgta	tcttggggaa	ttttttgaca	tccatttgtt	540
tgtcaatggt	accgtgacac	agggggacca	aagagtctcc	atgccctatg	cctccaaagg	600
gctgtatcta	gaaactgagg	ctgggtacta	caagctgtcc	ggtgaggcct	atggccttgt	660
ggccaggatc	gatggcagcg	gcaactttca	agtctgtctg	tcagacagat	acttcaacaa	720
gacctgcggg	ctgtgtggca	actttaacat	ctttgtctga	gatgacttta	tgaccaaga	780
agggggaatg	acctcggaac	cttatgactt	tgccaactca	tgggtctctga	gcagtggaga	840
acagtgtgtg	gaacgggcat	ctcctcccag	cagctcatgc	aacatctcct	ctggggaaat	900
gcagaagggc	ctgtgggagc	agtgccagct	tctgaagagc	acctcggtgt	ttgcccgtctg	960
ccacctctctg	gtggaccctg	agccttttgt	ggcctgtgt	gagaagactt	tgtgtgagtg	1020
tgtgtggggg	ctggagtgcg	cctgccttgc	cctcctggag	tacgcccga	cctgtgccc	1080
ggagggaatg	gtgctgtacg	gctggaccga	ccacagcgcg	tgacgcccag	tgtgccctgc	1140
tggatgtgag	tataggcagt	gtgtgtcccc	ttgcgccagg	acctgccaga	gcctgcacat	1200
caatgaatatg	tgtcaggagc	gatgcgtgga	tggctgcagc	tgccctgagg	gacagctcct	1260
ggatgaaggc	ctctgcgtgg	agagcaccca	gtgtccctgc	gtgcattccg	gaaagcgcta	1320
ccctcccggc	acctccctct	ctcgagactg	caacacctgc	atttgccgaa	acagccagtg	1380
gatctgcagc	aatgaagaat	gtccagggga	gtgccttgtc	acaggtcaat	cacacttcaa	1440
gagctttgac	aacagatact	tcaccttcag	tgggatctgc	cagtacctgc	tggcccggga	1500
ttgccaggac	cactccttct	ccattgtcat	tgagactgtc	cagtgtgctg	atgaccgcga	1560
cgctgtgtgc	acccgctccg	tcaccgtccg	gctgcctggc	ctgcacaaca	gccttgtgaa	1620
actgaaagcat	ggggcaggag	ttgccatgga	tgccaggac	gtccagctcc	ccctcctgaa	1680
aggtgacctc	cgcatccagc	atacagtgac	ggcctccgtg	cgctcagct	acggggagga	1740
cctgcagatg	gactgggatg	gccgcgggag	gctgctggtg	aagctgtccc	ccgtctatgc	1800
cgggaagacc	tgccgctgtg	gtgggaatta	caatggcaac	cagggcgacg	acttccctac	1860
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SEQ ID NO:2

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Gly	Thr	Val	Thr	Gln	Gly	Asp	Gln	Arg	Val	Ser	Met	Pro	Tyr	Ala	Ser
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Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala Leu Ser Ser Gly Glu Gln
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 Trp Cys Glu Arg Pro Ser Ser Ser Cys Asn Ile Ser Ser Gly Glu Met
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 Phe Ala Arg Cys His Pro Leu Val Asp Pro Glu Pro Phe Cys Glu Lys
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 Leu Glu Tyr Ala Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly
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 Trp Thr Asp His Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu
 275 280 285
 Tyr Arg Gln Cys Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His
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 Ile Asn Glu Met Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro
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 Glu Gly Gln Leu Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys
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 Pro Cys Val His Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser
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 Val Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly
 625 630 635 640
 Gln Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser
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 Leu Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys
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 Phe Cys Pro Pro Met Asp Glu Arg Gly Asp Cys Val Pro Lys Ala Gln
 675 680 685
 Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp Ile Phe
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 Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met His Cys
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 Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val Leu Ser
 725 730 735
 Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg Pro Pro
 740 745 750
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 770 775 780
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 Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr Cys Val Cys Arg Asp
 820 825 830
 Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp Ala Thr Cys Ser Thr
 835 840 845

Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe
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 865 870 875 880
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 Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu Val Glu Gly Gly Glu
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 Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys Arg Pro Met Lys Asp
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 Glu Thr His Phe Glu Val Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu
 930 935 940
 Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg His Leu Ser Ile Ser
 945 950 955 960
 Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly
 965 970 975
 Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln
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 995 1000 1005
 Gln Cys Ala Asp Thr Arg Lys Val Pro Leu Asp Ser Ser Pro Ala
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 1055 1060 1065
 Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys Phe Cys Asp Thr Ile
 1070 1075 1080
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 1100 1105 1110
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 Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu
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Lys	Lys	Val	Thr	Leu	Asn	Pro	Ser	Asp	Pro	Glu	His	Cys	Gln	Ile
1190						1195					1200			
Cys	His	Cys	Asp	Val	Val	Asn	Leu	Thr	Cys	Glu	Ala	Cys	Gln	Glu
1205						1210					1215			
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1220						1225					1230			
Thr	Thr	Leu	Tyr	Val	Glu	Asp	Ile	Ser	Glu	Pro	Pro	Leu	His	Asp
1235						1240					1245			
Phe	Tyr	Cys	Ser	Arg	Leu	Leu	Asp	Leu	Val	Phe	Leu	Leu	Asp	Gly
1250						1255					1260			
Ser	Ser	Arg	Leu	Ser	Glu	Ala	Glu	Phe	Glu	Val	Leu	Lys	Ala	Phe
1265						1270					1275			
Val	Val	Asp	Met	Met	Glu	Arg	Leu	Arg	Ile	Ser	Gln	Lys	Trp	Val
1280						1285					1290			
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1310						1315					1320			
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1325						1330					1335			
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1340						1345					1350			
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1355						1360					1365			
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1370						1375					1380			
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1415						1420					1425			
Arg	Asp	Glu	Ile	Val	Ser	Tyr	Leu	Cys	Asp	Leu	Ala	Pro	Glu	Ala
1430						1435					1440			
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1445						1450					1455			
Pro	Gly	Leu	Leu	Gly	Val	Ser	Thr	Leu	Gly	Pro	Lys	Arg	Asn	Ser
1460						1465					1470			
Met	Val	Leu	Asp	Val	Ala	Phe	Val	Leu	Glu	Gly	Ser	Asp	Lys	Ile
1475						1480					1485			

Gly	Glu	Ala	Asp	Phe	Asn	Arg	Ser	Lys	Glu	Phe	Met	Glu	Glu	Val
1490						1495					1500			
Ile	Gln	Arg	Met	Asp	Val	Gly	Gln	Asp	Ser	Ile	His	Val	Thr	Val
1505						1510					1515			
Leu	Gln	Tyr	Ser	Tyr	Met	Val	Thr	Val	Glu	Tyr	Pro	Phe	Ser	Glu
1520						1525					1530			
Ala	Gln	Ser	Lys	Gly	Asp	Ile	Leu	Gln	Arg	Val	Arg	Glu	Ile	Arg
1535						1540					1545			
Tyr	Gln	Gly	Gly	Asn	Arg	Thr	Asn	Thr	Gly	Leu	Ala	Leu	Arg	Tyr
1550						1555					1560			
Leu	Ser	Asp	His	Ser	Phe	Leu	Val	Ser	Gln	Gly	Asp	Arg	Glu	Gln
1565						1570					1575			
Ala	Pro	Asn	Leu	Val	Tyr	Met	Val	Thr	Gly	Asn	Pro	Ala	Ser	Asp
1580						1585					1590			
Glu	Ile	Lys	Arg	Leu	Pro	Gly	Asp	Ile	Gln	Val	Val	Pro	Ile	Gly
1595						1600					1605			
Val	Gly	Pro	Asn	Ala	Asn	Val	Gln	Glu	Leu	Glu	Arg	Ile	Gly	Trp
1610						1615					1620			
Pro	Asn	Ala	Pro	Ile	Leu	Ile	Gln	Asp	Phe	Glu	Thr	Leu	Pro	Arg
1625						1630					1635			
Glu	Ala	Pro	Asp	Leu	Val	Leu	Gln	Arg	Cys	Cys	Ser	Gly	Glu	Gly
1640						1645					1650			
Leu	Gln	Ile	Pro	Thr	Leu	Ser	Pro	Ala	Pro	Asp	Cys	Ser	Gln	Pro
1655						1660					1665			
Leu	Asp	Val	Ile	Leu	Leu	Leu	Asp	Gly	Ser	Ser	Ser	Phe	Pro	Ala
1670						1675					1680			
Ser	Tyr	Phe	Asp	Glu	Met	Lys	Ser	Phe	Ala	Lys	Ala	Phe	Ile	Ser
1685						1690					1695			
Lys	Ala	Asn	Ile	Gly	Pro	Arg	Leu	Thr	Gln	Val	Ser	Val	Leu	Gln
1700						1705					1710			
Tyr	Gly	Ser	Ile	Thr	Thr	Ile	Asp	Val	Pro	Trp	Asn	Val	Val	Pro
1715						1720					1725			
Glu	Lys	Ala	His	Leu	Leu	Ser	Leu	Val	Asp	Val	Met	Gln	Arg	Glu
1730						1735					1740			
Gly	Gly	Pro	Ser	Gln	Ile	Gly	Asp	Ala	Leu	Gly	Phe	Ala	Val	Arg
1745						1750					1755			
Tyr	Leu	Thr	Ser	Glu	Met	His	Gly	Ala	Arg	Pro	Gly	Ala	Ser	Lys
1760						1765					1770			
Ala	Val	Val	Ile	Leu	Val	Thr	Asp	Val	Ser	Val	Asp	Ser	Val	Asp
1775						1780					1785			
Ala	Ala	Ala	Asp	Ala	Ala	Arg	Ser	Asn	Arg	Val	Thr	Val	Phe	Pro
1790						1795					1800			

Ile Gly 1805	Ile Gly Asp Arg Tyr 1810	Asp Ala Ala Gln Leu 1815	Arg Ile Leu
Ala Gly 1820	Pro Ala Gly Asp Ser 1825	Asn Val Val Lys Leu 1830	Gln Arg Ile
Glu Asp 1835	Leu Pro Thr Met Val 1840	Thr Leu Gly Asn Ser 1845	Phe Leu His
Lys Leu 1850	Cys Ser Gly Phe Val 1855	Arg Ile Cys Met Asp 1860	Glu Asp Gly
Asn Glu 1865	Lys Arg Pro Gly Asp 1870	Val Trp Thr Leu Pro 1875	Asp Gln Cys
His Thr 1880	Val Thr Cys Gln Pro 1885	Asp Gly Gln Thr Leu 1890	Leu Lys Ser
His Arg 1895	Val Asn Cys Asp Arg 1900	Gly Leu Arg Pro Ser 1905	Cys Pro Asn
Ser Gln 1910	Ser Pro Val Lys Val 1915	Glu Glu Thr Cys Gly 1920	Cys Arg Trp
Thr Cys 1925	Pro Cys Val Cys Thr 1930	Gly Ser Ser Thr Arg 1935	His Ile Val
Thr Phe 1940	Asp Gly Gln Asn Phe 1945	Lys Leu Thr Gly Ser 1950	Cys Ser Tyr
Val Leu 1955	Phe Gln Asn Lys Glu 1960	Gln Asp Leu Glu Val 1965	Ile Leu His
Asn Gly 1970	Ala Cys Ser Pro Gly 1975	Ala Arg Gln Gly Cys 1980	Met Lys Ser
Ile Glu 1985	Val Lys His Ser Ala 1990	Leu Ser Val Glu Leu 1995	His Ser Asp
Met Glu 2000	Val Thr Val Asn Gly 2005	Arg Leu Val Ser Val 2010	Pro Tyr Val
Gly Gly 2015	Asn Met Glu Val Asn 2020	Val Tyr Gly Ala Ile 2025	Met His Glu
Val Arg 2030	Phe Asn His Leu Gly 2035	His Ile Phe Thr Phe 2040	Thr Pro Gln
Asn Asn 2045	Glu Phe Gln Leu Gln 2050	Leu Ser Pro Lys Thr 2055	Phe Ala Ser
Lys Thr 2060	Tyr Gly Leu Cys Gly 2065	Ile Cys Asp Glu Asn 2070	Gly Ala Asn
Asp Phe 2075	Met Leu Arg Asp Gly 2080	Thr Val Thr Thr Asp 2085	Trp Lys Thr
Leu Val 2090	Gln Glu Trp Thr Val 2095	Gln Arg Pro Gly Gln 2100	Thr Cys Gln
Pro Glu 2105	Gln Cys Leu Val Pro 2110	Asp Ser Ser His Cys 2115	Gln Val Leu

Leu	Leu	Pro	Leu	Phe	Ala	Glu	Cys	His	Lys	Val	Leu	Ala	Pro	Ala
2120						2125					2130			
Thr	Phe	Tyr	Ala	Ile	Cys	Gln	Gln	Asp	Ser	Cys	His	Gln	Glu	Gln
2135						2140					2145			
Val	Cys	Glu	Val	Ile	Ala	Ser	Tyr	Ala	His	Leu	Cys	Arg	Thr	Asn
2150						2155					2160			
Gly	Val	Cys	Val	Asp	Trp	Arg	Thr	Pro	Asp	Phe	Cys	Ala	Met	Ser
2165						2170					2175			
Cys	Pro	Pro	Ser	Leu	Val	Tyr	Asn	His	Cys	Glu	His	Gly	Cys	Pro
2180						2185					2190			
Arg	His	Cys	Asp	Gly	Asn	Val	Ser	Ser	Cys	Gly	Asp	His	Pro	Ser
2195						2200					2205			
Glu	Gly	Cys	Phe	Cys	Pro	Pro	Asp	Lys	Val	Met	Leu	Glu	Gly	Ser
2210						2215					2220			
Cys	Val	Pro	Glu	Glu	Ala	Cys	Thr	Gln	Cys	Ile	Gly	Glu	Asp	Gly
2225						2230					2235			
Val	Gln	His	Gln	Phe	Leu	Glu	Ala	Trp	Val	Pro	Asp	His	Gln	Pro
2240						2245					2250			
Cys	Gln	Ile	Cys	Thr	Cys	Leu	Ser	Gly	Arg	Lys	Val	Asn	Cys	Thr
2255						2260					2265			
Thr	Gln	Pro	Cys	Pro	Thr	Ala	Lys	Ala	Pro	Thr	Cys	Gly	Leu	Cys
2270						2275					2280			
Glu	Val	Ala	Arg	Leu	Arg	Gln	Asn	Ala	Asp	Gln	Cys	Cys	Pro	Glu
2285						2290					2295			
Tyr	Glu	Cys	Val	Cys	Asp	Pro	Val	Ser	Cys	Asp	Leu	Pro	Pro	Val
2300						2305					2310			
Pro	His	Cys	Glu	Arg	Gly	Leu	Gln	Pro	Thr	Leu	Thr	Asn	Pro	Gly
2315						2320					2325			
Glu	Cys	Arg	Pro	Asn	Phe	Thr	Cys	Ala	Cys	Arg	Lys	Glu	Glu	Cys
2330						2335					2340			
Lys	Arg	Val	Ser	Pro	Pro	Ser	Cys	Pro	Pro	His	Arg	Leu	Pro	Thr
2345						2350					2355			
Leu	Arg	Lys	Thr	Gln	Cys	Cys	Asp	Glu	Tyr	Glu	Cys	Ala	Cys	Asn
2360						2365					2370			
Cys	Val	Asn	Ser	Thr	Val	Ser	Cys	Pro	Leu	Gly	Tyr	Leu	Ala	Ser
2375						2380					2385			
Thr	Ala	Thr	Asn	Asp	Cys	Gly	Cys	Thr	Thr	Thr	Thr	Cys	Leu	Pro
2390						2395					2400			
Asp	Lys	Val	Cys	Val	His	Arg	Ser	Thr	Ile	Tyr	Pro	Val	Gly	Gln
2405						2410					2415			
Phe	Trp	Glu	Glu	Gly	Cys	Asp	Val	Cys	Thr	Cys	Thr	Asp	Met	Glu
2420						2425					2430			

Asp	Ala	Val	Met	Gly	Leu	Arg	Val	Ala	Gln	Cys	Ser	Gln	Lys	Pro
2435						2440					2445			
Cys	Glu	Asp	Ser	Cys	Arg	Ser	Gly	Phe	Thr	Tyr	Val	Leu	His	Glu
2450						2455					2460			
Gly	Glu	Cys	Cys	Gly	Arg	Cys	Leu	Pro	Ser	Ala	Cys	Glu	Val	Val
2465						2470					2475			
Thr	Gly	Ser	Pro	Arg	Gly	Asp	Ser	Gln	Ser	Ser	Trp	Lys	Ser	Val
2480						2485					2490			
Gly	Ser	Gln	Trp	Glu	Asn	Pro	Cys	Leu	Ile	Asn	Glu	Cys	Val	Arg
2495						2500					2505			
Val	Lys	Glu	Glu	Val	Phe	Ile	Gln	Gln	Arg	Asn	Val	Ser	Cys	Pro
2510						2515					2520			
Gln	Leu	Glu	Val	Pro	Val	Cys	Pro	Ser	Gly	Phe	Gln	Leu	Ser	Cys
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Lys	Thr	Ser	Ala	Cys	Cys	Pro	Ser	Cys	Arg	Cys	Glu	Arg	Met	Glu
2540						2545					2550			
Ala	Cys	Met	Leu	Asn	Gly	Thr	Val	Ile	Gly	Pro	Gly	Lys	Thr	Val
2555						2560					2565			
Met	Ile	Asp	Val	Cys	Thr	Thr	Cys	Arg	Cys	Met	Val	Gln	Val	Gly
2570						2575					2580			
Val	Ile	Ser	Gly	Phe	Lys	Leu	Glu	Cys	Arg	Lys	Thr	Thr	Cys	Asn
2585						2590					2595			
Pro	Cys	Pro	Leu	Gly	Tyr	Lys	Glu	Glu	Asn	Asn	Thr	Gly	Glu	Cys
2600						2605					2610			
Cys	Gly	Arg	Cys	Leu	Pro	Thr	Ala	Cys	Thr	Ile	Gln	Leu	Arg	Gly
2615						2620					2625			
Gly	Gln	Ile	Met	Thr	Leu	Lys	Arg	Asp	Glu	Thr	Leu	Gln	Asp	Gly
2630						2635					2640			
Cys	Asp	Thr	His	Phe	Cys	Lys	Val	Asn	Glu	Arg	Gly	Glu	Tyr	Phe
2645						2650					2655			
Trp	Glu	Lys	Arg	Val	Thr	Gly	Cys	Pro	Pro	Phe	Asp	Glu	His	Lys
2660						2665					2670			
Cys	Leu	Ala	Glu	Gly	Gly	Lys	Ile	Met	Lys	Ile	Pro	Gly	Thr	Cys
2675						2680					2685			
Cys	Asp	Thr	Cys	Glu	Glu	Pro	Glu	Cys	Asn	Asp	Ile	Thr	Ala	Arg
2690						2695					2700			
Leu	Gln	Tyr	Val	Lys	Val	Gly	Ser	Cys	Lys	Ser	Glu	Val	Glu	Val
2705						2710					2715			
Asp	Ile	His	Tyr	Cys	Gln	Gly	Lys	Cys	Ala	Ser	Lys	Ala	Met	Tyr
2720						2725					2730			
Ser	Ile	Asp	Ile	Asn	Asp	Val	Gln	Asp	Gln	Cys	Ser	Cys	Cys	Ser

2735 2740 2745
 Pro Thr Arg Thr Glu Pro Met Gln His Cys Thr Asn Gly Ser Val
 2750 2755 2760
 Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys Ser Pro
 2765 2770 2775
 Arg Lys Cys Ser Lys
 2780

SEQ ID NO:3

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 Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr
 20 25 30
 Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
 35 40 45
 Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
 50 55 60
 Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
 65 70 75 80
 Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
 85 90 95
 Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr
 100 105 110
 Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr
 115 120 125
 Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile
 130 135 140
 Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys
 145 150 155 160
 Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly
 165 170 175
 Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val
 180 185 190
 Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser
 195 200 205
 Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr
 210 215 220
 Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln
 225 230 235 240
 Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val
 245 250 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg
 260 265 270
 Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met
 275 280 285
 Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val
 290 295 300
 Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val
 305 310 315 320
 Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys
 325 330 335
 Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly
 340 345 350
 Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu
 355 360 365
 Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn
 370 375 380
 Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu
 385 390 395 400
 Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro
 405 410 415
 Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp
 420 425 430
 Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys
 435 440 445
 Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys
 450 455 460
 Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu
 465 470 475 480
 Val Val Pro Pro Thr Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val
 485 490 495
 Glu Asp Ile Ser Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu
 500 505 510
 Leu Asp Leu Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala
 515 520 525
 Glu Phe Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu
 530 535 540
 Arg Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp
 545 550 555 560
 Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser Glu
 565 570 575
 Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln Val Ala
 580 585 590

Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile Phe Ser Lys
 595 600 605
 Ile Asp Arg Pro Glu Ala Ser Arg Ile Thr Leu Leu Leu Met Ala Ser
 610 615 620
 Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val Arg Tyr Val Gln Gly
 625 630 635 640
 Leu Lys Lys Lys Lys Val Ile Val Ile Pro Val Gly Ile Gly Pro His
 645 650 655
 Ala Asn Leu Lys Gln Ile Arg Leu Ile Glu Lys Gln Ala Pro Glu Asn
 660 665 670
 Lys Ala Phe Val Leu Ser Ser Val Asp Glu Leu Glu Gln Gln Arg Asp
 675 680 685
 Glu Ile Val Ser Tyr Leu Cys Asp Leu Ala Pro Glu Ala Pro Pro Pro
 690 695 700
 Thr Leu Pro Pro Asp Met Ala Gln Val Thr Val Gly Pro Gly Leu Leu
 705 710 715 720
 Gly Val Ser Thr Leu Gly Pro Lys Arg Asn Ser Met Val Leu Asp Val
 725 730 735
 Ala Phe Val Leu Glu Gly Ser Asp Lys Ile Gly Glu Ala Asp Phe Asn
 740 745 750
 Arg Ser Lys Glu Phe Met Glu Glu Val Ile Gln Arg Met Asp Val Gly
 755 760 765
 Gln Asp Ser Ile His Val Thr Val Leu Gln Tyr Ser Tyr Met Val Thr
 770 775 780
 Val Glu Tyr Pro Phe Ser Glu Ala Gln Ser Lys Gly Asp Ile Leu Gln
 785 790 795 800
 Arg Val Arg Glu Ile Arg Tyr Gln Gly Gly Asn Arg Thr Asn Thr Gly
 805 810 815
 Leu Ala Leu Arg Tyr Leu Ser Asp His Ser Phe Leu Val Ser Gln Gly
 820 825 830
 Asp Arg Glu Gln Ala Pro Asn Leu Val Tyr Met Val Thr Gly Asn Pro
 835 840 845
 Ala Ser Asp Glu Ile Lys Arg Leu Pro Gly Asp Ile Gln Val Val Pro
 850 855 860
 Ile Gly Val Gly Pro Asn Ala Asn Val Gln Glu Leu Glu Arg Ile Gly
 865 870 875 880
 Trp Pro Asn Ala Pro Ile Leu Ile Gln Asp Phe Glu Thr Leu Pro Arg
 885 890 895
 Glu Ala Pro Asp Leu Val Leu Gln Arg Cys Cys Ser Gly Glu Gly Leu
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 Gln Ile Pro Thr Leu Ser Pro Ala Pro Asp Cys Ser Gln Pro Leu Asp
 915 920 925

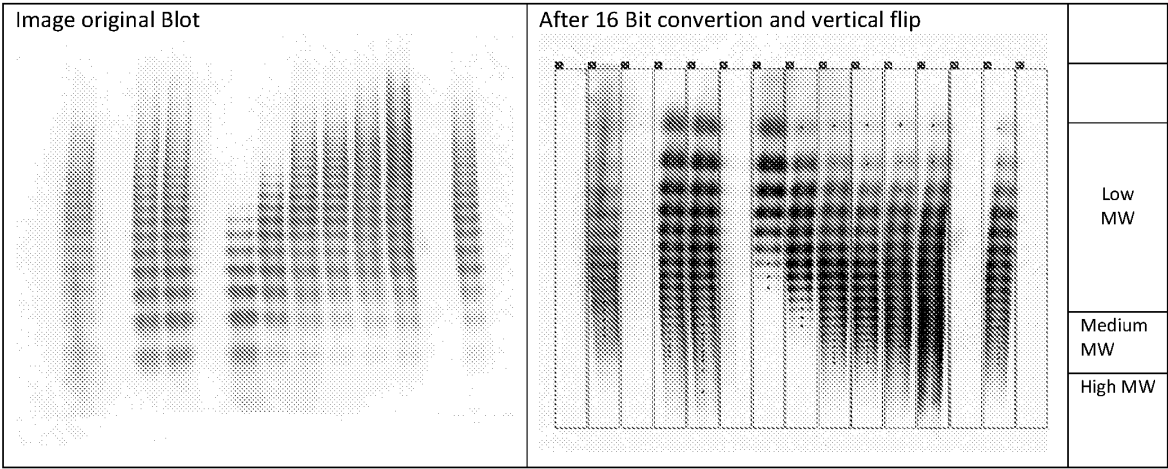
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 945 950 955 960
 Gly Pro Arg Leu Thr Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr
 965 970 975
 Thr Ile Asp Val Pro Trp Asn Val Val Pro Glu Lys Ala His Leu Leu
 980 985 990
 Ser Leu Val Asp Val Met Gln Arg Glu Gly Gly Pro Ser Gln Ile Gly
 995 1000 1005
 Asp Ala Leu Gly Phe Ala Val Arg Tyr Leu Thr Ser Glu Met His
 1010 1015 1020
 Gly Ala Arg Pro Gly Ala Ser Lys Ala Val Val Ile Leu Val Thr
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 Asp Val Ser Val Asp Ser Val Asp Ala Ala Ala Asp Ala Ala Arg
 1040 1045 1050
 Ser Asn Arg Val Thr Val Phe Pro Ile Gly Ile Gly Asp Arg Tyr
 1055 1060 1065
 Asp Ala Ala Gln Leu Arg Ile Leu Ala Gly Pro Ala Gly Asp Ser
 1070 1075 1080
 Asn Val Val Lys Leu Gln Arg Ile Glu Asp Leu Pro Thr Met Val
 1085 1090 1095
 Thr Leu Gly Asn Ser Phe Leu His Lys Leu Cys Ser Gly Phe Val
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 Arg Ile Cys Met Asp Glu Asp Gly Asn Glu Lys Arg Pro Gly Asp
 1115 1120 1125
 Val Trp Thr Leu Pro Asp Gln Cys His Thr Val Thr Cys Gln Pro
 1130 1135 1140
 Asp Gly Gln Thr Leu Leu Lys Ser His Arg Val Asn Cys Asp Arg
 1145 1150 1155
 Gly Leu Arg Pro Ser Cys Pro Asn Ser Gln Ser Pro Val Lys Val
 1160 1165 1170
 Glu Glu Thr Cys Gly Cys Arg Trp Thr Cys Pro Cys Val Cys Thr
 1175 1180 1185
 Gly Ser Ser Thr Arg His Ile Val Thr Phe Asp Gly Gln Asn Phe
 1190 1195 1200
 Lys Leu Thr Gly Ser Cys Ser Tyr Val Leu Phe Gln Asn Lys Glu
 1205 1210 1215
 Gln Asp Leu Glu Val Ile Leu His Asn Gly Ala Cys Ser Pro Gly
 1220 1225 1230
 Ala Arg Gln Gly Cys Met Lys Ser Ile Glu Val Lys His Ser Ala
 1235 1240 1245

Leu 1250	Val	Glu	Leu	His	Ser 1255	Asp	Met	Glu	Val	Thr 1260	Val	Asn	Gly	
Arg 1265	Leu	Val	Ser	Val	Pro 1270	Tyr	Val	Gly	Gly	Asn 1275	Met	Glu	Val	Asn
Val 1280	Tyr	Gly	Ala	Ile	Met 1285	His	Glu	Val	Arg	Phe 1290	Asn	His	Leu	Gly
His 1295	Ile	Phe	Thr	Phe	Thr 1300	Pro	Gln	Asn	Asn	Glu	Phe 1305	Gln	Leu	Gln
Leu 1310	Ser	Pro	Lys	Thr	Phe 1315	Ala	Ser	Lys	Thr	Tyr	Gly 1320	Leu	Cys	Gly
Ile 1325	Cys	Asp	Glu	Asn	Gly 1330	Ala	Asn	Asp	Phe	Met 1335	Leu	Arg	Asp	Gly
Thr 1340	Val	Thr	Thr	Asp	Trp 1345	Lys	Thr	Leu	Val	Gln	Glu 1350	Trp	Thr	Val
Gln 1355	Arg	Pro	Gly	Gln	Thr 1360	Cys	Gln	Pro	Ile	Leu	Glu 1365	Glu	Gln	Cys
Leu 1370	Val	Pro	Asp	Ser	Ser 1375	His	Cys	Gln	Val	Leu	Leu 1380	Leu	Pro	Leu
Phe 1385	Ala	Glu	Cys	His	Lys 1390	Val	Leu	Ala	Pro	Ala	Thr 1395	Phe	Tyr	Ala
Ile 1400	Cys	Gln	Gln	Asp	Ser 1405	Cys	His	Gln	Glu	Gln	Val 1410	Cys	Glu	Val
Ile 1415	Ala	Ser	Tyr	Ala	His 1420	Leu	Cys	Arg	Thr	Asn	Gly 1425	Val	Cys	Val
Asp 1430	Trp	Arg	Thr	Pro	Asp 1435	Phe	Cys	Ala	Met	Ser	Cys 1440	Pro	Pro	Ser
Leu 1445	Val	Tyr	Asn	His	Cys 1450	Glu	His	Gly	Cys	Pro	Arg 1455	His	Cys	Asp
Gly 1460	Asn	Val	Ser	Ser	Cys 1465	Gly	Asp	His	Pro	Ser	Glu 1470	Gly	Cys	Phe
Cys 1475	Pro	Pro	Asp	Lys	Val 1480	Met	Leu	Glu	Gly	Ser	Cys 1485	Val	Pro	Glu
Glu 1490	Ala	Cys	Thr	Gln	Cys 1495	Ile	Gly	Glu	Asp	Gly	Val 1500	Gln	His	Gln
Phe 1505	Leu	Glu	Ala	Trp	Val 1510	Pro	Asp	His	Gln	Pro	Cys 1515	Gln	Ile	Cys
Thr 1520	Cys	Leu	Ser	Gly	Arg 1525	Lys	Val	Asn	Cys	Thr	Thr 1530	Gln	Pro	Cys
Pro 1535	Thr	Ala	Lys	Ala	Pro 1540	Thr	Cys	Gly	Leu	Cys	Glu 1545	Val	Ala	Arg
Leu 1550	Arg	Gln	Asn	Ala	Asp 1555	Gln	Cys	Cys	Pro	Glu	Tyr 1560	Glu	Cys	Val

Cys Asp 1565	Pro Val Ser Cys Asp 1570	Leu Pro Pro Val Pro 1575	His Cys Glu
Arg Gly 1580	Leu Gln Pro Thr Leu 1585	Thr Asn Pro Gly Glu 1590	Cys Arg Pro
Asn Phe 1595	Thr Cys Ala Cys Arg 1600	Lys Glu Glu Cys Lys 1605	Arg Val Ser
Pro Pro 1610	Ser Cys Pro Pro His 1615	Arg Leu Pro Thr Leu 1620	Arg Lys Thr
Gln Cys 1625	Cys Asp Glu Tyr Glu 1630	Cys Ala Cys Asn Cys 1635	Val Asn Ser
Thr Val 1640	Ser Cys Pro Leu Gly 1645	Tyr Leu Ala Ser Thr 1650	Ala Thr Asn
Asp Cys 1655	Gly Cys Thr Thr Thr 1660	Thr Cys Leu Pro Asp 1665	Lys Val Cys
Val His 1670	Arg Ser Thr Ile Tyr 1675	Pro Val Gly Gln Phe 1680	Trp Glu Glu
Gly Cys 1685	Asp Val Cys Thr Cys 1690	Thr Asp Met Glu Asp 1695	Ala Val Met
Gly Leu 1700	Arg Val Ala Gln Cys 1705	Ser Gln Lys Pro Cys 1710	Glu Asp Ser
Cys Arg 1715	Ser Gly Phe Thr Tyr 1720	Val Leu His Glu Gly 1725	Glu Cys Cys
Gly Arg 1730	Cys Leu Pro Ser Ala 1735	Cys Glu Val Val Thr 1740	Gly Ser Pro
Arg Gly 1745	Asp Ser Gln Ser Ser 1750	Trp Lys Ser Val Gly 1755	Ser Gln Trp
Ala Ser 1760	Pro Glu Asn Pro Cys 1765	Leu Ile Asn Glu Cys 1770	Val Arg Val
Lys Glu 1775	Glu Val Phe Ile Gln 1780	Gln Arg Asn Val Ser 1785	Cys Pro Gln
Leu Glu 1790	Val Pro Val Cys Pro 1795	Ser Gly Phe Gln Leu 1800	Ser Cys Lys
Thr Ser 1805	Ala Cys Cys Pro Ser 1810	Cys Arg Cys Glu Arg 1815	Met Glu Ala
Cys Met 1820	Leu Asn Gly Thr Val 1825	Ile Gly Pro Gly Lys 1830	Thr Val Met
Ile Asp 1835	Val Cys Thr Thr Cys 1840	Arg Cys Met Val Gln 1845	Val Gly Val
Ile Ser 1850	Gly Phe Lys Leu Glu 1855	Cys Arg Lys Thr Thr 1860	Cys Asn Pro
Cys Pro 1865	Leu Gly Tyr Lys Glu 1870	Glu Asn Asn Thr Gly 1875	Glu Cys Cys

Gly Arg Cys Leu Pro Thr Ala Cys Thr Ile Gln Leu Arg Gly Gly	1880	1885	1890
Gln Ile Met Thr Leu Lys Arg Asp Glu Thr Leu Gln Asp Gly Cys	1895	1900	1905
Asp Thr His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Phe Trp	1910	1915	1920
Glu Lys Arg Val Thr Gly Cys Pro Pro Phe Asp Glu His Lys Cys	1925	1930	1935
Leu Ala Glu Gly Gly Lys Ile Met Lys Ile Pro Gly Thr Cys Cys	1940	1945	1950
Asp Thr Cys Glu Glu Pro Glu Cys Asn Asp Ile Thr Ala Arg Leu	1955	1960	1965
Gln Tyr Val Lys Val Gly Ser Cys Lys Ser Glu Val Glu Val Asp	1970	1975	1980
Ile His Tyr Cys Gln Gly Lys Cys Ala Ser Lys Ala Met Tyr Ser	1985	1990	1995
Ile Asp Ile Asn Asp Val Gln Asp Gln Cys Ser Cys Cys Ser Pro	2000	2005	2010
Thr Arg Thr Glu Pro Met Gln Val Ala Leu His Cys Thr Asn Gly	2015	2020	2025
Ser Val Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys	2030	2035	2040
Ser Pro Arg Lys Cys Ser Lys	2045	2050	

FIG. 61



Order of samples per lane

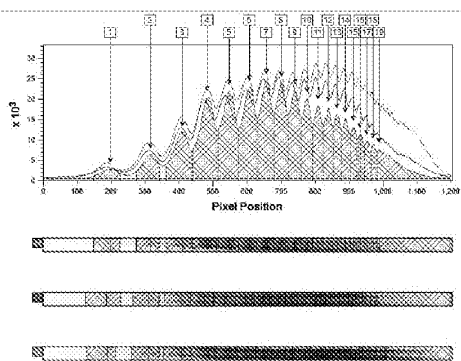
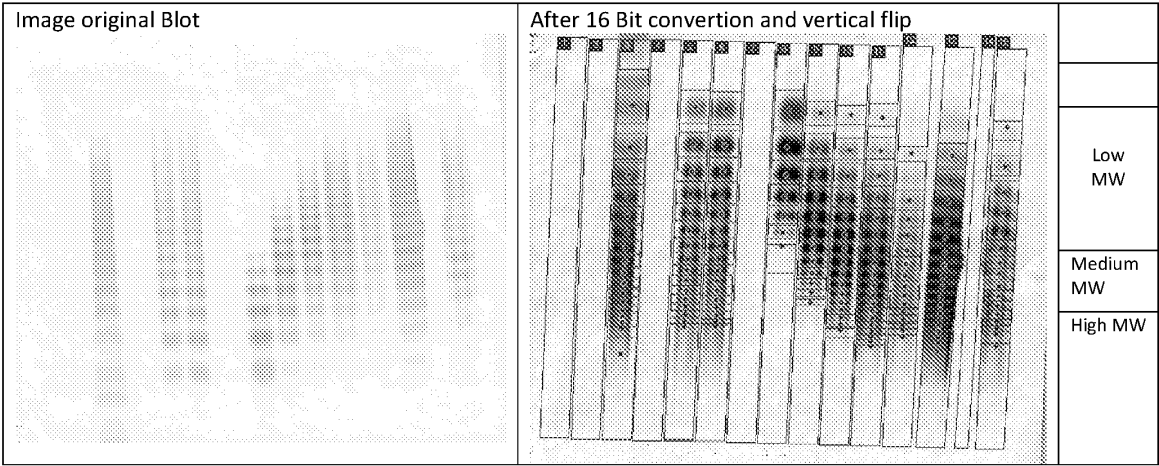
LANE	SAMPLE	Step	Overlay Lane 11+12 + 14
1	SB		
2	Control: Standard Human Plasma Dade Behring		
3	SB		
4	VW_USS_04 L prior SD treatment		
5	VW_USS_04 L		
6	SB		
7	VW_USS_04 FT		
8	VW_USS_04 W1		
9	VW_USS_04 W2		
10	VW_USS_04 VE		
11	VW_USS_04 E		
12	VW_USS_04 NE		
13	SB		
14	ORVWSEC16070F (530 µg/ml Antigen content)	Pos. Control	
15	SB		

FIG. 62



Order of samples per lane

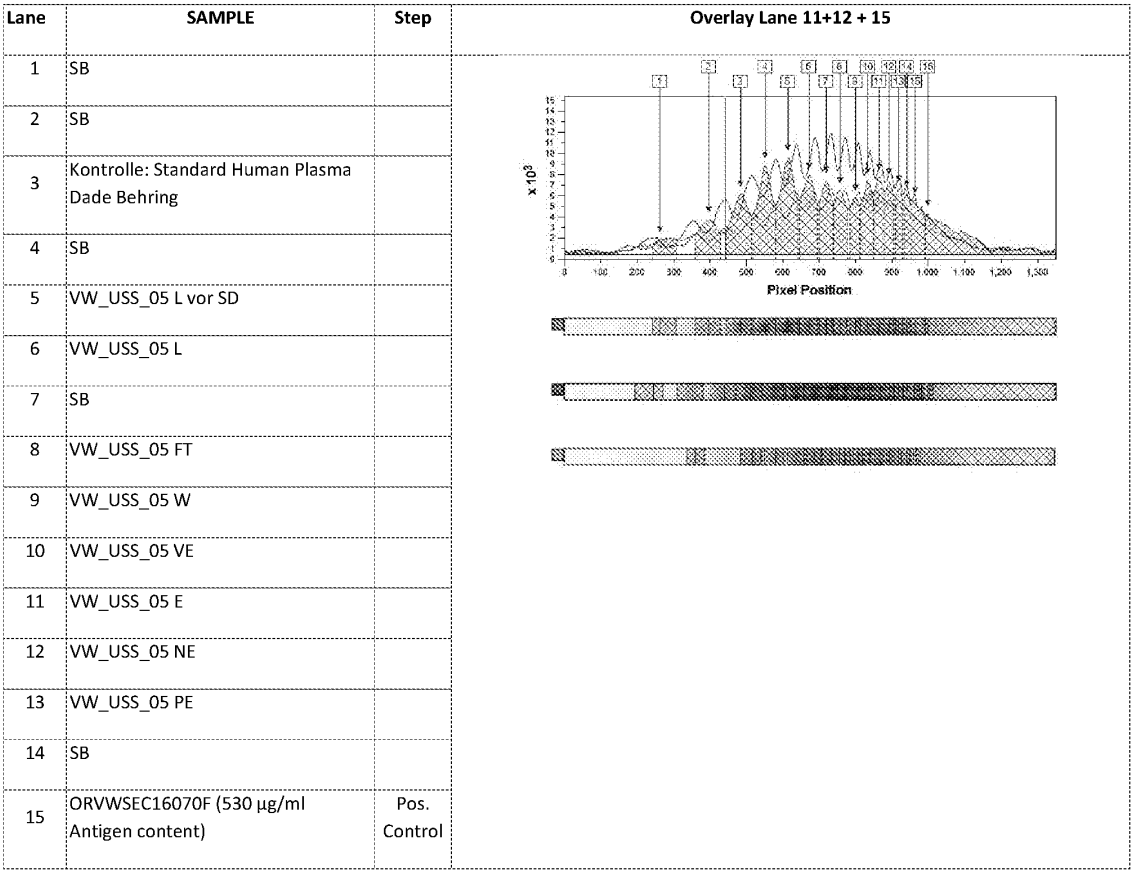
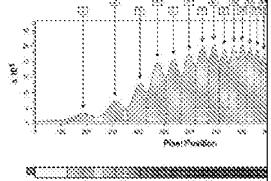
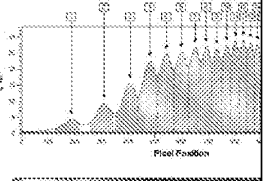
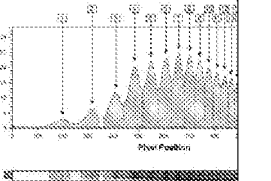
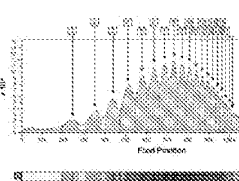
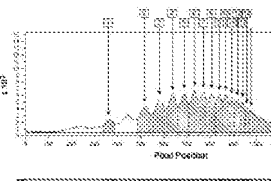
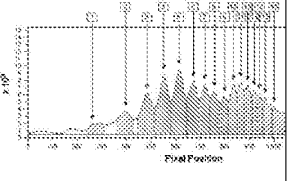


FIG. 63

RESULTS: EVALUATION

		VW_USS_04 E	VW_USS_04 NE	ORVWSEC16070F Positiv control
Lane Profiles:				
		Lane 11	Lane 12	Lane 14
		Band %	Band %	Band %
SUM Band 1-6	Low MW	34,91	32,93	40,86
SUM Band 7-12	Medium MW	39	33,81	40,27
SUM Band >12	High MW	26,08	33,24	18,87

DF3362/023

		VW_USS_05 E	VW_USS_05 NE	ORVWSEC16070F Positive control
Lane Profiles:				
		Lane 11	Lane 12	Lane 15
		Band %	Band %	Band %
SUM Band 1-6	Low MW	38,39	29,65	37,21
SUM Band 7-12	Medium MW	36,87	36,97	29,51
SUM Band >12	High MW	24,74	33,38	33,27
Data of ORVWSEC16070F Positivkontrol are not valid due deformation of the blot (see image on Original Blot and Lane Profile)				
Remark : Usually lane 15 is not used because the border zone is not easy to handle				

764	770		780		790		800	
SLSCRPP	MVKLVCPADN	LRAEGLECTK	TCQNYDLECM					
810	820	830	840	850				
SMGCVSGCLC	PPGMVRHENR	CVALERCPCF	HQGKEYAPGE	TVKIGCNTCV				
860	870	880	890	900				
CQDRKWNTCTD	HVCDATCSTI	GMAHYLTFDG	LKYLFPGECQ	YVLVQDYCGS				
910	920	930	940	950				
NPGTFRILVG	NKGCSHPSVK	CKKRVTILVE	GGEIELFDGE	VNVKRPMPKDE				
960	970	980	990	1000				
THFEVVESGR	YIILLGKAL	SVVWDRHLIS	SVVLKQTYQE	KVCGLCGNFD				
1010	1020	1030	1040	1050				
GIQNNDLTSS	NLQVEEDPVD	FGNSWKVSSQ	CADTRKVPLD	SSPATCHNNI				
1060	1070	1080	1090	1100				
MKQTMVDSSC	RILTSDVFQD	CNKLVDPPEPY	LDVCIYDTCS	CESIGDCACF				
1110	1120	1130	1140	1150				
CDTIAAYAHV	CAQHGVVITW	RTATLCPQSC	EERNLRENGY	ECEWRYNSCA				
1160	1170	1180	1190	1200				
PACQVTCQHP	EPLACPVCV	EGCHAHCPPG	KILDELLQTC	VPEDPCPVCE				
1210	1220	1230	1240	1250				
VAGRRFASGK	KVTLNPSDPE	HCQICHCDVV	NLTCEACQEP	GGLVVPPTDA				
1260	1270	1280	1290	1300				
PVSPTTLTYE	DISEPPLHDF	YCSRLLDLVF	LLDGSSRLSE	AEFEVLKAFV				
1310	1320	1330	1340	1350				
VDMMERLRIS	QKWWRVAVVE	YHDGSHAYIG	LKD RKR	YYLGAVE	LSWDYMQS	DSL	GELPVDARFP	
60	70	80	90	100				
PRVPKSFFPN	TSVVYKKTLF	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY				
110	120	130	140	150				
DTVVITLKMN	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG				
160	170	180	190	200				
GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE				
210	220	230	240	250				
GSLAKEKTQT	HKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM				
260	270	280	290	300				
HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH				
310	320	330	340	350				
RQASLEISPI	TFLTAAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE				
360	370	380	390	400				

EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 410 420 430 440 450
 WWHYIAEEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 460 470 480 490 500
 TDETFKTREA IQHESGILGP LLYGEVGD TL LIFKNQASR PYNIYPHGIT
 510 520 530 540 550
 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 560 570 580 590 600
 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 610 620 630 640 650
 NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 660 670 680 690 700
 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 710 720 730 740 750
 MENPGLWLIG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 ⇐FVIII 760 VWF⇒(2218) VWF2230 VWF2240 VWF2250
 SKNNAIEPRS RHC DGNVSSCGDH PSEGCFCPPD KVMLEGSCVP
 VWF2260 VWF2270 VWF2280 VWF2290 VWF2300
 EEACTQCIGE DGVQHQFLEA WVPDQHQCQI CTCLSGRKVN CTTQPCPTAK
 VWF2310 VWF2320 VWF2330 VWF2340 VWF2350
 APTCGLCEVA RLRQNADQCC PEYECVCDPV SCDLPPVPHC ERGLQPTLTN
 VWF2360 VWF2370 VWF2380 VWF2390 VWF2400
 PGECPNFTC ACRKEECKRV SPPSCPPHRL PTLRKTQCCD EYECACNCVN
 VWF2410 VWF2420 VWF2430 VWF2440 VWF2450
 STVSCPLGYL ASTATND CGC TTTTCLPDKV CVHRSTIYPV GQFWEEGCDV
 VWF2460 VWF2470 VWF2480 VWF2490 VWF2500
 CTCTDMEDAV MGLRVAQCSQ KPCEDSCRSG FTYVLHEGEC CGRCLPSACE
 VWF2510 VWF2520 VWF2530 VWF2540 VWF2550
 VVTGSPRGDS QSSWKS VGSQ WASPENPCLI NECVRVKEEV FIQQRNVSCP
 VWF2560 VWF2570 VWF2580 VWF2590 VWF2593
 QLEVPVCPSG FQLSCKTSAC CPSCRCERME ACMLNGTVIG PGK
FVIII ⇐(LIGHT CHAIN)
 1333 1340 1350
 ALKQFRLP LEETELEKRI
 1360 1370 1380 1390 1400
 IVDDTSTQWS KNMKHLTPST LTQIDYNEKE KGAITQSPLS DCLTRSHSIP
 1410 1420 1430 1440 1450
 QANRSPLPIA KVSSFPSIRP IYLTRVLFQD NSSHLPAASY RKKDSGVQES
 1460 1470 1480 1490 1500
 SHFLQGAKKN NLSLAILTLE MTGDQREVGS LGTSATNSVT YKKVENTVLP
 1510 1520 1530 1540 1550
 KPDLPKTSGK VELLPKVHIY QKDLFPTETS NGSPGHLDLV EGSL LQGTEG
 1560 1570 1580 1590 1600
 AIKWNEANRP GKVPFLRVAT ESSAKTPSKL LDPLAWDNHY GTQIPKEEWK
 1610 1620 1630 1640 1650
 SKEKSPEKTA FKKKDTILSL NACESNHAIA AINEGQNKPE IEVTWAKQGR
 1660 1670 1680 1690 1700
 TERLCSQNPP VLKRHQREIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1710 1720 1730 1740 1750
 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
 1760 1770 1780 1790 1800
 KVVFEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR

1810	1820	1830	1840	1850
PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
1860	1870	1880	1890	1900
CKAWAYFSDV	DLEKDVHSGL	IGPLLVCHTN	TLNPAHGRQV	TVQEFALFFT
1910	1920	1930	1940	1950
IFDETKSWYF	TENMERN CRA	PCNIQMEDPT	FKENYRFHAI	NGYIMDTLPG
1960	1970	1980	1990	2000
LVMAQDQRIR	WYLLSMGSNE	NIHSIHFSGH	VFTVRKKEEY	KMALYNLYPG
2010	2020	2030	2040	2050
VFETVEMLP SK	AGIWRVECL	IGEHLHAGMS	TLFLVYSNKC	QTPLGMASGH
2060	2070	2080	2090	2100
IRDFQITASG	QYGQWAPKLA	RLHYSGSINA	WSTKEPFSWI	KVDLLAPMII
2110	2120	2130	2140	2150
HGIKTQGARQ	KFSSLYISQF	IIMYSLDGKK	WQTYRGNSTG	TLMVFFGNVD
2160	2170	2180	2190	2200
SSGIKHNIFN	PPIIARYIRL	HPTHYSIRST	LRMELMGCDL	NSCSMPLGME
2210	2220	2230	2240	2250
SKAISDAQIT	ASSYFTNMFA	TWSPSKARLH	LQGRSNAWRP	QVNNPKEWLQ
2260	2270	2280	2290	2300
VDFQKTMKVT	GVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFFQNGKVK
2310	2320	2330	2340	2350
VFQGNQDSFT	PVNSLDPPL	LTRYLRIHPQ	SWWHQIALRM	EVLGCEAQDL
2351 ⇐FVIII	WVF ⇒2620	2630	2640	2650
Y	R	KTTCNPCPLG	YKEENNTGEC	CGRCLPTACT
2660	2670	2680	2690	2700
IQLRGGQIMT	LKRDETLQDG	CDTHFCKVNE	RGEYFWEKRV	TGCPPFDEHK
2710	2720	2730	2740	2750
CLAEGGKIMK	IPGTCCDTCE	EPECNDITAR	LQYVKVGSCK	SEVEVDIHYC
2760	2770	2780	2790	2800
QGKCASKAMY	SIDINDVQDQ	CSCCSPTRTE	PMQVALHCTN	GSVVYHEVLN
2810				
AMECKCSPRK	CSK			

FIG. 65

A VWF-FVIII FUSION PROTEIN WHEREIN THE N -GLYCOSYLATION RICH DOMAIN REPLACES THE FVIII-B-DOMAIN

FVIII heavy chain 19 to 760 – vWF 2218 to 2593 – FVIII light chain 1333 to 2351

REMARK 1-19 SIGNAL PEPTIDE

10	20	30	40	50
MQIELSTCFF	LCLLRFCFS	A TRRYYLGA	VE LSWDYMQSDL	GELPVDARFP
60	70	80	90	100
PRVPKSFPPN	TSVVYKKTLF	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
110	120	130	140	150
DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
160	170	180	190	200
GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
210	220	230	240	250
GSLAKEKTQT	HKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
260	270	280	290	300
HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
310	320	330	340	350
RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
360	370	380	390	400
EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
410	420	430	440	450
WWHYIAEEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
460	470	480	490	500
TDETFKTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
510	520	530	540	550
DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
560	570	580	590	600
YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
610	620	630	640	650
NRSWYL TENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
660	670	680	690	700
HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
710	720	730	740	750
MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
⇄FVIII	760 VWF⇒(2218)	VWF2230	VWF2240	VWF2250
SKNNAIEPRS	RHC DGNVSSCGDH	PSEGCFCPPD	KVMLEGSCVP	
	VWF2260	VWF2270	VWF2280	VWF2290
				VWF2300

EEACTQCIGE	DGVQHQFLEA	WVPDHQPCQI	CTCLSGRKVN	CTTQPCPTAK
VWF2310	VWF2320	VWF2330	VWF2340	VWF2350
APTCGLCEVA	RLRQNADQCC	PEYECVCDPV	SCDLPPVPHC	ERGLQPTLTN
VWF2360	VWF2370	VWF2380	VWF2390	VWF2400
PGECRPNFTC	ACRKEECKRV	SPPSCPPHRL	PTLRKTQCCD	EYECACNCVN
VWF2410	VWF2420	VWF2430	VWF2440	VWF2450
STVSCPLGYL	ASTATNDCGC	TTTTCLPDKV	CVHRSTIYPV	GQFWEEGCDV
VWF2460	VWF2470	VWF2480	VWF2490	VWF2500
CTCTDMEDAV	MGLRVAQCSQ	KPCEDSCRSG	FTYVLHEGEC	CGRCLPSACE
VWF2510	VWF2520	VWF2530	VWF2540	VWF2550
VVTGSPRGDS	QSSWKSQSGS	WASPENPCLI	NECVRVKEEV	FIQQRNVSCP
VWF2560	VWF2570	VWF2580	VWF2590	VWF2593↔VWF
QLEVPVCPSPG	FQLSCKTSAC	CPSCRCERME	ACMLNGTVIG	PGK

FVIII ⇨

1333	1340	1350		
ALKQFRLP	LEETELKRI			
	1360	1370	1380	1390
IVDDTSTQWS	KNMKHLTPST	LTQIDYNEKE	KGAITQSPLS	DCLTRSHSIP
	1410	1420	1430	1440
QANRSPLPIA	KVSSFPSIRP	IYLRVLFQD	NSSHLPAASY	RKKDSGVQES
	1460	1470	1480	1490
SHFLQGAKKN	NLSLAILTLE	MTGDQREVGS	LGTSATNSVT	YKKVENTVLP
	1510	1520	1530	1540
KPDLPKTSGK	VELLPKVHIY	QKDLFPTETS	NGSPGHLDLV	EGSLLQGTEG
	1560	1570	1580	1590
AIKWNEANRP	GKVPFLRVAT	ESSAKTPSKL	LDPLAWDNHY	GTQIPKEEWK
	1610	1620	1630	1640
SQEKSPKTA	FKKKDTILSL	NACESNHAIA	AINEGQNKPE	IEVTWAKQGR
	1660	1670	1680	1690
TERLCSQNPP	VLKRHQREIT	RTTLQSDQEE	IDYDDTISVE	MKKEDFDIYD
	1710	1720	1730	1740
EDENQSPRSF	QKKTRHYFIA	AVERLWDYGM	SSSPHVLNRN	AQSGSVPQFK
	1760	1770	1780	1790
KVVFQEFTDG	SFTQPLYRGE	LNEHLGLLGP	YIRAEVEDNI	MVTFRNQASR
	1810	1820	1830	1840
PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
	1860	1870	1880	1890
CKAWAYFSDV	DLEKDVHSGL	IGPLLVCHTN	TLNPAHGRQV	TVQEFALFFT
	1910	1920	1930	1940
IFDETKSWYF	TENMERN CRA	PCNIQMEDPT	FKENYRFHAI	NGYIMDTLPG
	1960	1970	1980	1990
LVMAQDQRIR	WYLLSMGSNE	NIHSIHFGSH	VFTVRKKEEY	KMALYNLYPG
				2000

2010	2020	2030	2040	2050
VFETVEMLPSK	AGIWRVECL	IGEHLHAGMS	TLFLVYSNKC	QTPLGMASGH
2060	2070	2080	2090	2100
IRDFQITASG	QYGQWAPKLA	RLHYSGSINA	WSTKEPFSWI	KVDLLAPMII
2110	2120	2130	2140	2150
HGIKTQGARQ	KFSSLYISQF	IIMYSLDGKK	WQTYRGNSTG	TLMVFFGNVD
2160	2170	2180	2190	2200
SSGIKHNIFN	PPIIARYIRL	HPTHYSIRST	LRMELMGCDL	NSCSMPLGME
2210	2220	2230	2240	2250
SKAISDAQIT	ASSYFTNMFA	TWSPSKARLH	LQGRSNAWRP	QVNNPKEWLQ
2260	2270	2280	2290	2300
VDFQKTMKVT	GVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFFQNGKVK
2310	2320	2330	2340	2350
VFQGNQDSFT	PVNSLDPPL	LTRYLRIHPQ	SWWHQIALRM	EVLGCEAQDL
2351	∅FVIII			
Y				

FIG. 66

Nr.	Composition	pH	Purpose / Remark
1.	60mM NaCitrate	7.6 ±0.2	Dilution buffer
2.	30mM Na-Citrat, 180mM NaCl	7.5 ±0.2	Equilibration and wash buffer for r-vWF-Propeptide depletion
3.	30mM Na-Citrat, 180mM NaCl, + SD_VI On column 25g/kg 18.0g Polysorbate 80 3.5g DMSO 3.5g InBP	7.5 ±0.2	WSD_Buffer Potential buffer for inactivation of lipid enveloped viruses on column/on resin
4.	50mM Glycine 10mM Taurine 10% Sucrose 0.1% Polysorbate 80	5.5 ±0.2	Buffer exchange equilibration buffer
5.	50mM Glycine 10mM Taurine 5% (w/w) Sucrose 5% (w/w) D-Mannitol 0.1% Polysorbate 80 2mM CaCl ₂ 150mM NaCl	7.4 ±0.2	Pre_formulation_Buffer + FVIII Con buffer (addition)
6.	50mM Glycine 10mM Taurine 5% (w/w) Sucrose 5% (w/w) D-Mannitol 0.1% Polysorbate 80 2mM CaCl ₂ 600mM NaCl	7.4 ±0.2	Elution buffer
7.	50mM Glycine 10mM Taurine 5% (w/w) Sucrose 5% (w/w) D-Mannitol 0.1% Polysorbate 80 2mM CaCl ₂	7.4 ±0.2	Formulation Adjustment buffer

FIG. 67

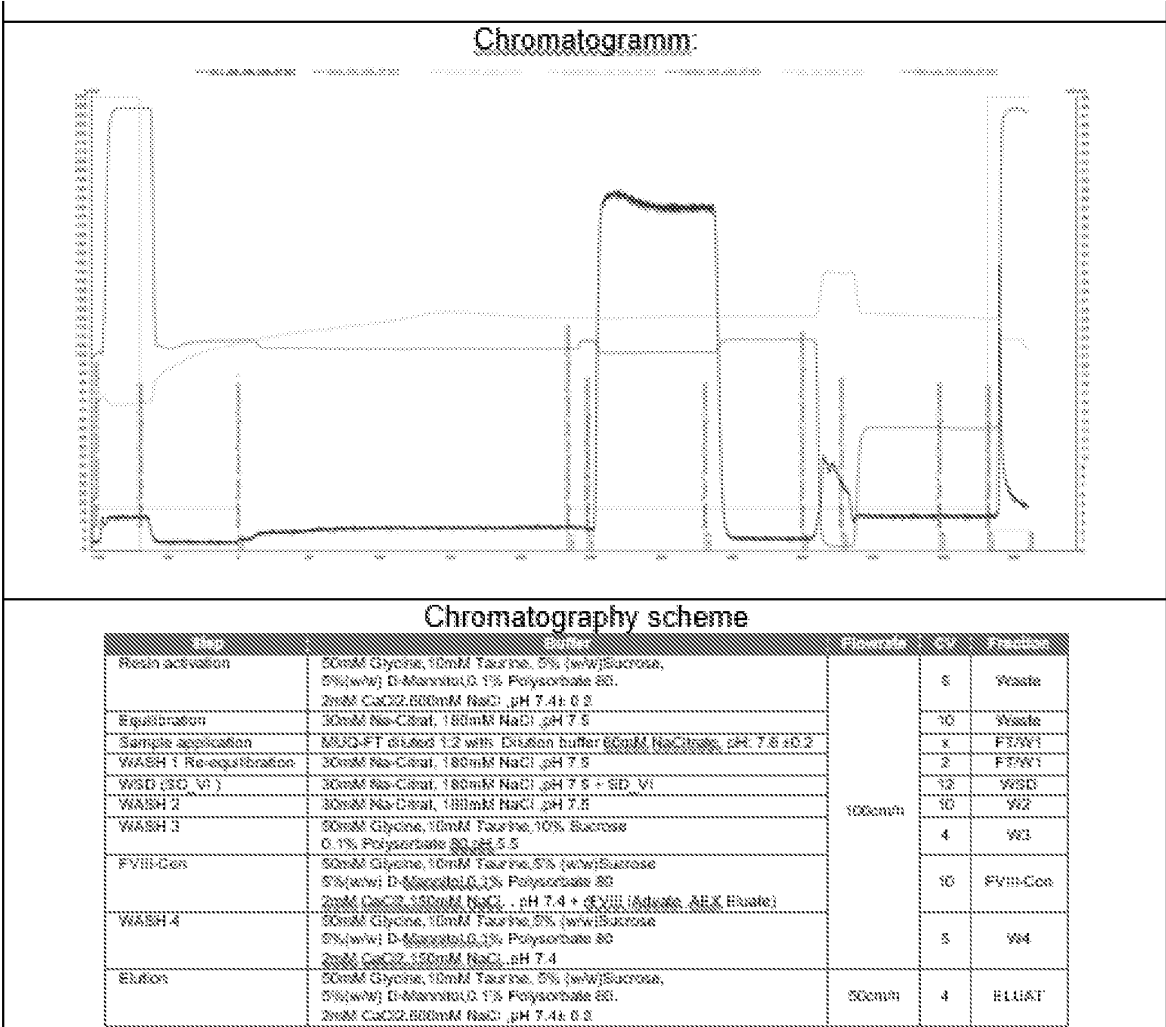


FIG. 68

Step	Amount	vWF:AG [µg/ml]	Total vWF Antigen [µg]	% vWF Antigen	FVIII:C [U/ml]	Total FVIII:C [U]	% FVIII:C
LOAD	231.64	55.2	12786.5	100.0%	<0.2	<46.33	-
FT / WASH 1	248.95	19.5	4854.5	38.0%	<0.2	<49.79	-
Elution	28.88	88.5	2324.84	18.2%	34.28	990.01	100.0%
BDS	119.23	20.2	2408.44	18.8%	8.14	970.53	98.0%

Step	Amount	vWF:PP [µg/ml]	Total vWF Propeptide [µg]	% vWF Propeptide	CHO:HCP [µg/ml]	Total CHO:HCP [µg]	% CHO:HCP
LOAD	231.64	16.44	3807.74	100.0%	930	215.43	100.0%
FT / WASH 1	248.95	9.18	2284.41	58.0%	711	177.00	82.2%
Elution	28.88	0.089	2.59	0.07%	40	1.10	0.5%
BDS	119.23	0.014	2.88	0.08%	<30	<1	<0.5%

Step	Amount	RICoF [U/ml]	Total RICoF [U]	% RICoF	Ratio RICoF/ mg vWF:AG	Ratio FVIII:C/ mg vWF:AG
LOAD	231.64	4.38	1014.58	100.0%	43.80	<3.6U/mg
FT / WASH 1	248.95	0.23	57.26	5.6%	11.79	<10.3U/mg
Elution	28.88	7.37	212.85	21.0%	91.55	425.8U/mg
BDS	119.23	1.77	211.04	20.8%	87.62	403.0U/mg

FIG. 69

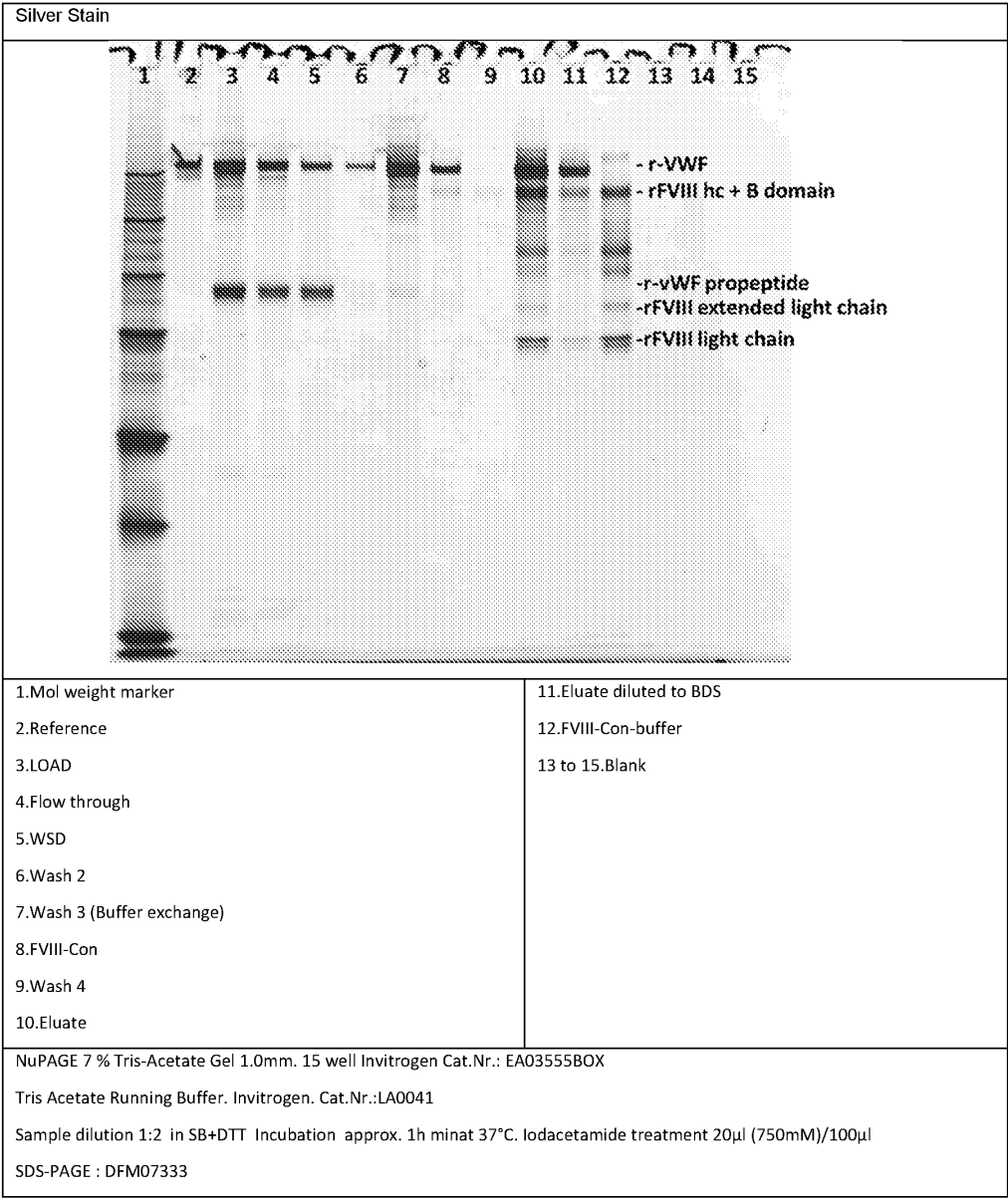


FIG. 70

Nr:	Composition	pH	Purpose / Remark
1.	60mM NaCitrate	7.6 ±0.2	Dilution buffer
2.	30mM Na-Citrat 180mM NaCl	7.5 ±0.2	Equilibration and wash buffer for r-vWF-Propeptide depletion
3.	11mg CMP NANA C8271-25mg Lot.Nr.: SLBV 7777 dissolved in 154.29g 30mM Na-Citrat, 180mM NaCl	7.5 ±0.2	Cytidine-5' -monophospho-N- acetylneuraminic acid sodium salt Reagent A
4.	0.5U alpha 2,6 Sialyltransferase in 30mM Na-Citrat, 180mM NaCl	7.5 ±0.2	alpha 2,6 Sialyltransferase Reagent B
5.	50mM HEPES 150mM NaCl	6.0 ±0.2	Pre_elution_buffer
6.	50mM HEPES 500mM NaCl	7.5 ±0.2	Elution_buffer

FIG. 71

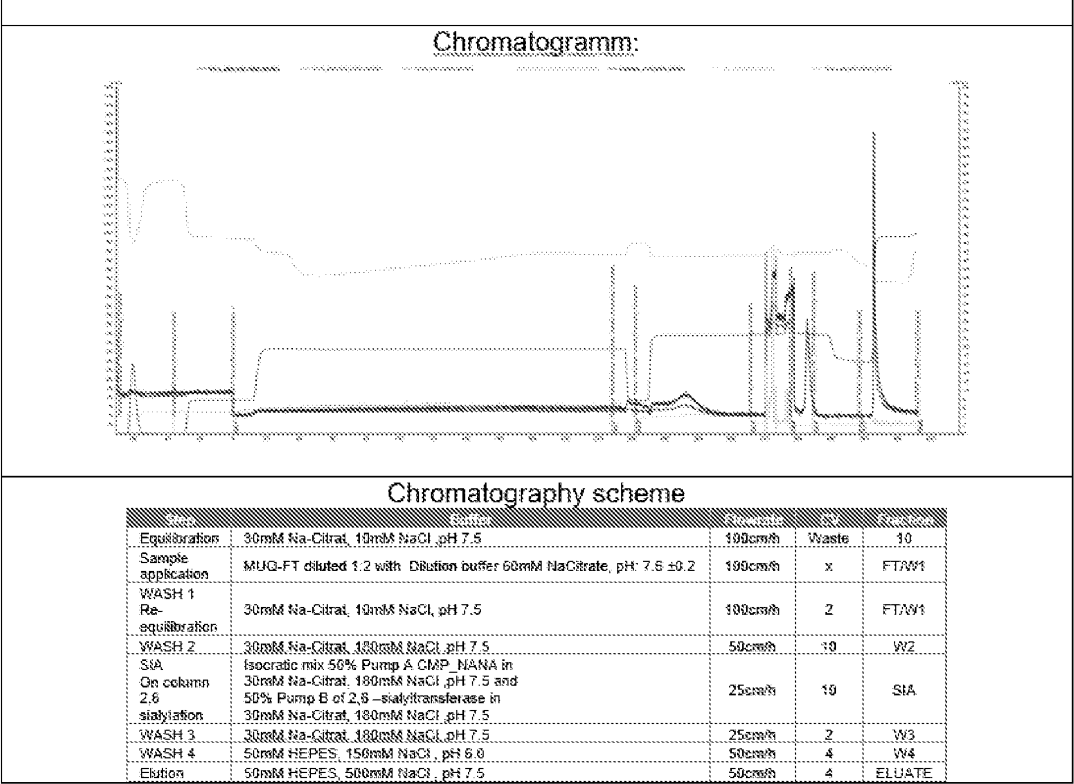


FIG. 72

YIELD							
Step	Amount	vWF AG [µg/ml]	Total vWF Antigen [µg]	% vWF Antigen	vWF-PP [µg/ml]	Total vWF Propeptide [µg]	% vWF Propeptide
LOAD	228.02	50.6	11437	100%	13.96	3156.14	100%
E	34.84	46.4	1617	14.1%	<0.006	<217.75	<0.01%

YIELD SIALYLATION

STEP	Amount	2,3 Sialylation	2,6 Sialylation
LOAD	228.02	x	x
E	34.84	x	x

FIG. 73

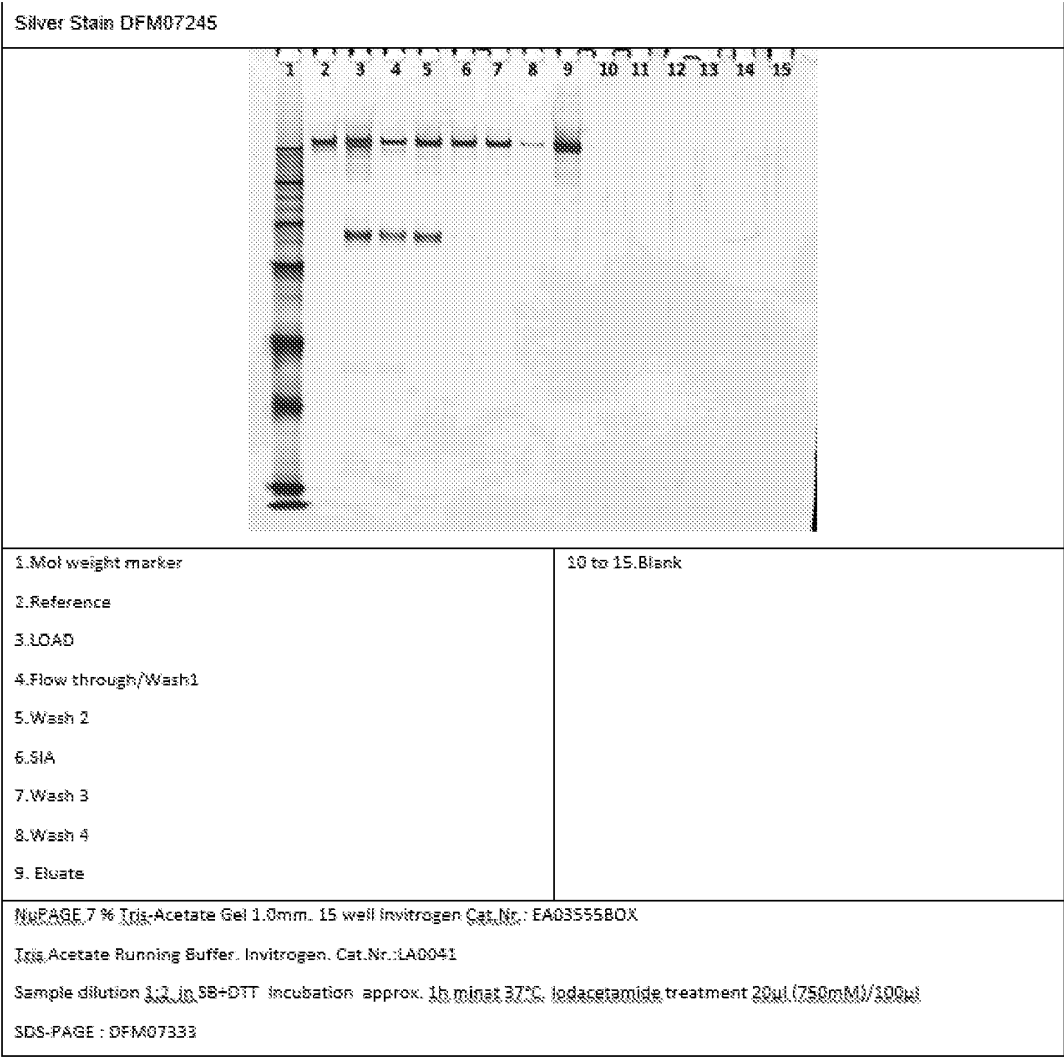


FIG. 74

Nr.	Composition	pH	Purpose / Remark
1.	60mM NaCitrate	7.6 ±0.2	Dilution buffer
2.	30mM Na-Citrat 180mM NaCl	7.5 ±0.2	Equilibration and wash buffer for r-vWF-Propeptide depletion
3.	14mg CMP_NANA C8271-25mg Lot.Nr.: SLBV 7777 dissolved in 121.57g 30mM Na-Citrat, 180mM NaCl	7.5 ±0.2	Cytidine-5'-monophospho-N- acetylneuraminic acid sodium salt Reagent A
4.	1.0U alpha 2,6 Sialyltransferase in 121.10g 30mM Na-Citrat, 180mM NaCl	7.5 ±0.2	alpha 2,6 Sialyltransferase Reagent B
5.	50mM HEPES 150mM NaCl	6.0 ±0.2	Pre_elution_buffer
6.	50mM HEPES 500mM NaCl	7.5 ±0.2	Elution_buffer

FIG. 75

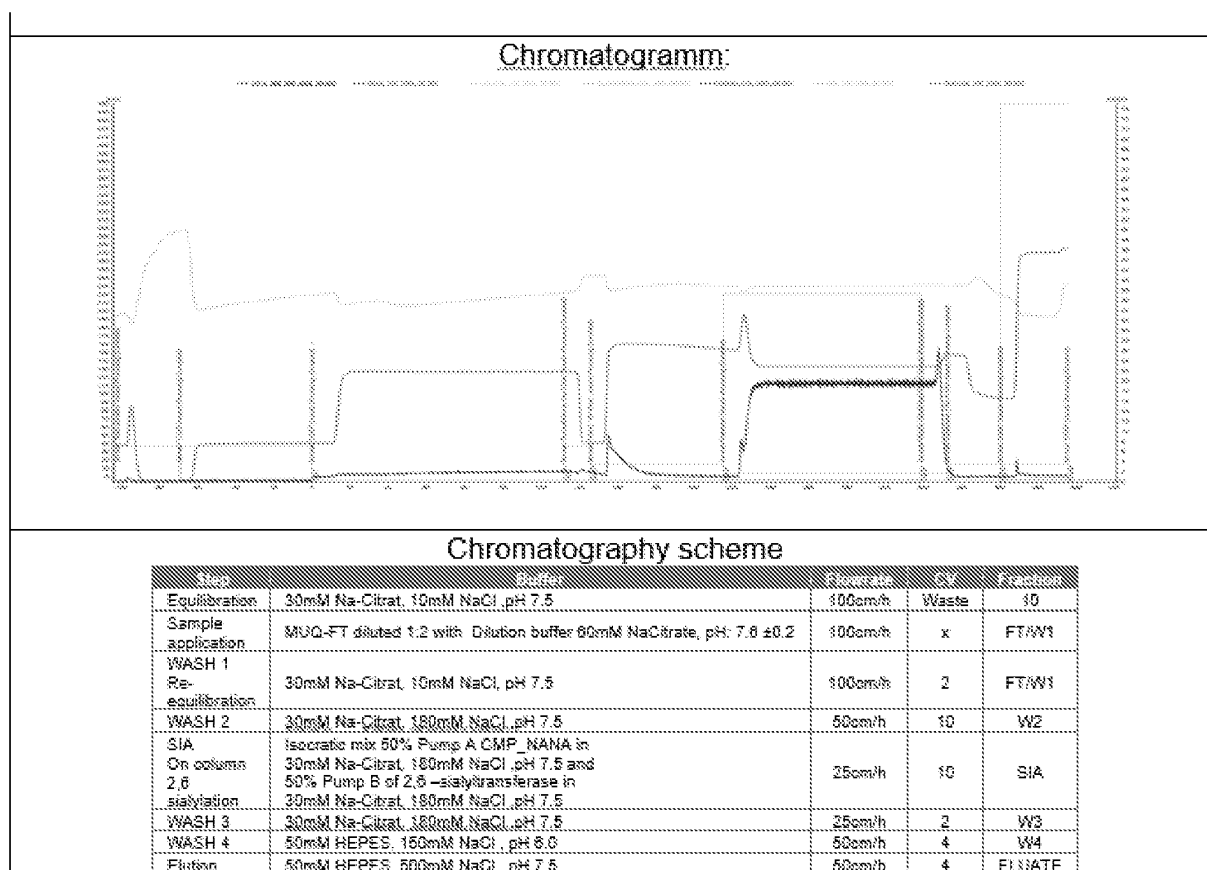


FIG. 76

YIELD							
Step	Amount	vWF:Ag IU/ml	Total vWF Antigen IU/ml	% vWF Antigen	vWF:PP IU/ml	Total vWF Propeptide IU/ml	% vWF Propeptide
LOAD	312.15	57.40	17917.41	100%	13.96	3156.14	100%
E	56.85	1.70	96.65	0.5%	<0.006	<217.75	<0.01%

YIELD SIALYLATION

STEP	Amount	2,3 Sialylation	2,6 Sialylation
LOAD	312.15	x	x
E	56.85	x	x

FIG. 77

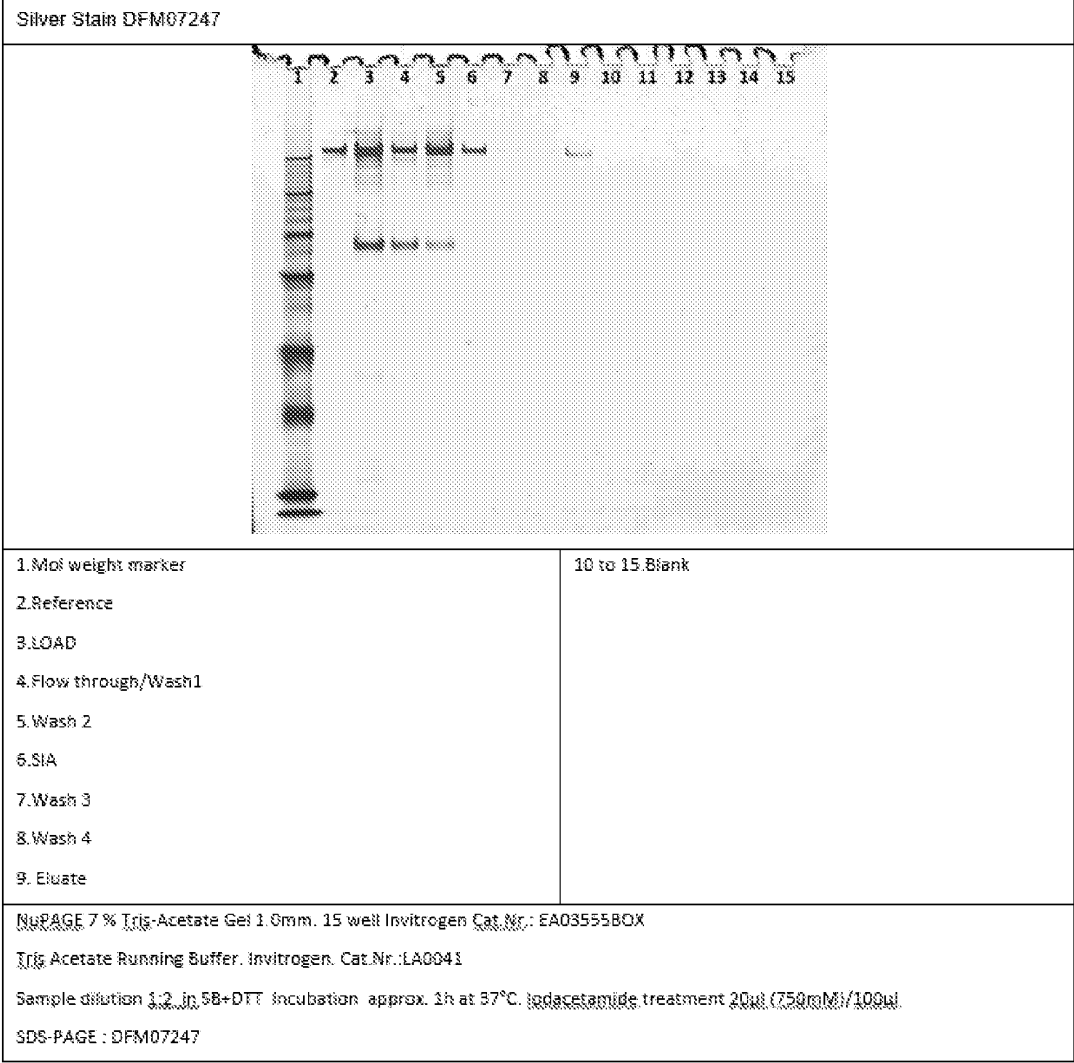
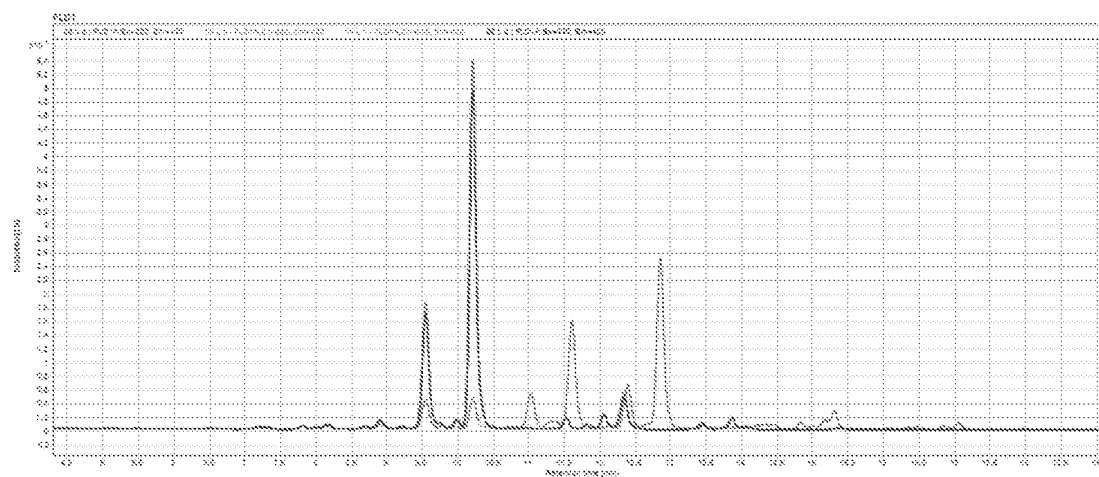


FIG. 78

Run Nr. VW_USS_08

Emf-File



Run Nr. VW_USS_06

Emf-File

