Fig. 1A. RSV F Protein Primary Structure

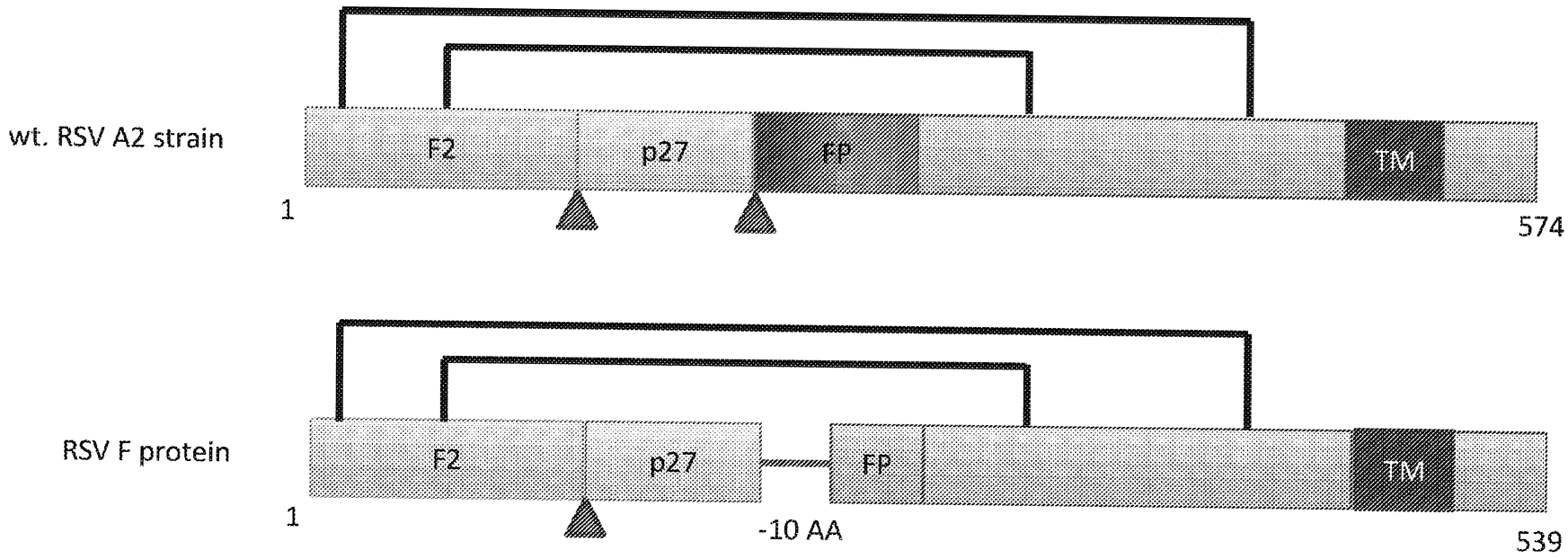
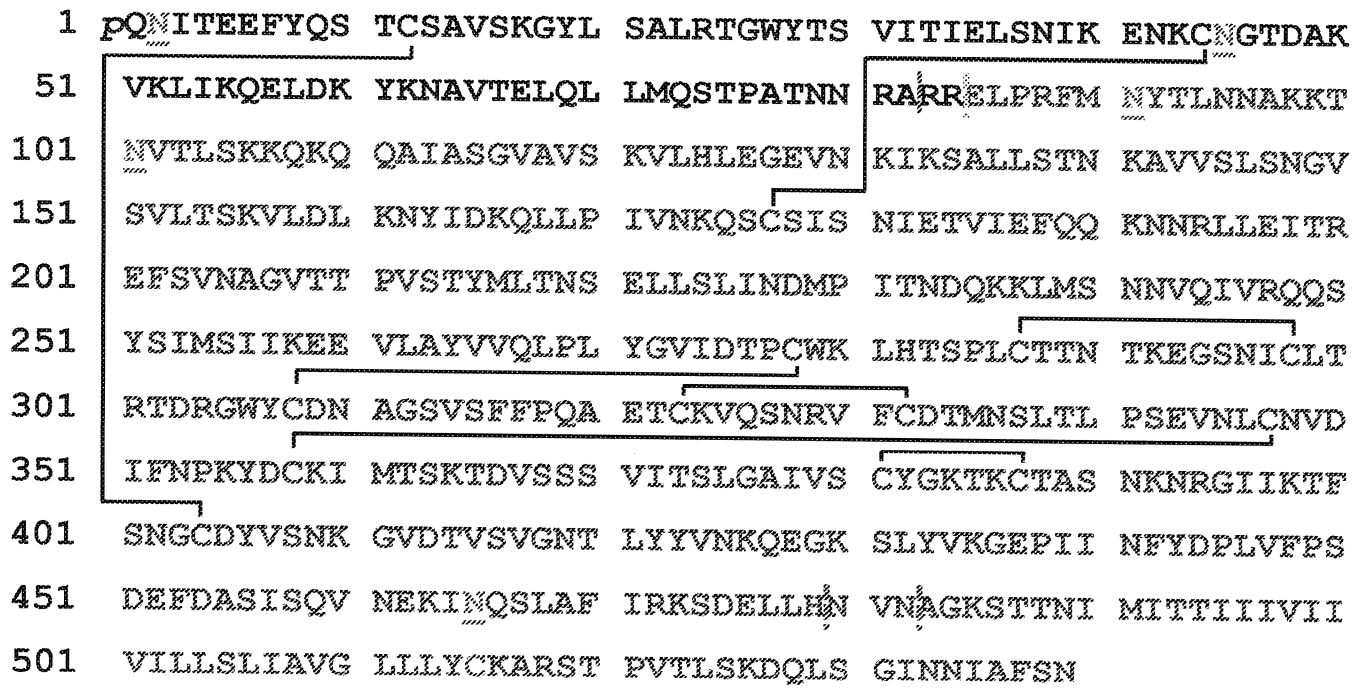


Fig. 1B. RSV F Protein Functional Sites



Legend:

F1 domain in blue, F2 domain in black, N-Linked glycosylation site, -Furin cleavage site, - major cleavage site
- Disulfide bond

Fig. 2. Separation of RSV F Proteins by RP-HPLC

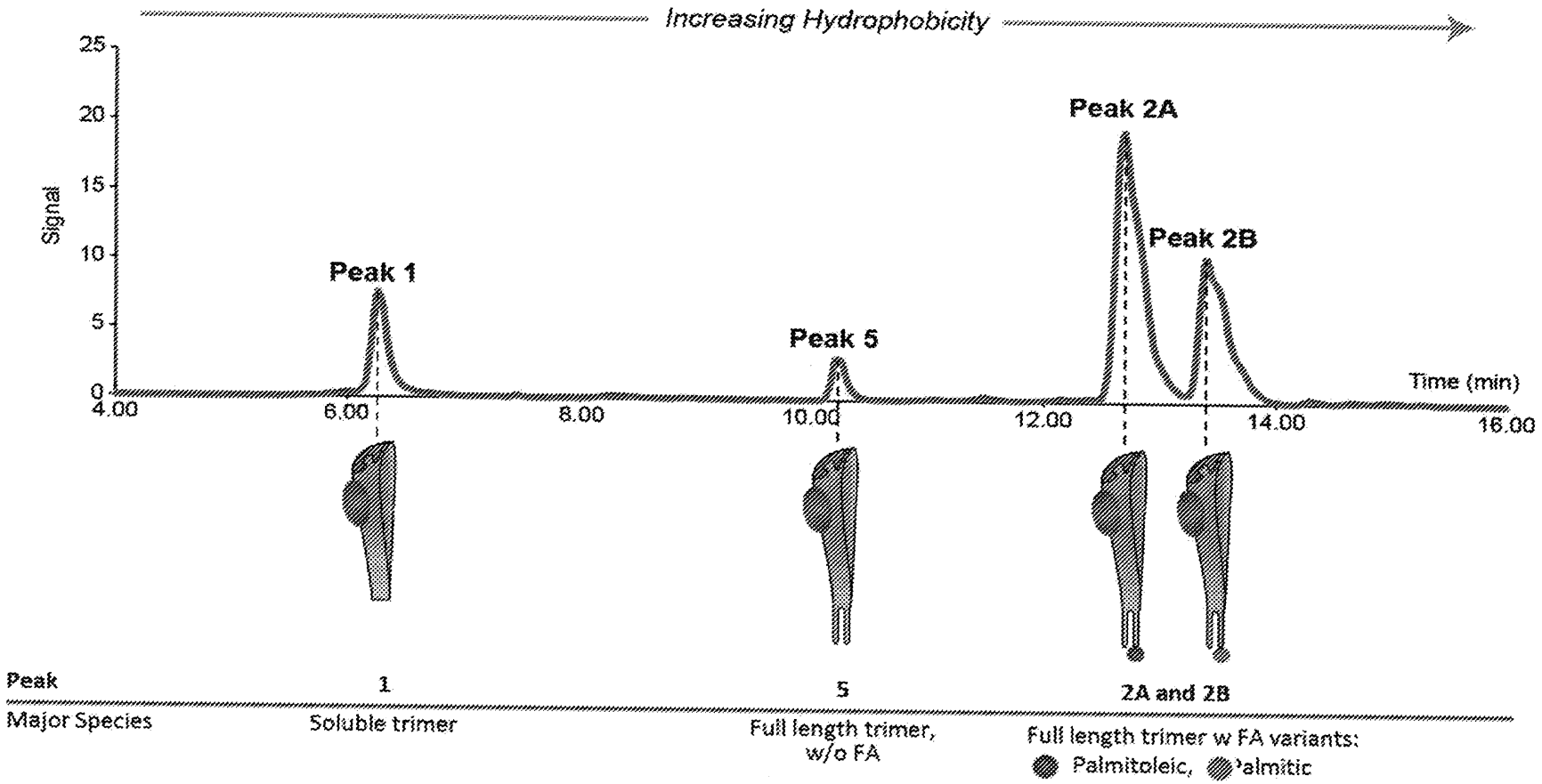


Fig. 3. Separation of RSV F Proteins by rSDS-PAGE

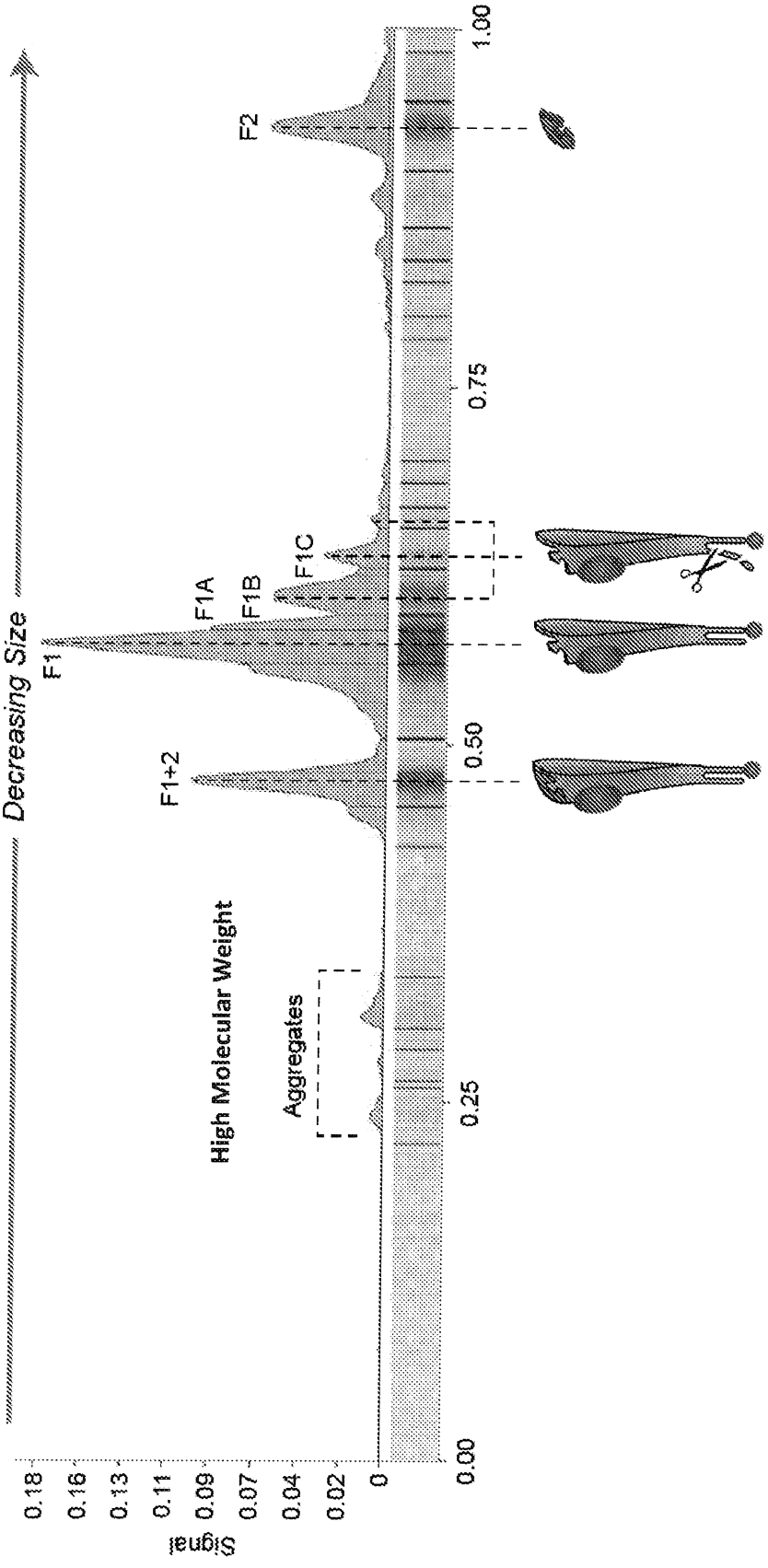
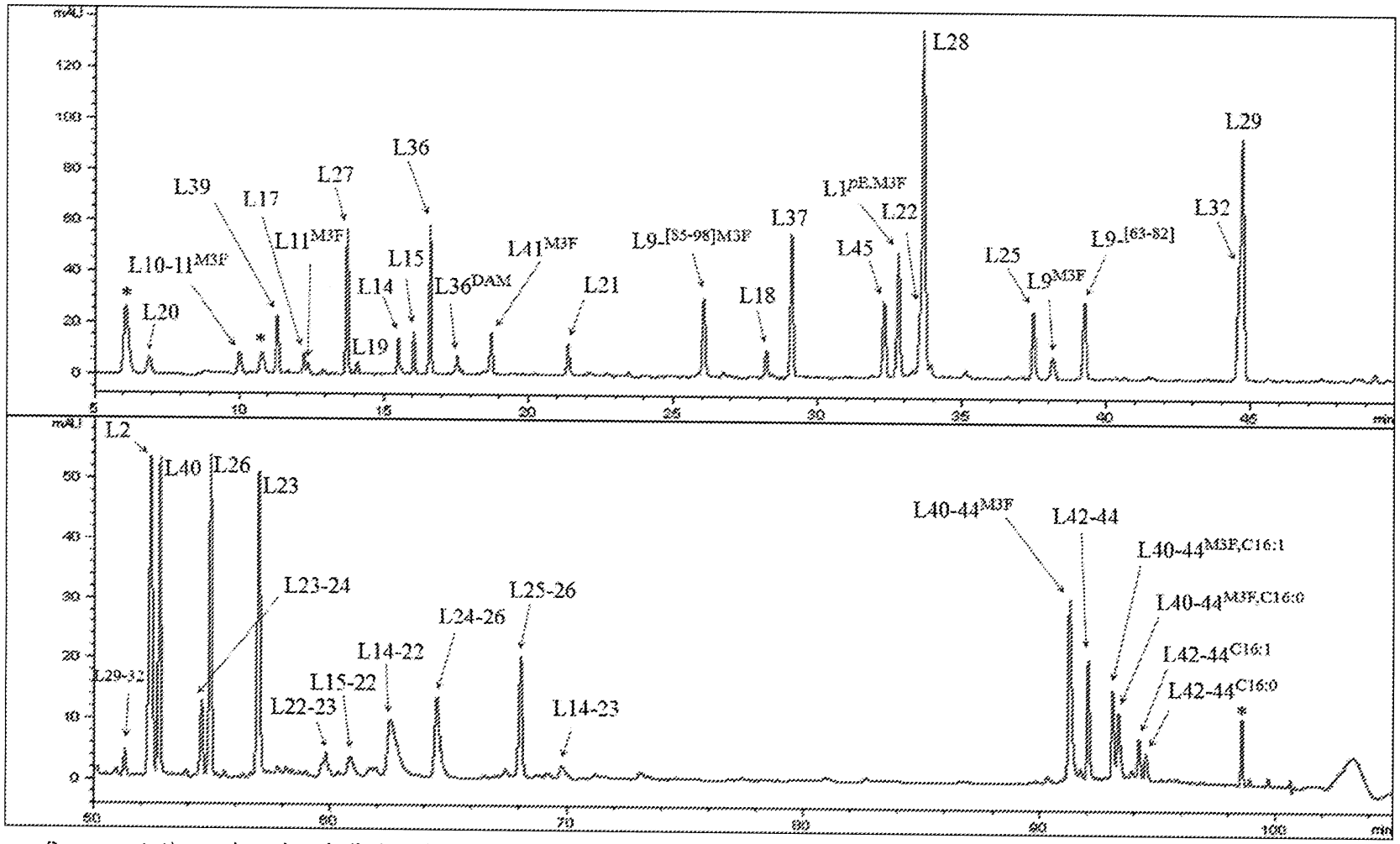


Fig. 4. Primary Structure by Peptide Mapping



Representative reduced and alkylated endoproteinase Lys-C peptide map of RSV F
* = System peak, DAM – Deamidation, M3F – Fucosylated Mannose 3 glycan.

Fig. 5. Glycoanalysis using HPLC-FLD

- Major glycan structures are fucosylated Man3

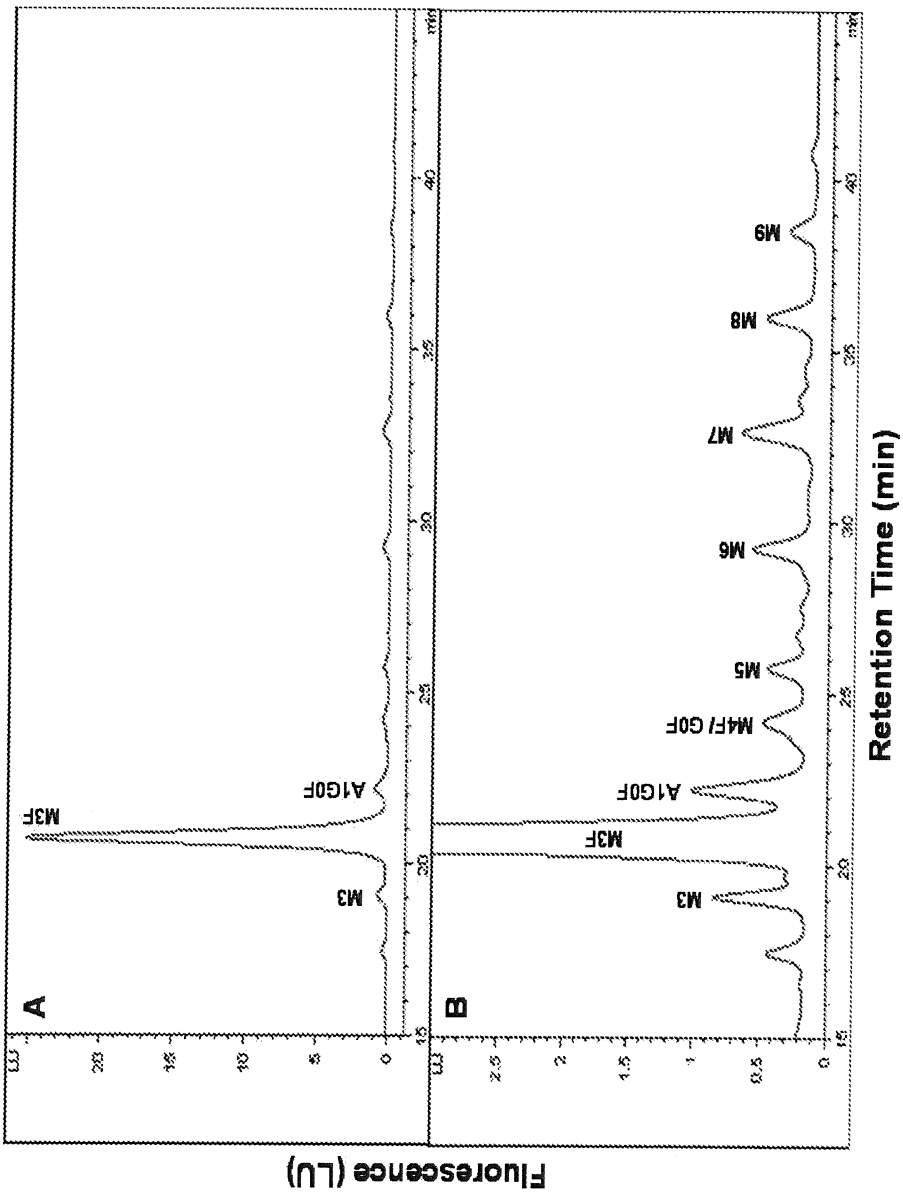
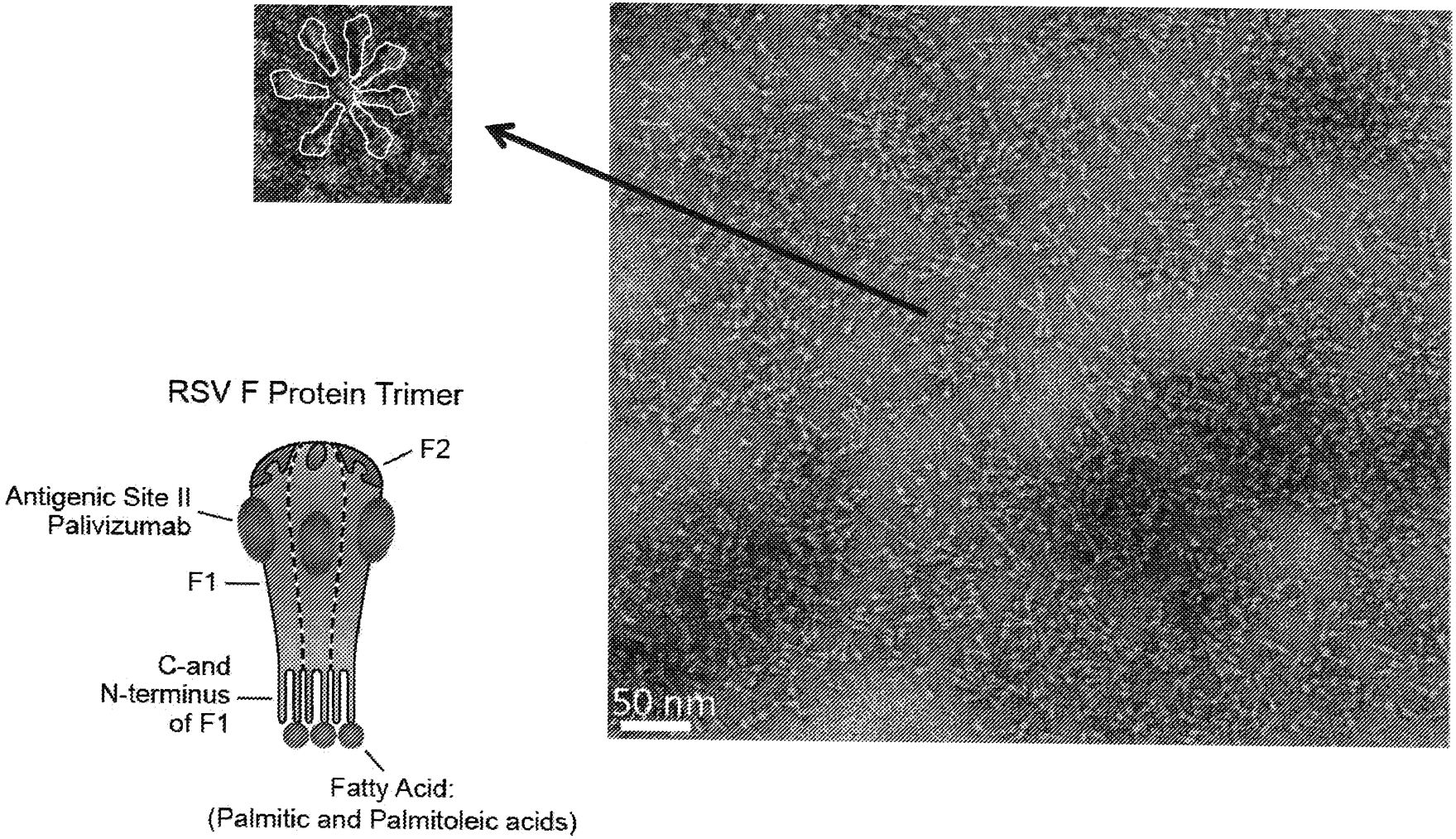


Fig. 6. Electron Micrograph of RSV F Nanoparticles showing RSV F Protein Trimers attached to the PS-80 core



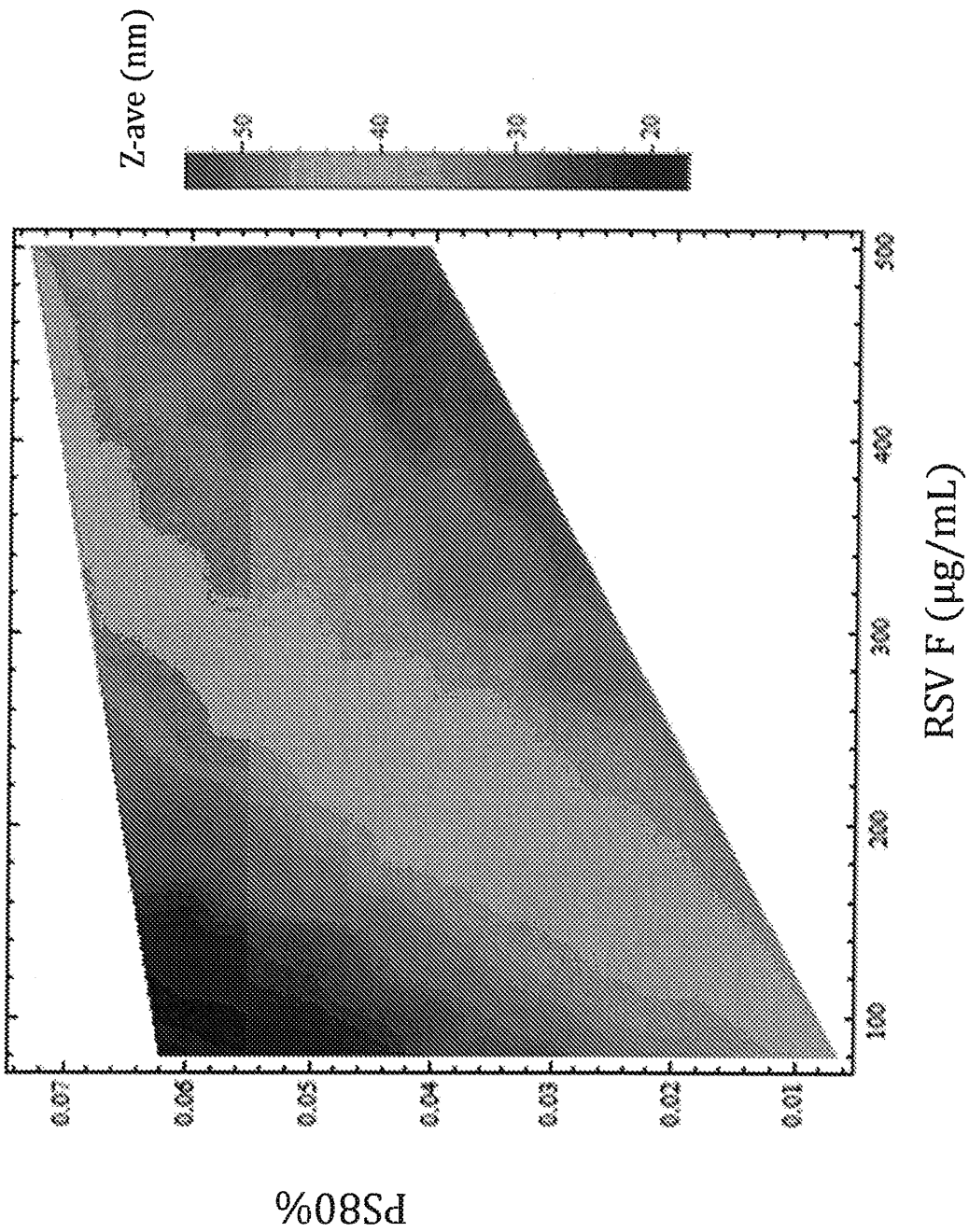
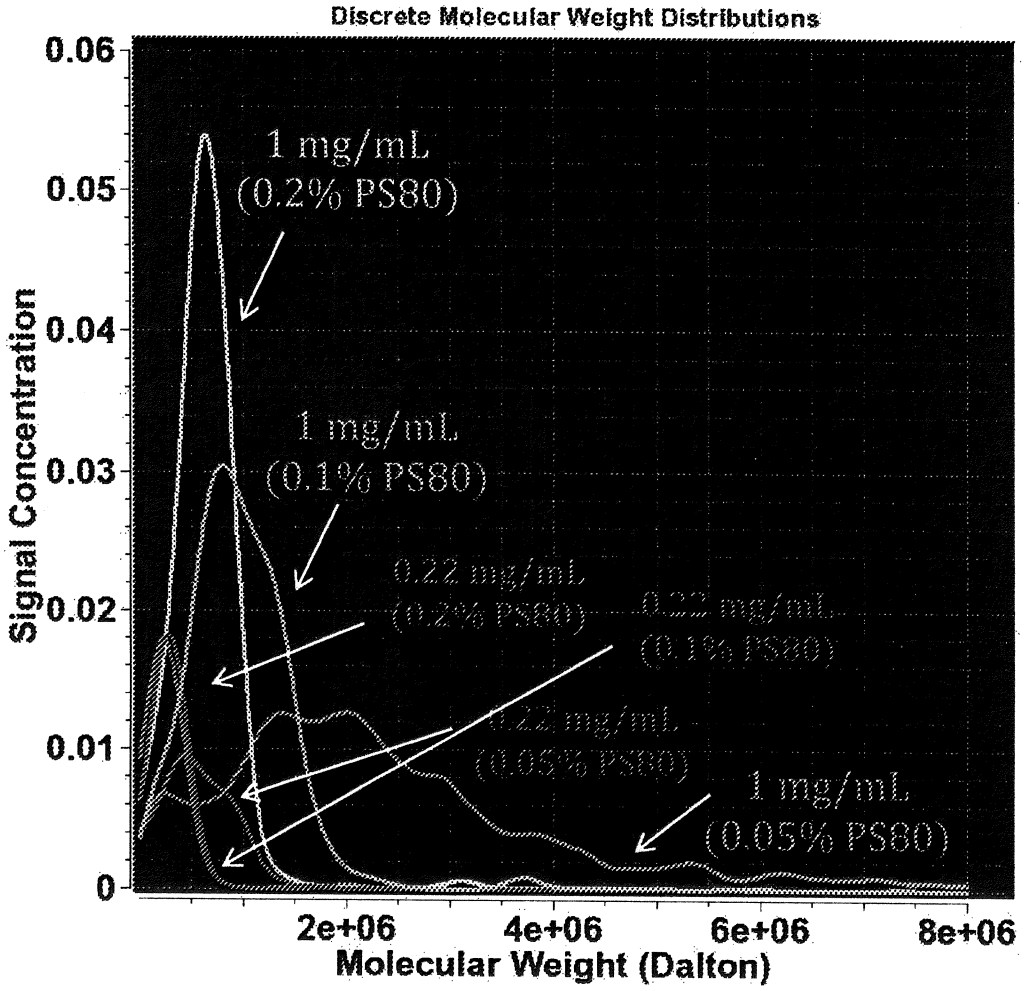


Fig. 7. Dynamic Light Scattering (DLS) measurement of particle size of RSV-F nanoparticles

Fig. 8. Particle size determined by Analytical Ultracentrifugation (AUC)



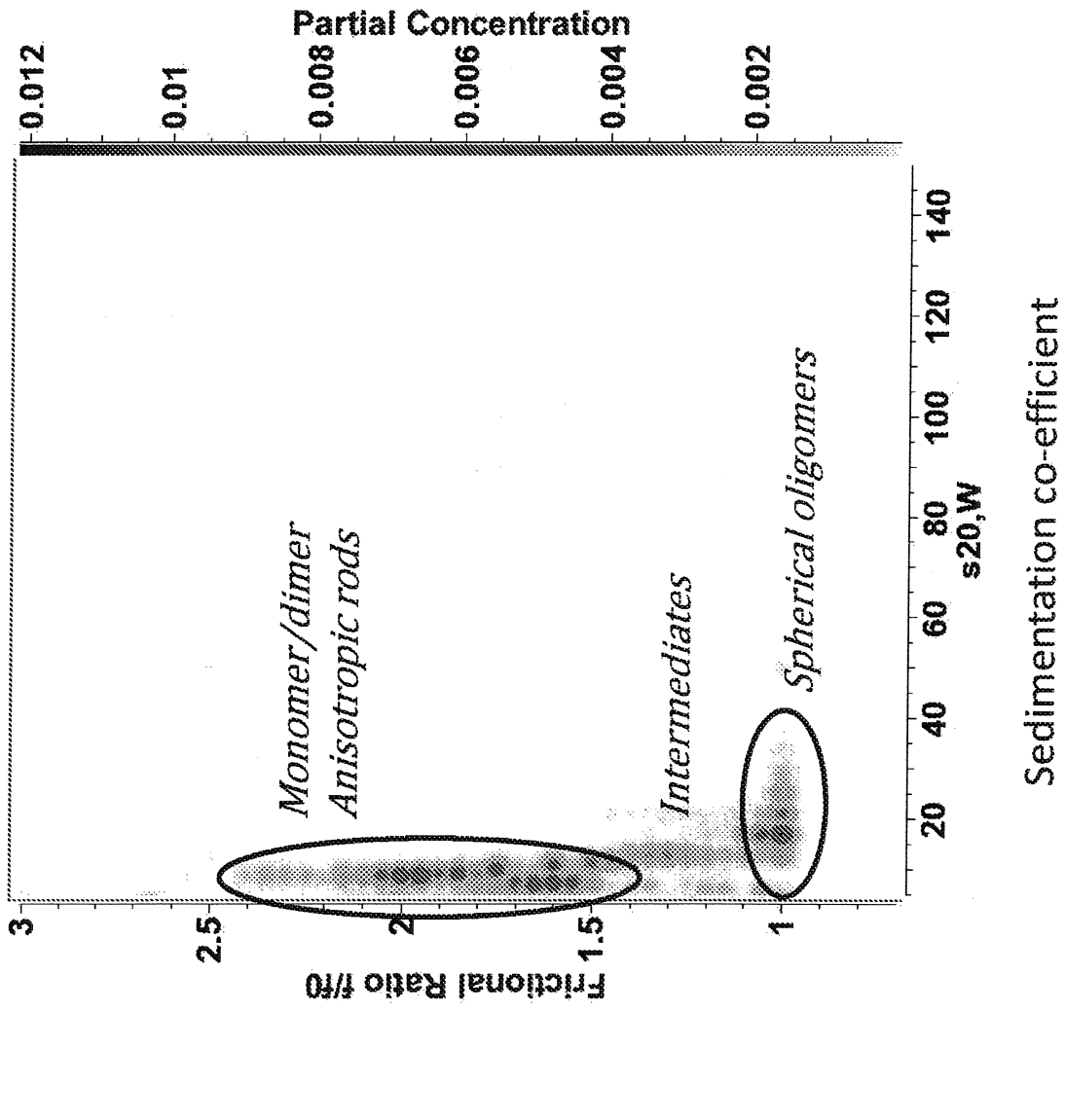


Fig. 9A. Shape of RSV F particles produced with 0.2% PS80, 0.22 mg/mL RSV F

Fig. 9B. Shape of RSV F particles produced with 0.5% PS80, 0.22 mg/mL RSV F or 1 mg/ml RSV F

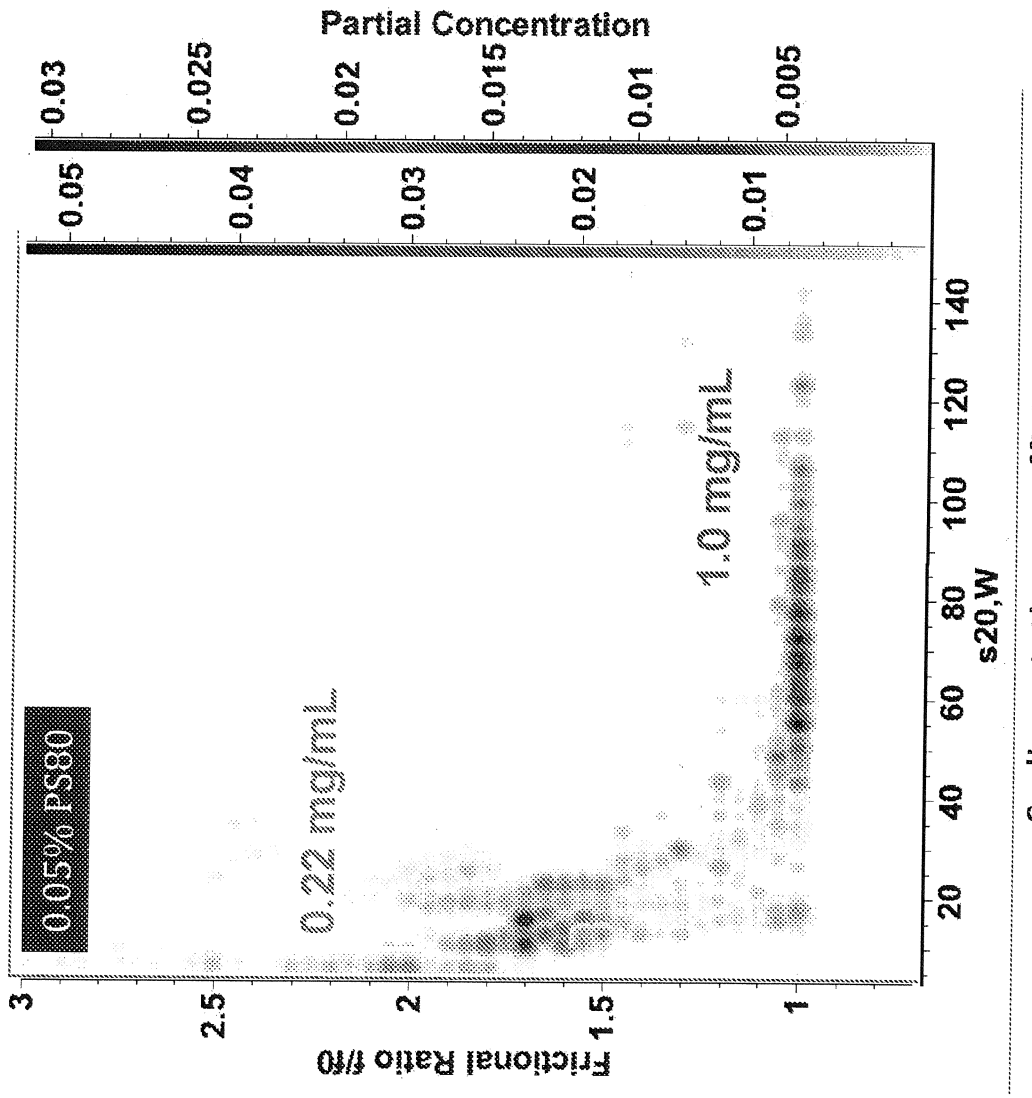


Fig. 10. Stability Studies: Primary Structure Differences after Stress Test

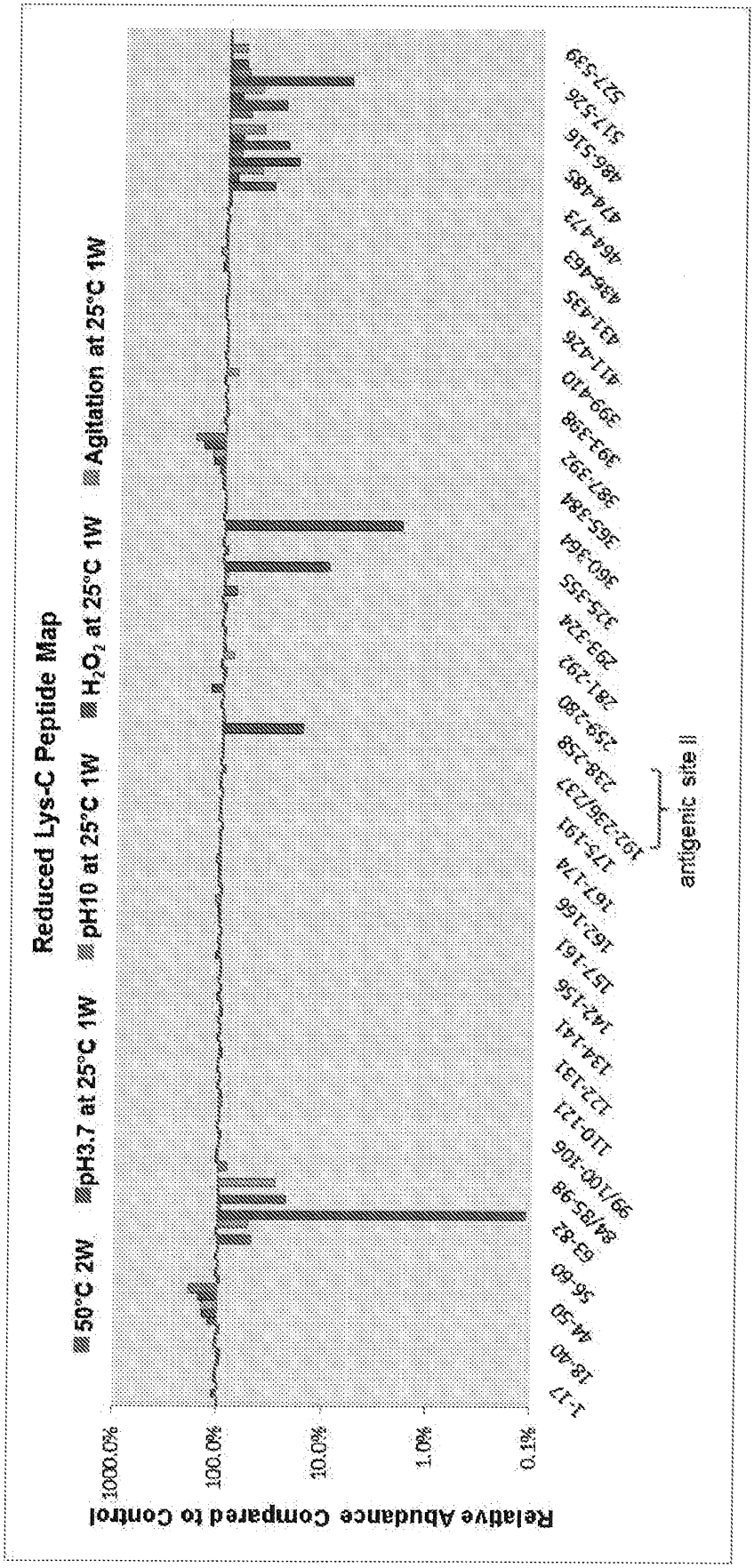


Fig. 11. Stability Studies: Antigenic site 2 (palivizumab site) is robust with respect to stress conditions

Residue	192–236/237	238–258
Sequence	...NSELLSLINDMPITNDQK/K	LMSNN...
Thermal Stress	98.80%	97.00%
Low pH	102.80%	95.90%
High pH	101.50%	95.90%
Oxidation	90.20%	16.90%
Agitation	102.10%	93.40%

Fig. 12A Stability Studies: Preservation of Mouse Immunogenicity

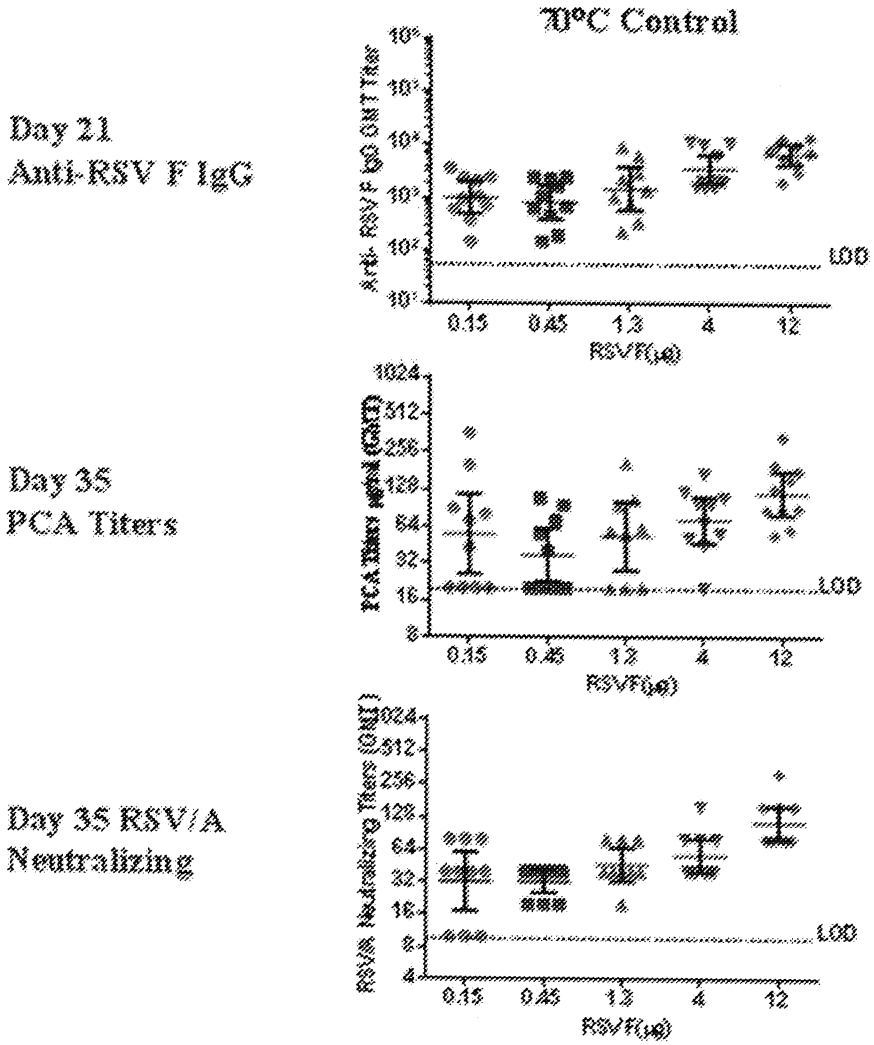


Fig. 12B Stability Studies: Preservation of Mouse Immunogenicity

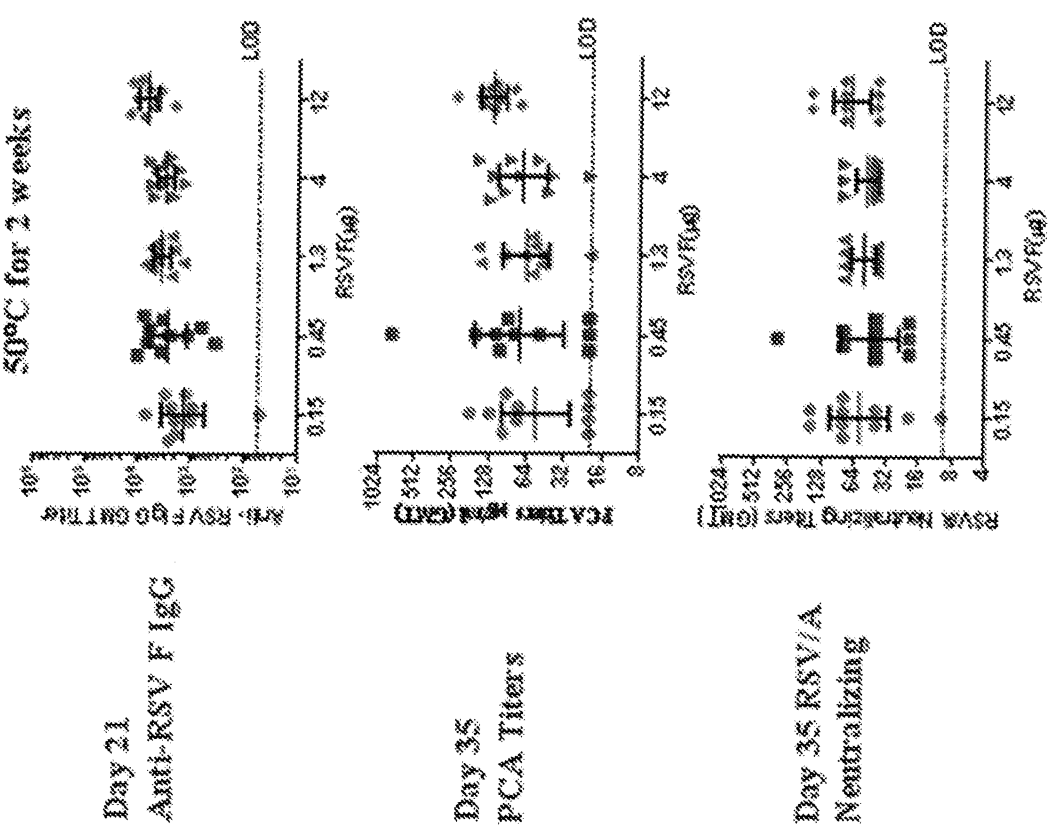


Fig. 12C Stability Studies: Preservation of Mouse Immunogenicity

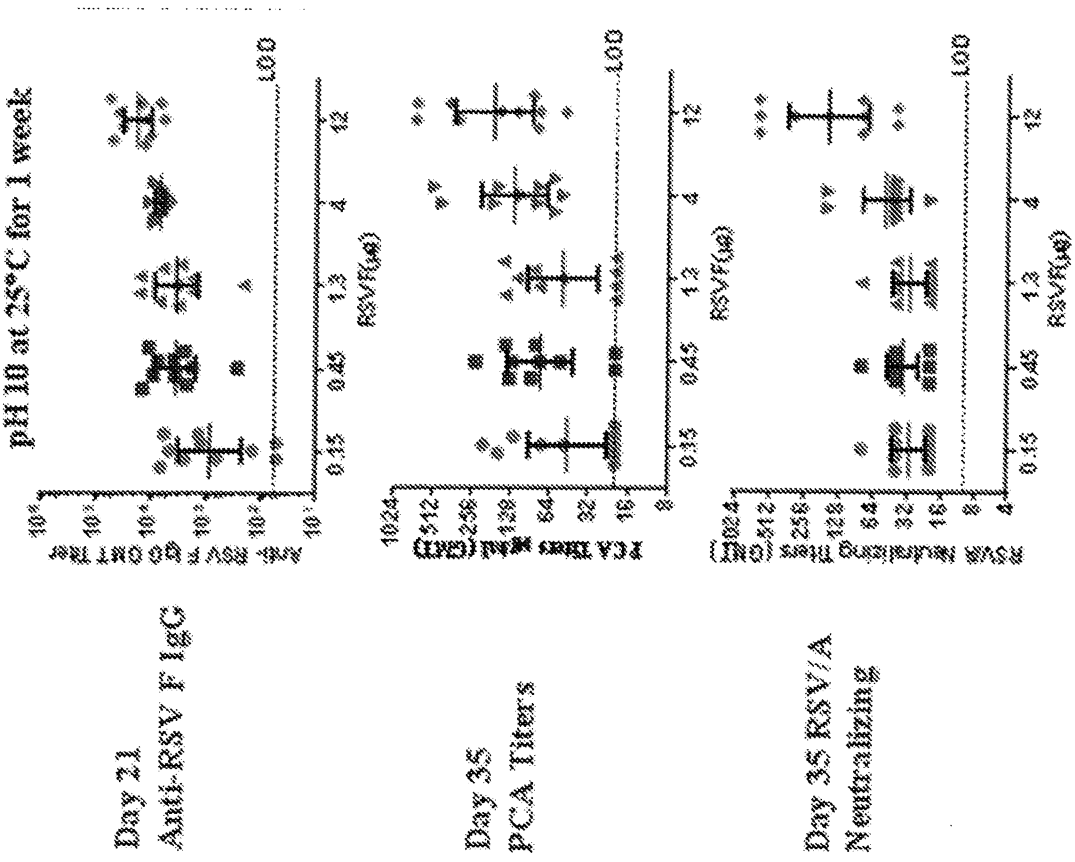


Fig. 12D Stability Studies: Preservation of Mouse Immunogenicity

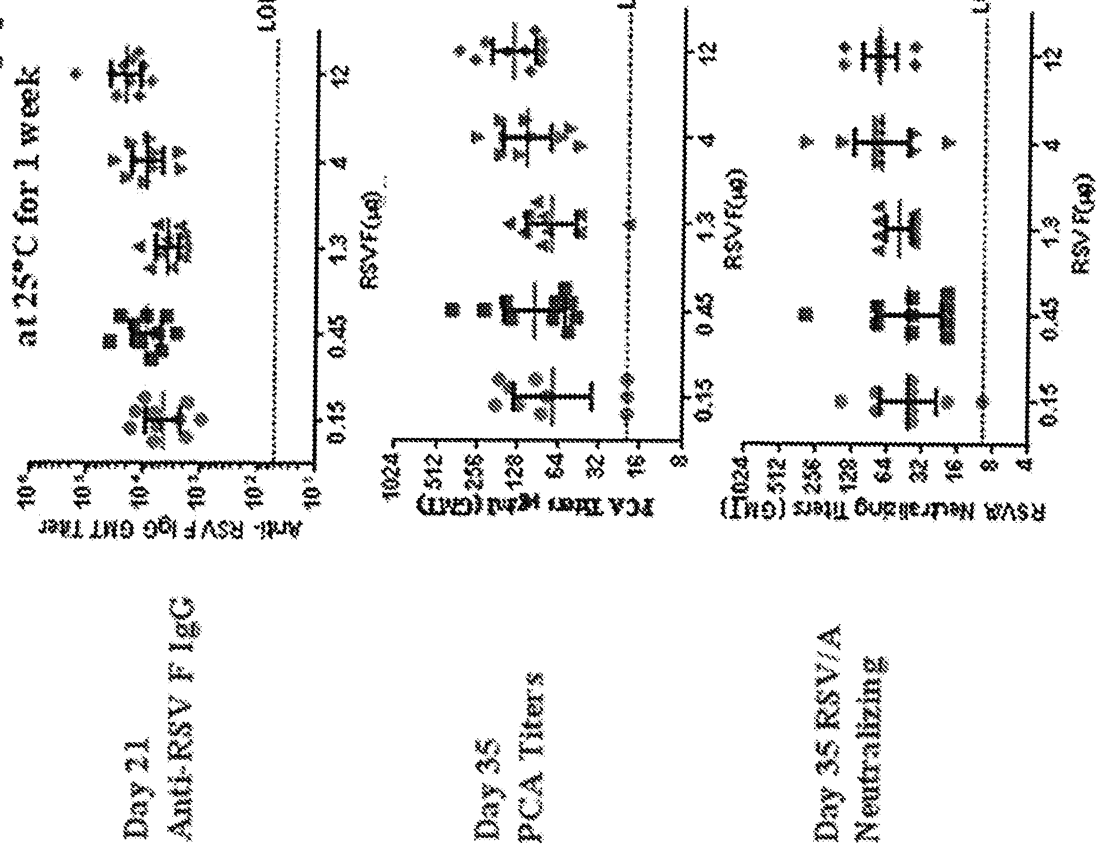


Fig. 13. Enhanced Protease Resistance of Nanoparticles Having Higher PS80

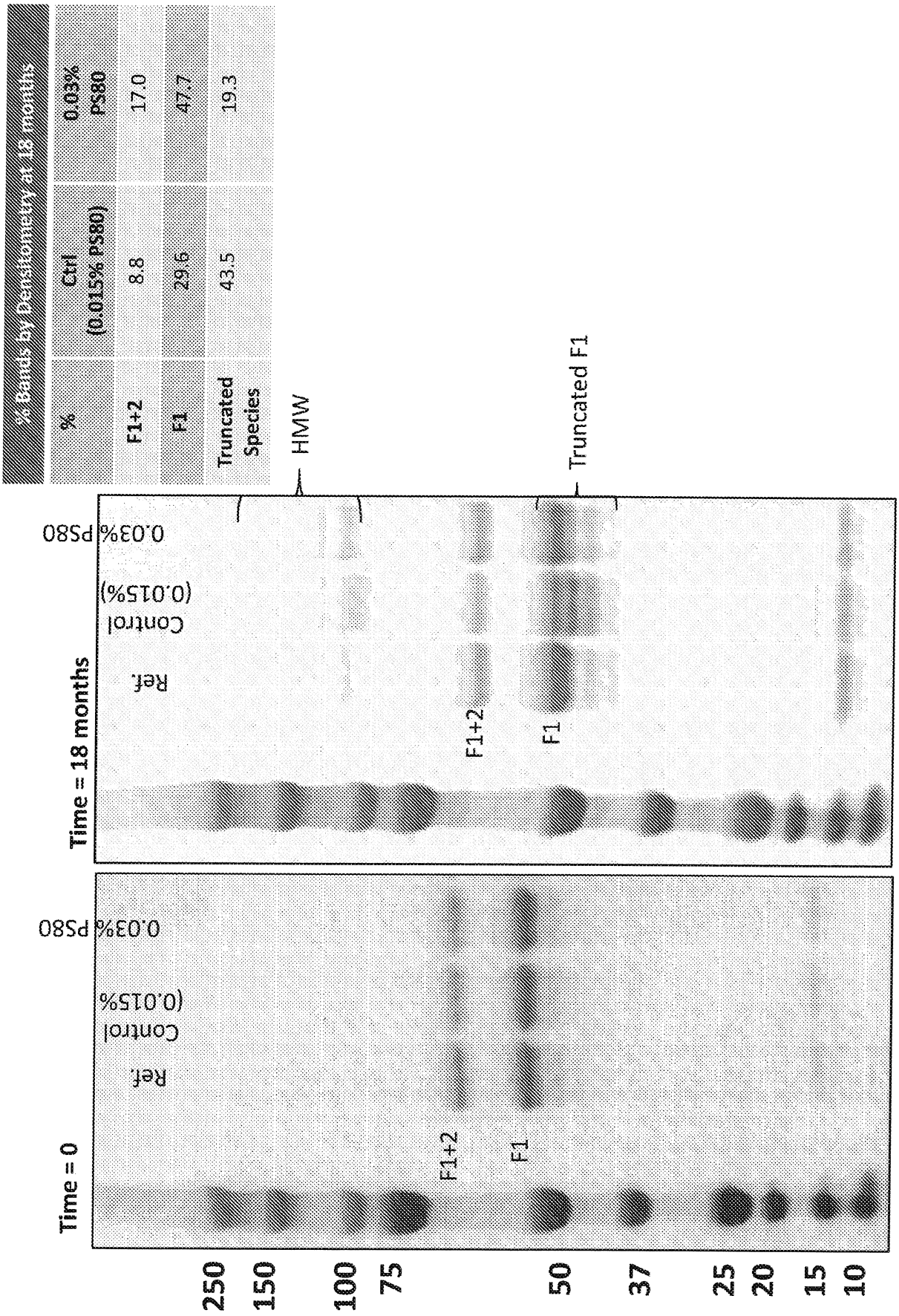


Fig. 14. RSV F Nanoparticles Bind To Site I, II, and IV Neutralizing mAbs

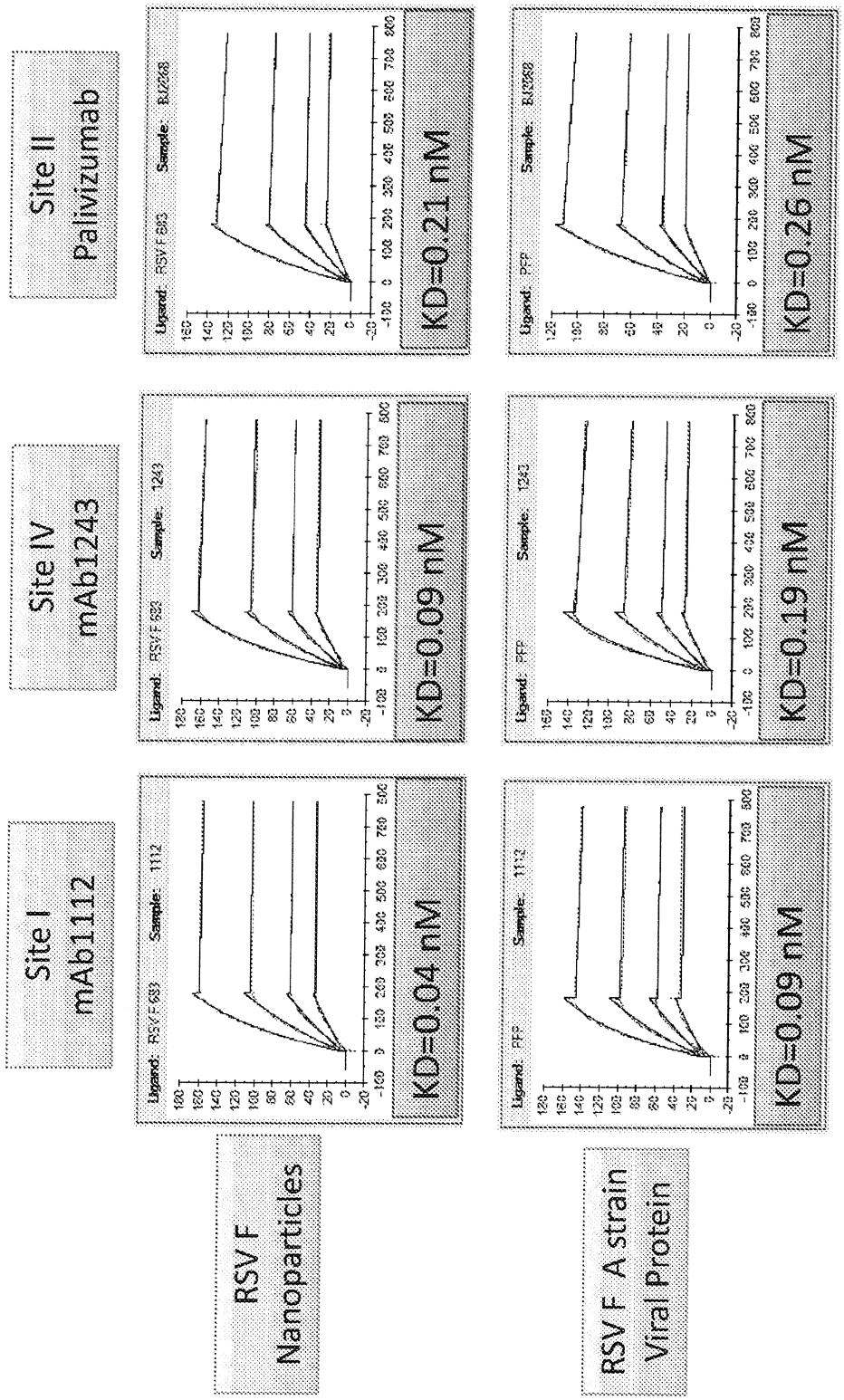


Fig. 15. RSV F Nanoparticle Vaccine Induces Antibody Competitive with mAb Binding to Sites I, IV, and II in Cotton Rats

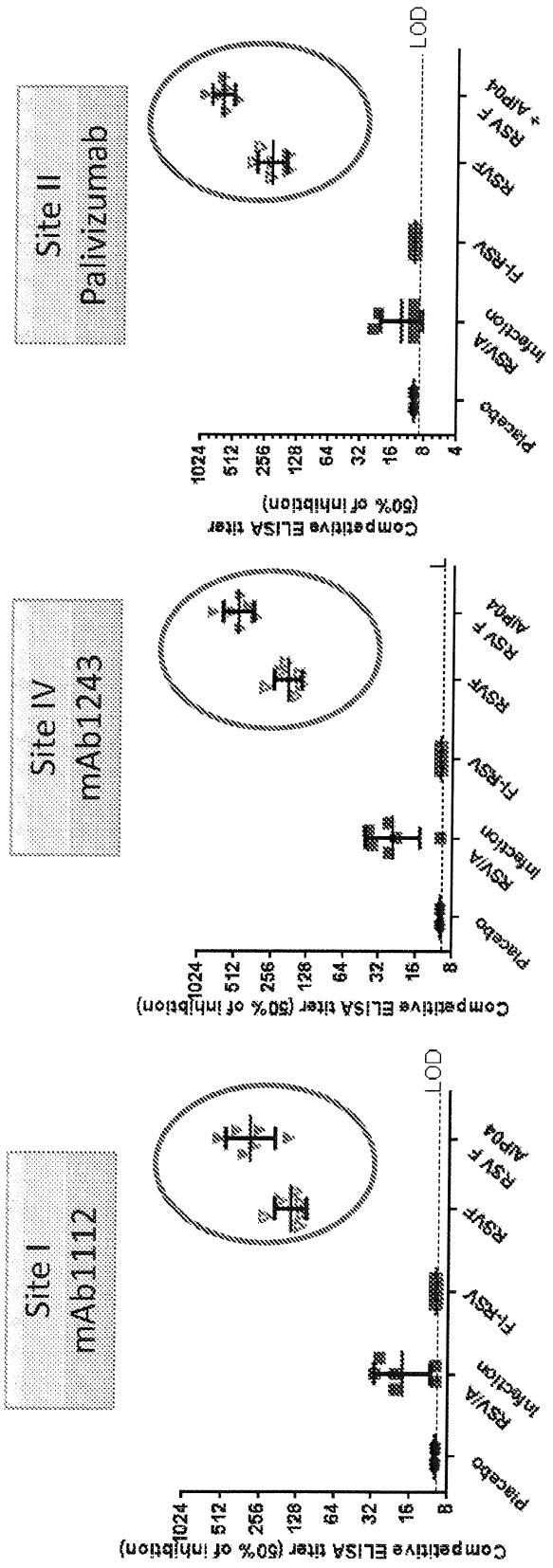


Fig. 16. Preparation of RSV F Nanoparticles from Baculovirus to Drug Substance

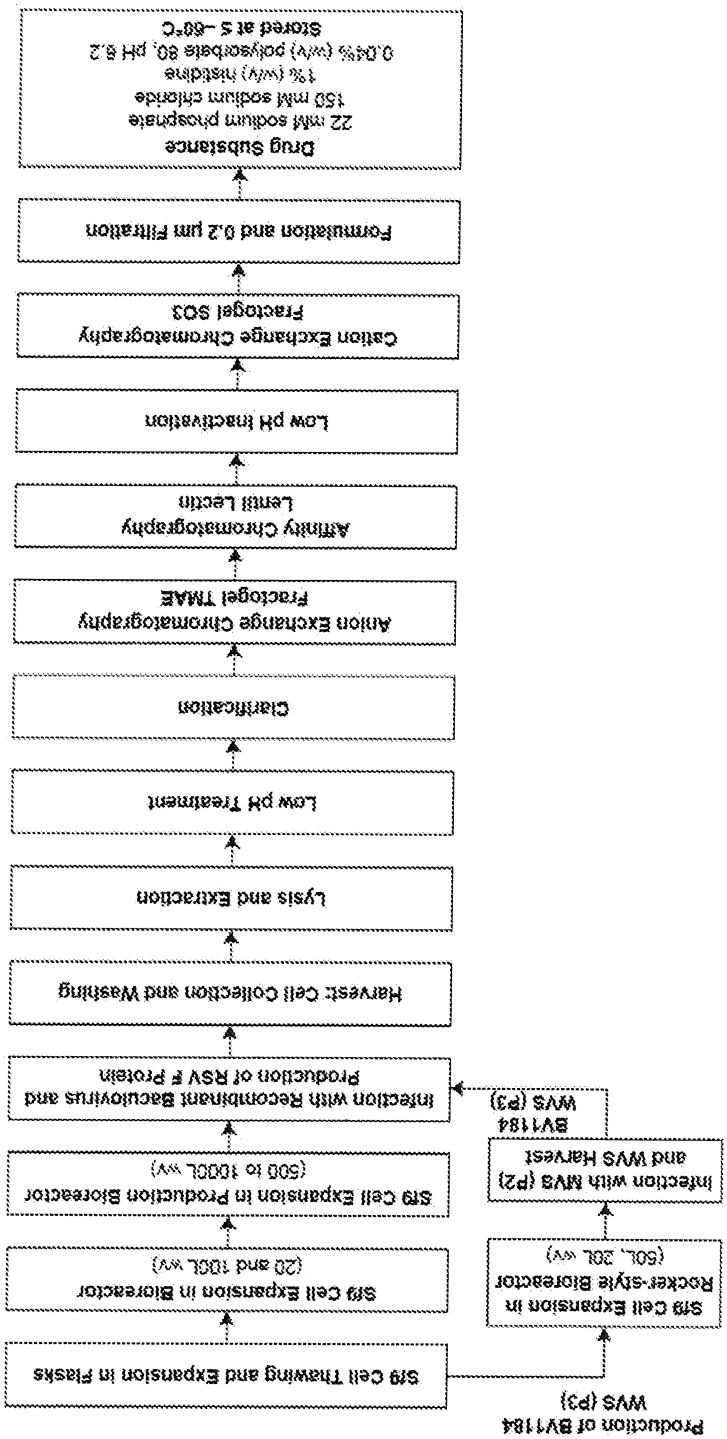
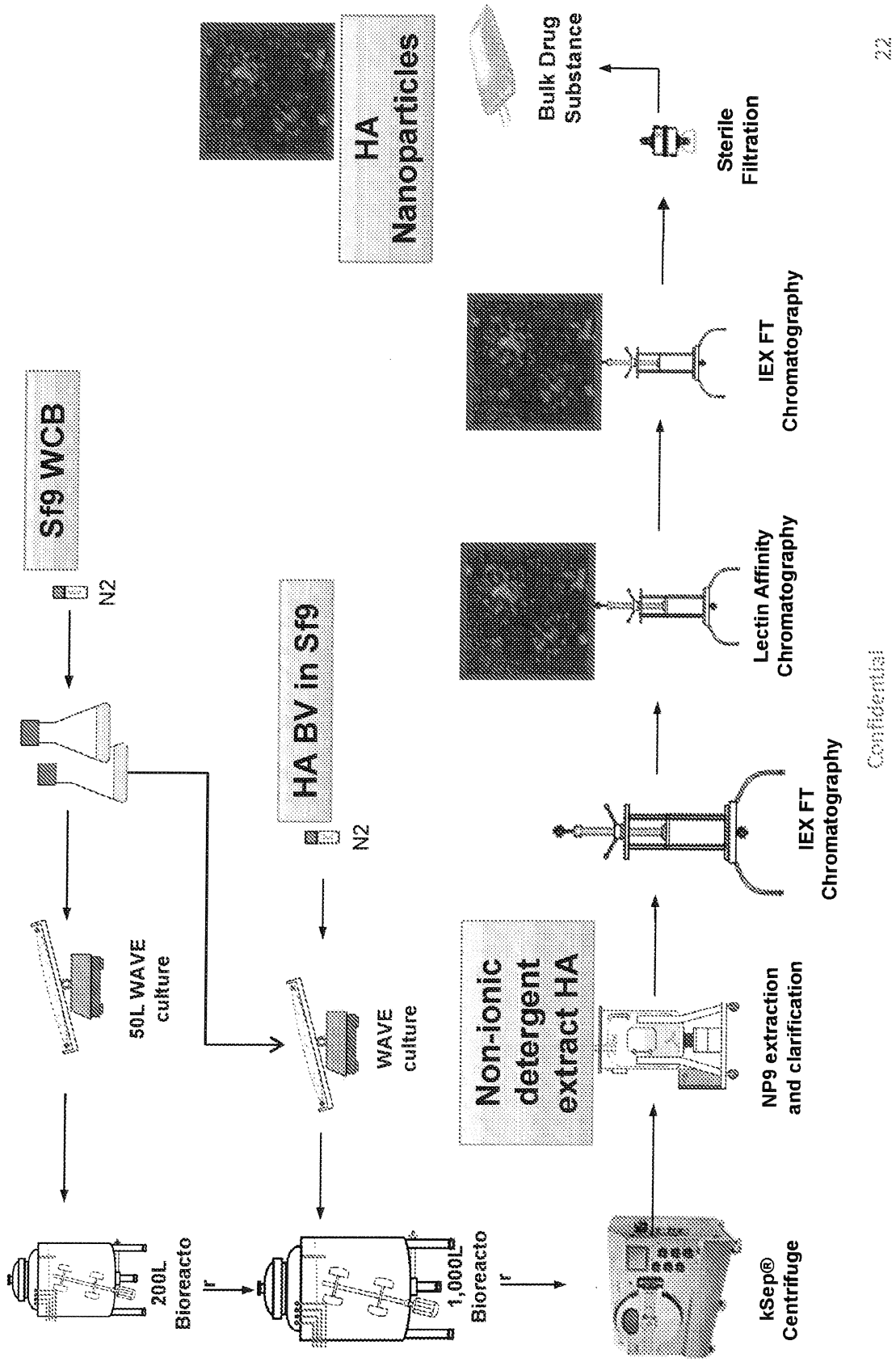


Fig. 17 Preparation of HA Nanoparticles from Baculovirus to Drug Substance



Confidential

Fig. 18A: HA Nanoparticle Purification flow chart

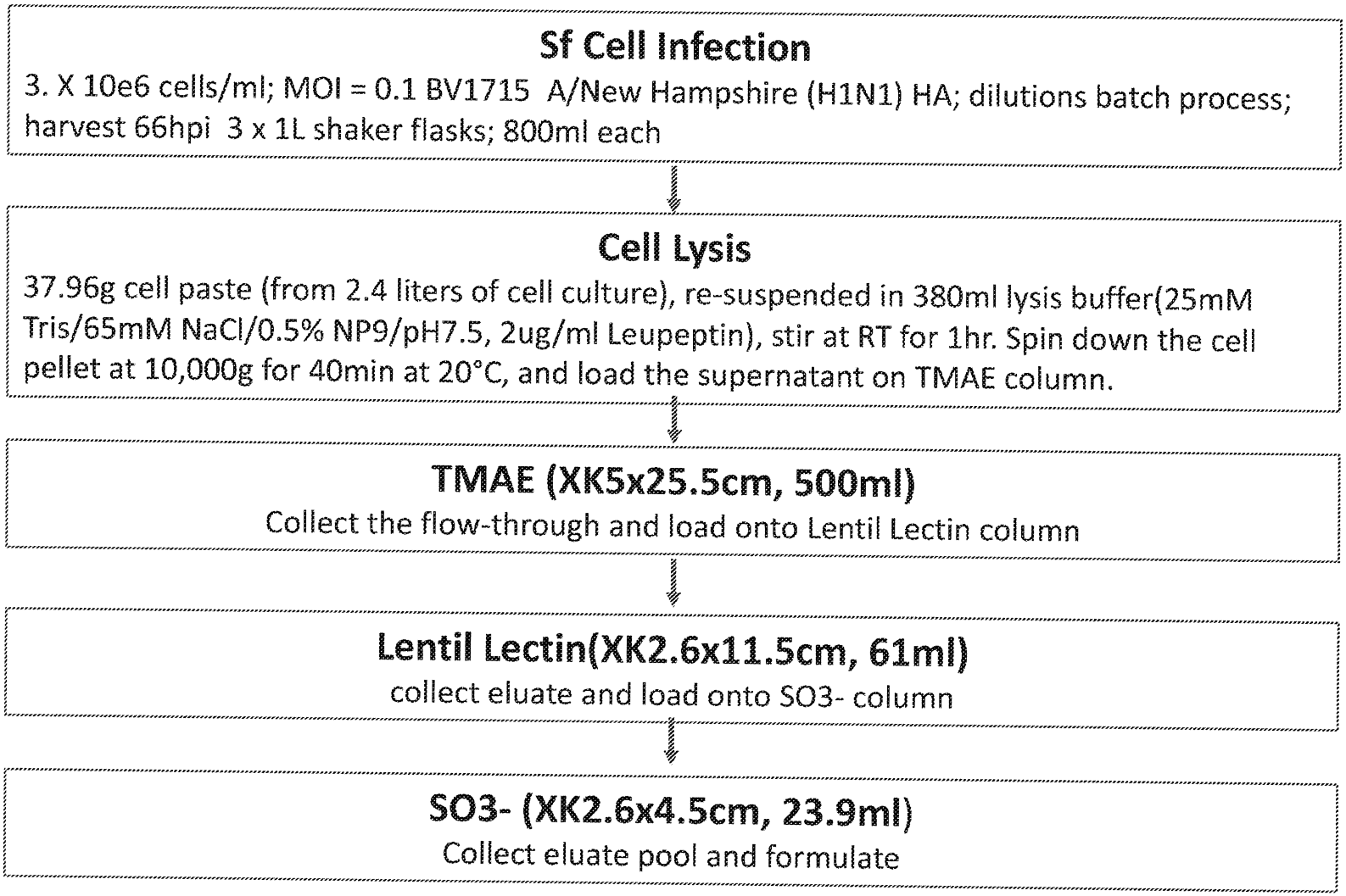


Fig. 18B: HA Nanoparticle : TMAE column chromatogram

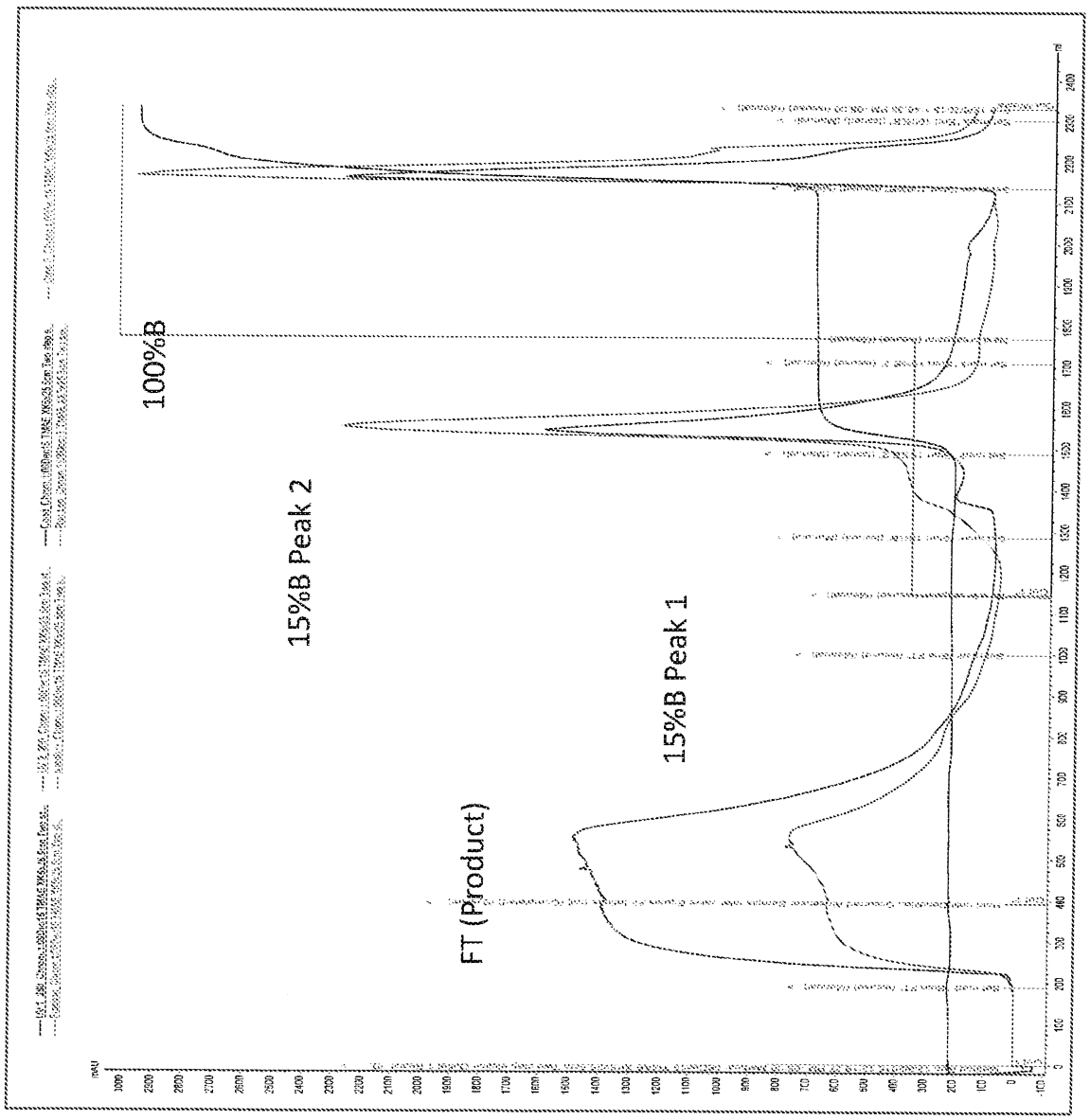


Fig. 18C: HA Nanoparticle: Lentil Lectin Chromatogram

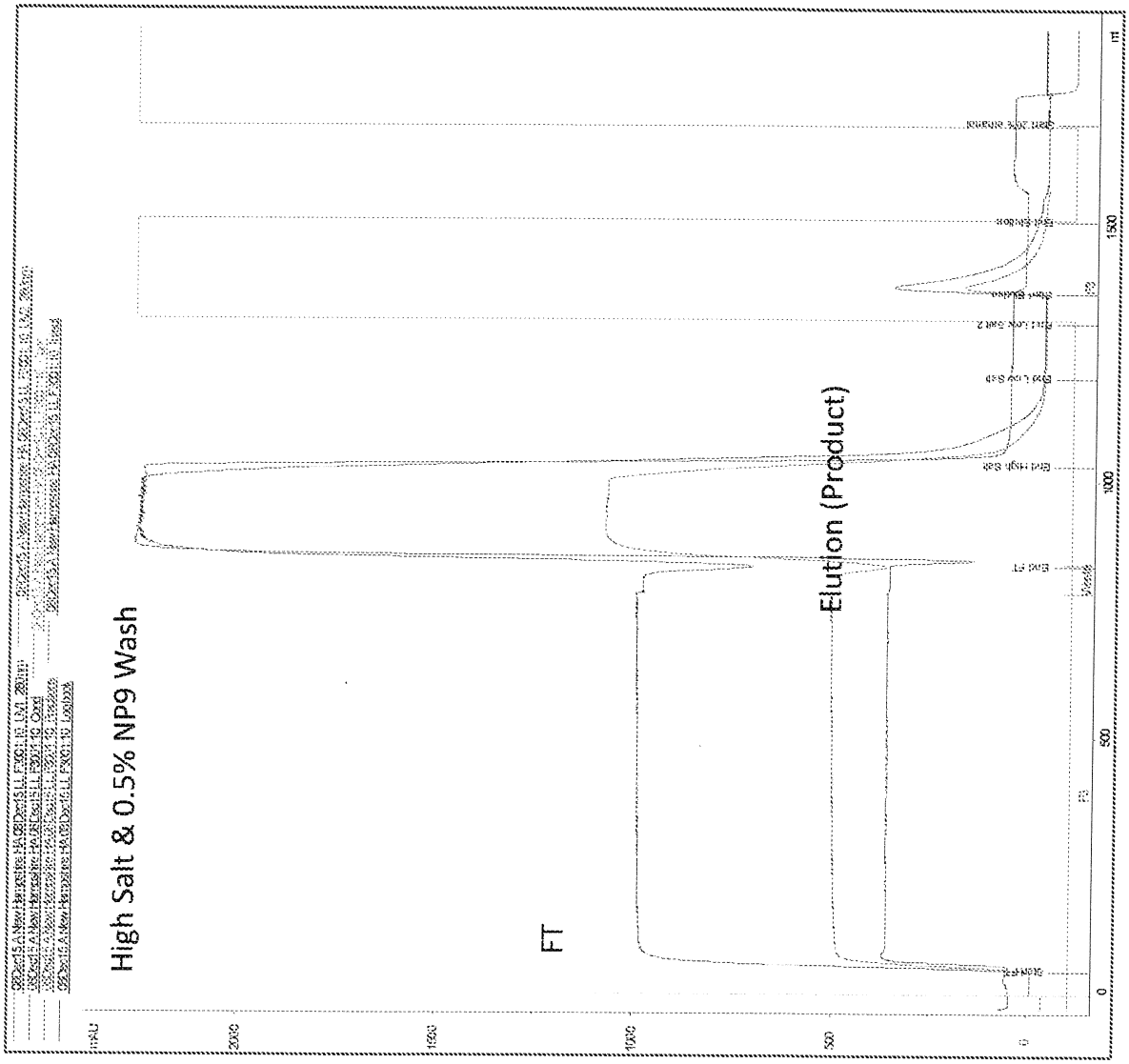
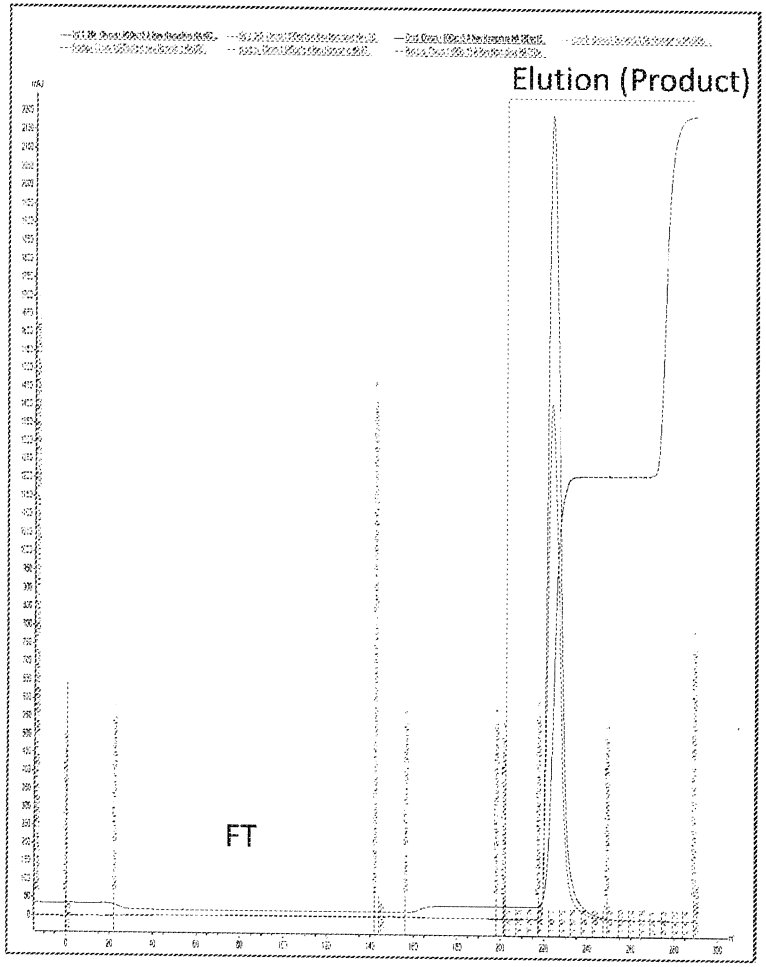


Fig. 18D: HA Nanoparticle: Sulfate (SO3-) Chromatogram



SO3 XK2.6x4.5cm 23.9ml, New
Flow Rate: 113cm/h 10ml/MIN
Equilibrate column with 3CV Buffer A1: 25mM NaPi pH6.0/10mM NaCl/0.05% ps80

Loading sample: 5ml/min 56.5cm/h

Wash with 2CV Buffer A1: 25mM NaPi pH6.0/10mM NaCl/0.05% ps80

Elution: 5ml/min—56.5cm/hcm/h
100%B1 25mM NaPi pH7.5/500mM NaCl/ 0.05% PS80 2cv

Formulation: 25 ml SO3- pool + 25 ml 50 mM NaPi pH 9, sterile filtration.
Final pH 7.2, conductivity 21 mS/cm.

Fig. 18E: HA Nanoparticle: SDS PAGE showing TMAE and LL columns

Lane	Mcl gel	Mcl WB	Description/Comments
1	6ul	6ul	Standard
2	2.5	2.5	Cell Mixture 417.96 ml
3	2.5	2.5	Lysis Sup 391.48 ml
4	5	5	TMAE FT 909.33 ml
5	5	5	TMAE 15%B 1 200.24ml
6	5	5	TMAE 15%B 2 223.5 ml
7	5	5	TMAE 100%B 169.63 ml
8	10	10	LL FT 763.69 ml
9	10	10	LL High Salt 199.95ml
10	10	10	LL Low Salt 1, 169.55 ml
11	10	10	LL Low salt 2, 108.29ml
12	10	10	LL Elution Pool 144.99ml

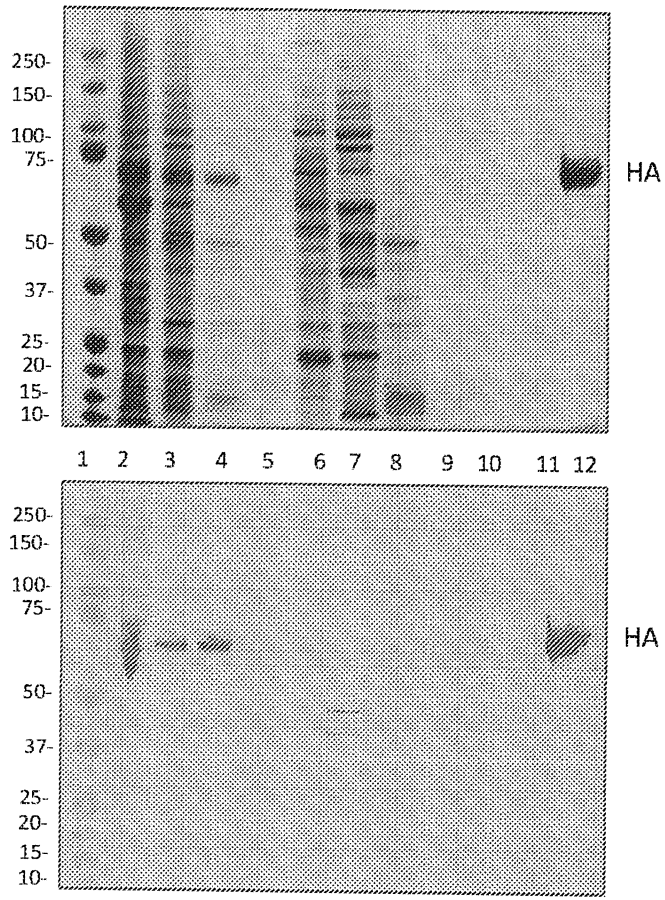


Fig. 18F: HA Nanoparticle: SDS PAGE for SO3- column

Lane	Material	Description/Comments
1	5ul	Standard
2	9	II Pool 1in4 5ml
3	9	SO3 Load 1in4 95ml
4	9	SO3 FT 1in1 98ml
5	9	1A2 5ml
6	9	1A3
7	9	1A5
8	9	1B1
9	9	1B2
10	9	1B3
11	9	1B4
12	9	1B5
13	9	1C1
14	9	2A1
15	9	2A2

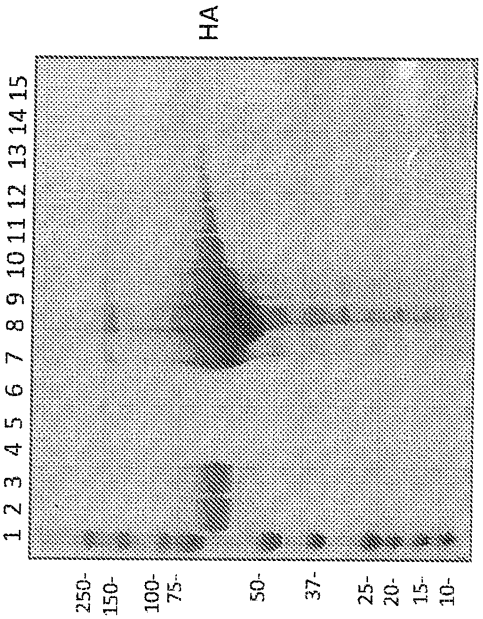


Fig. 19A: HA Nanoparticle Analysis A/New Hampshire/1/2015 (H1N1)

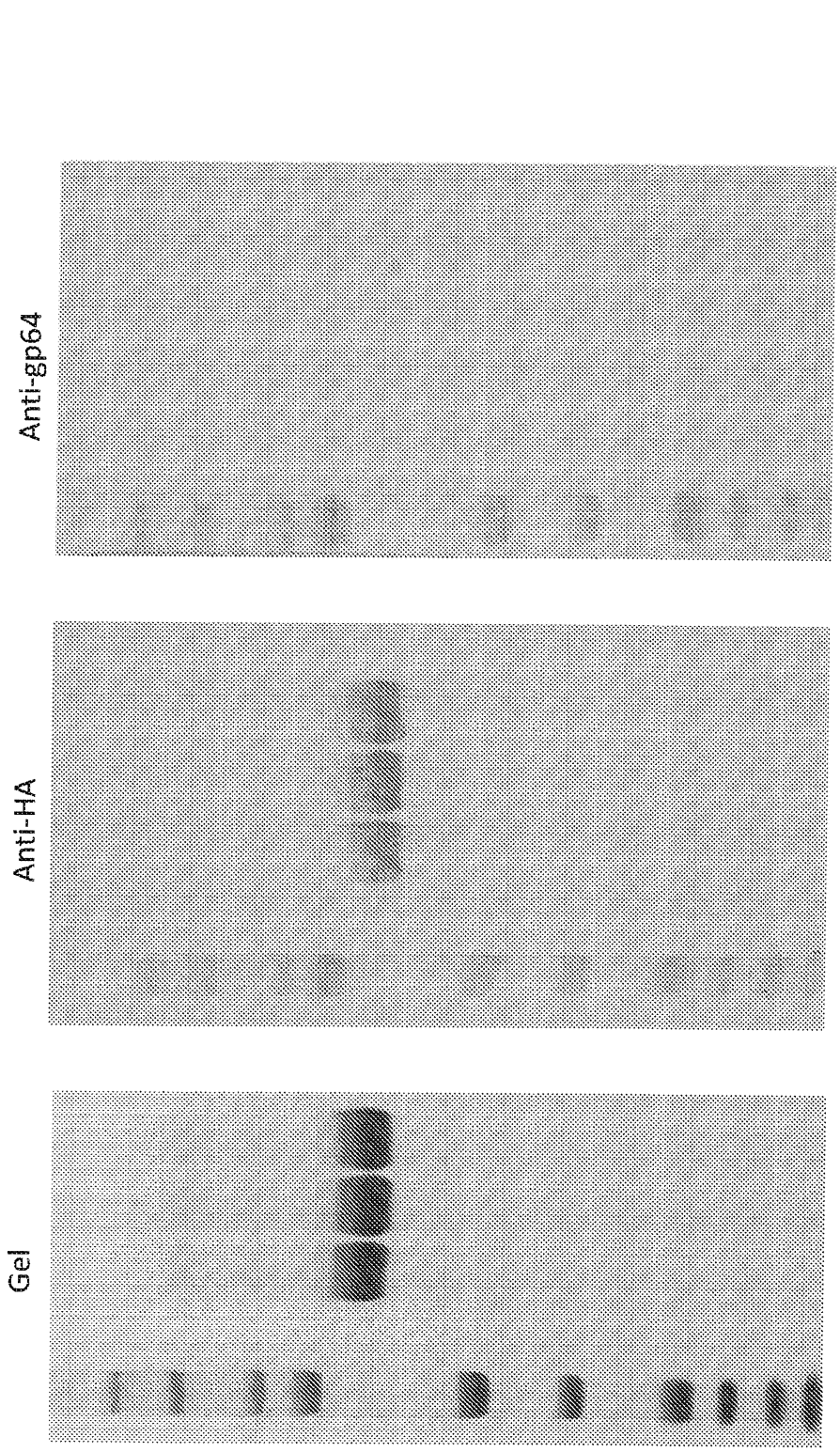
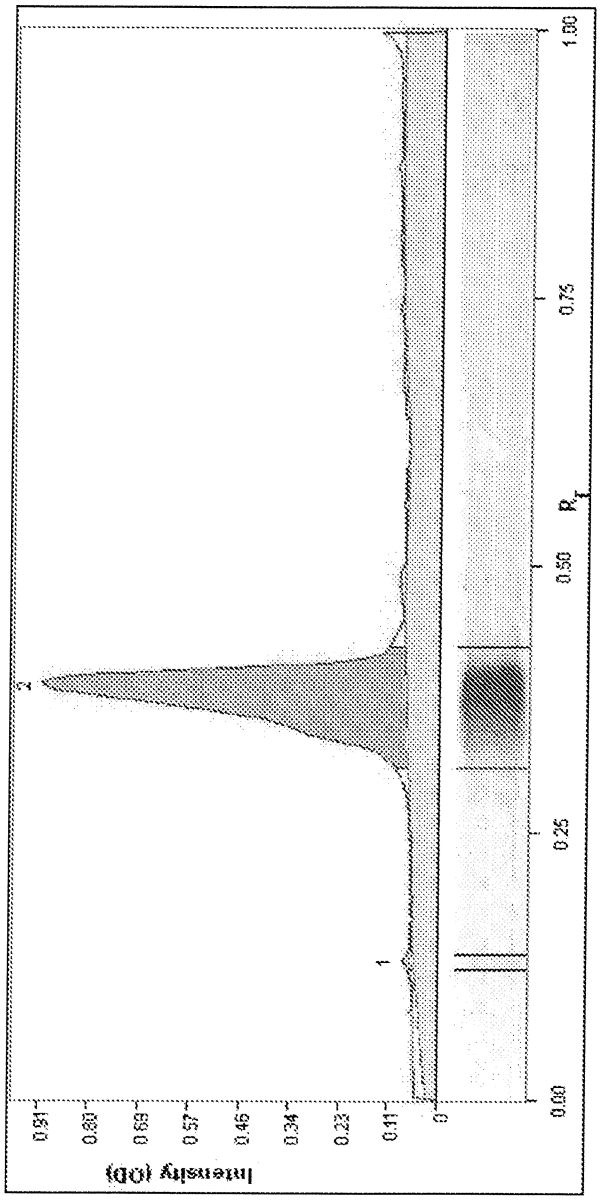


Fig. 19B: HA Nanoparticle Analysis A/New Hampshire/1/2015 (H1N1)



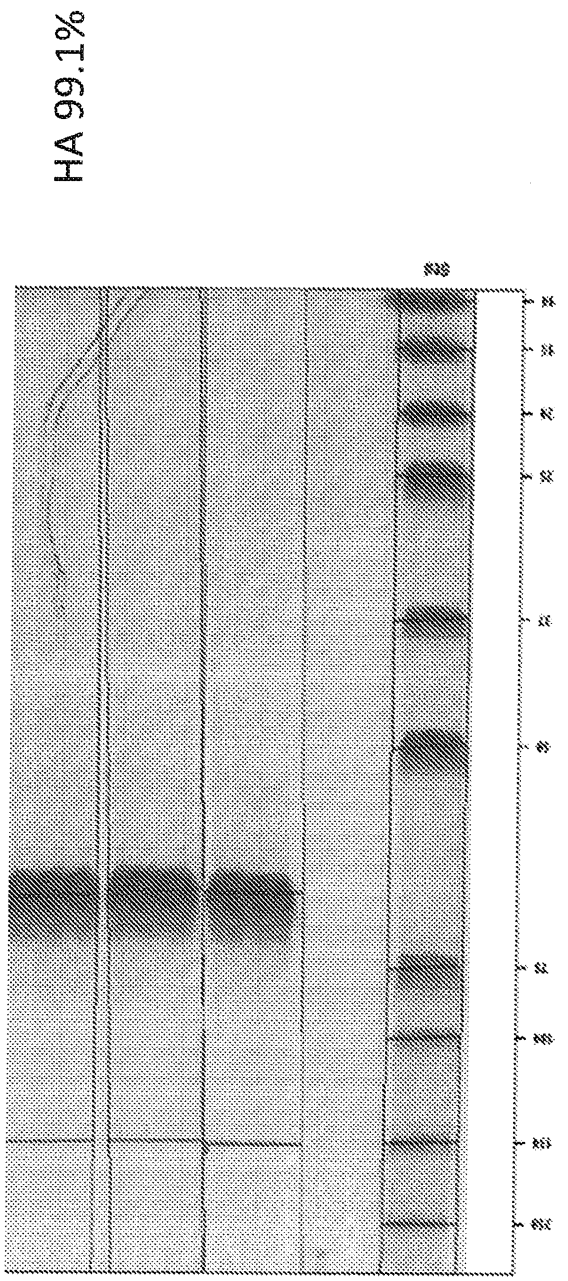


Fig. 19C: HA Nanoparticle Analysis (A/Switzerland/9715293/2013 (H3N2))

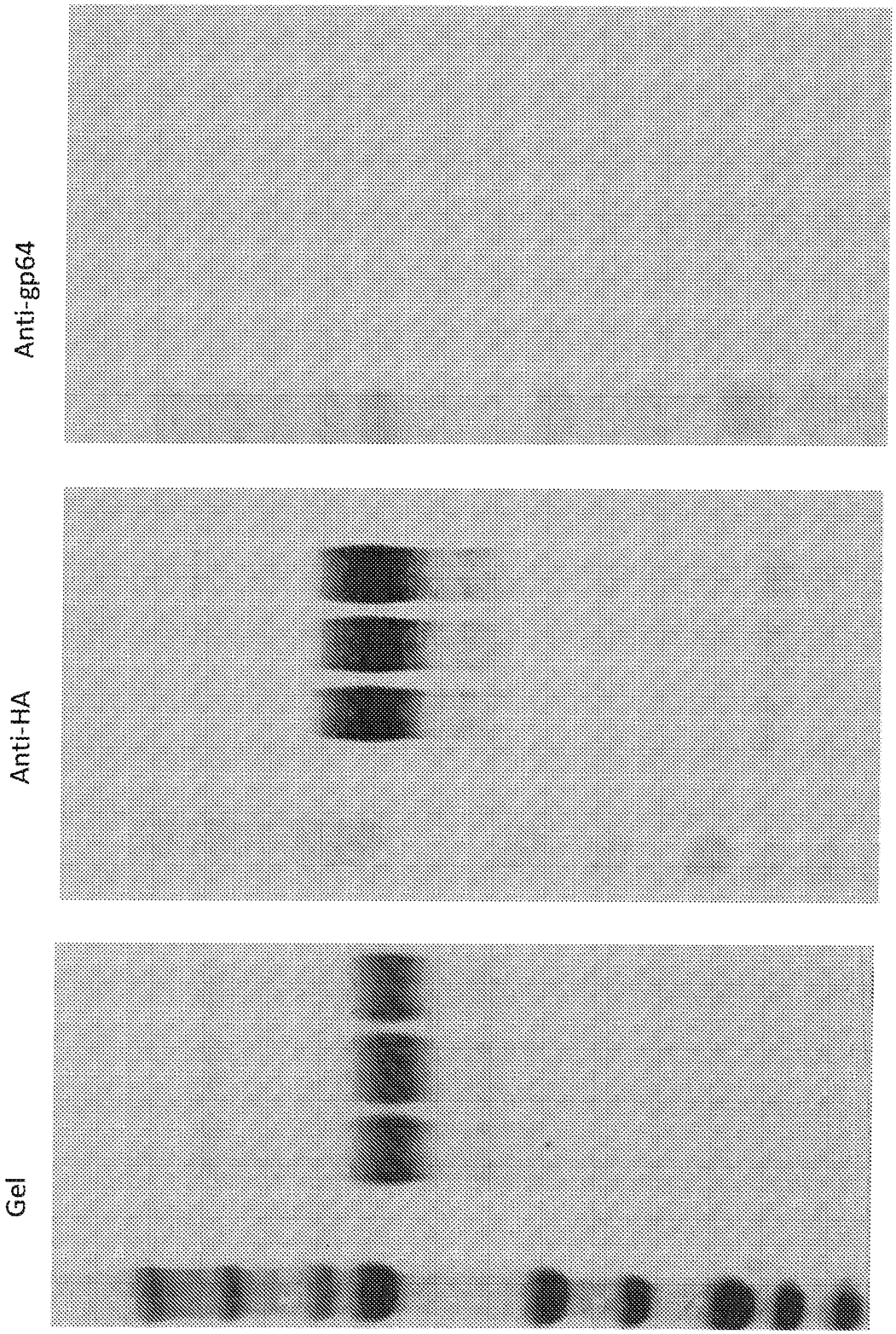


Fig. 19D: HA Nanoparticle Analysis (A/Switzerland/9715293/2013 (H3N2))

Fig. 19E: HA Nanoparticle Analysis A/Hong Kong/4801/2014 (H3N2)

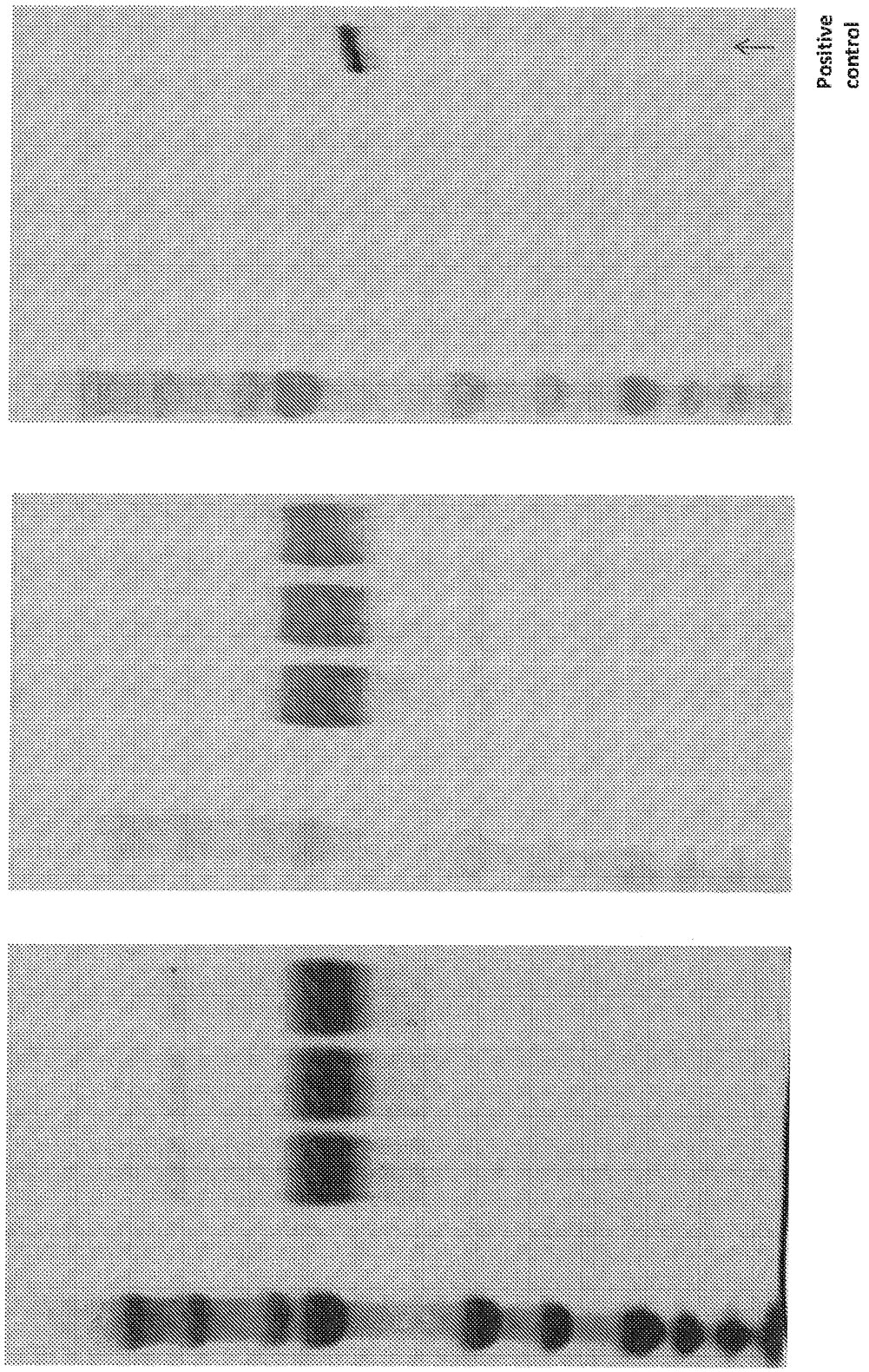
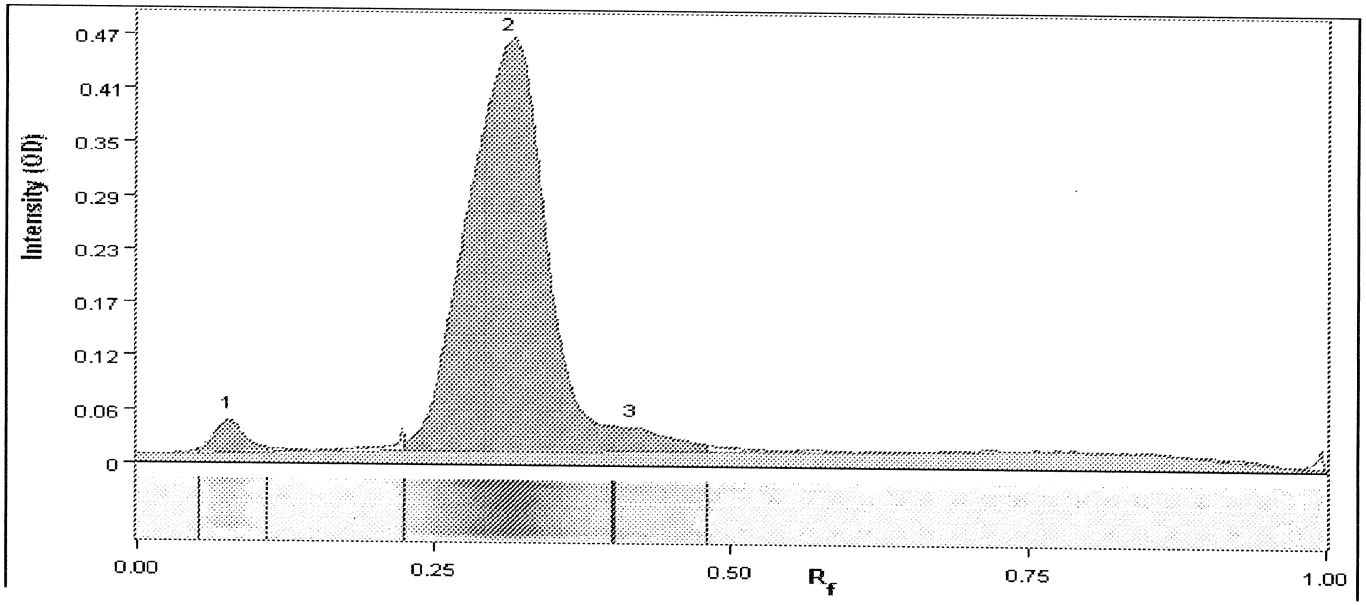
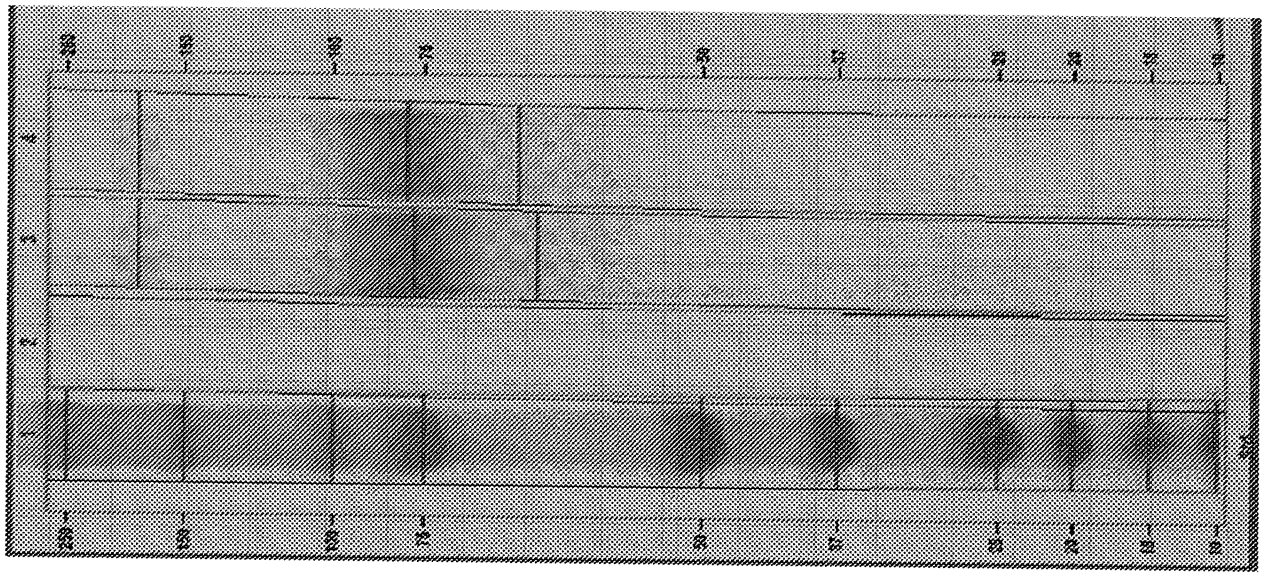


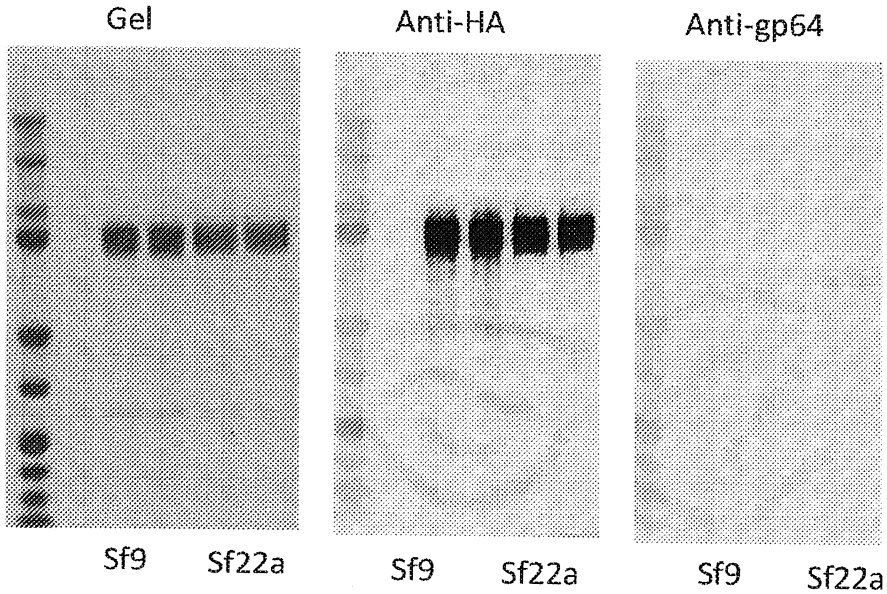
Fig. 19F: HA Nanoparticle Analysis (A/Hong Kong/4801/2014 (H3N2)



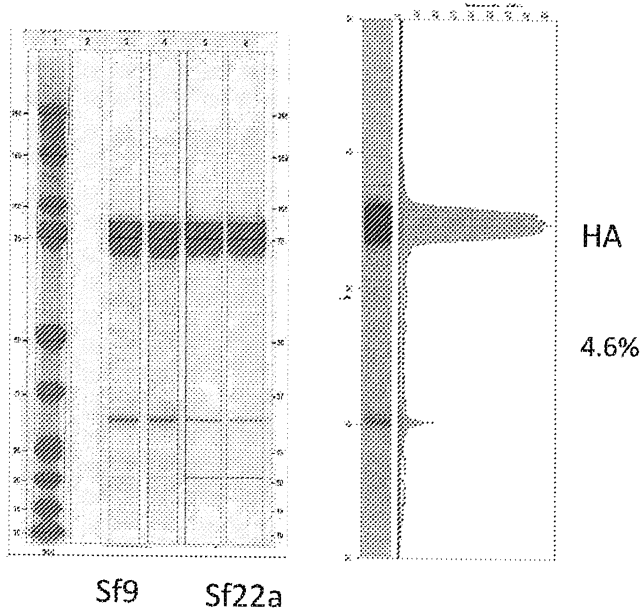


HA: 93.3%

Fig. 19G: HA Nanoparticle Analysis (B/Phuket/3073/2013) in Sf9 and Sf22a cells

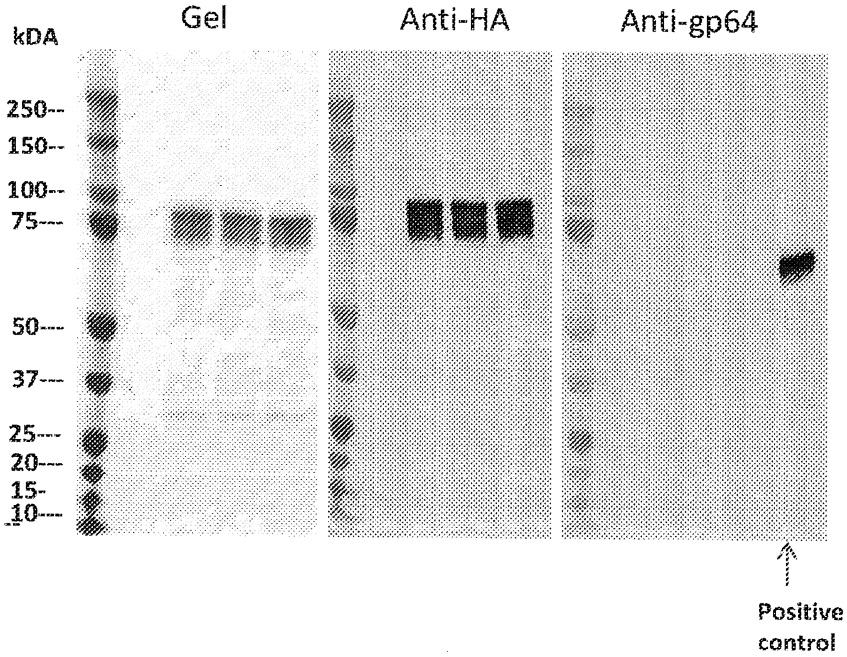


20 mg/L A280

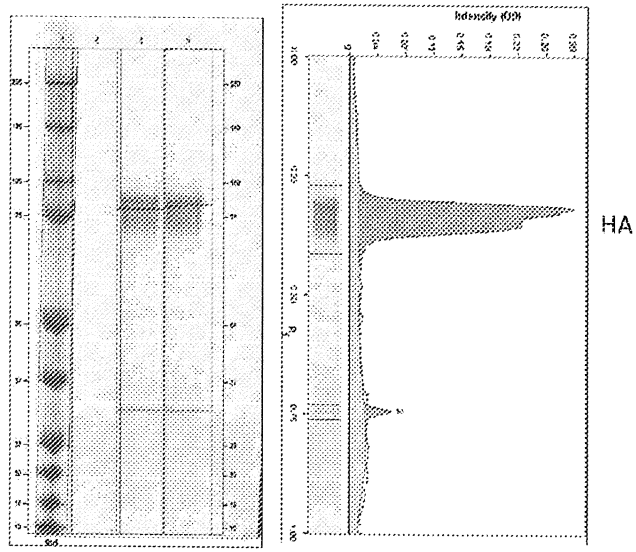


HA: 95.4%

Fig. 19H: HA Nanoparticle Analysis (B/Brisbane/60/2008 HA nanoparticles)



29 mg/L A280



HA: 96.7%

Fig. 19I: HA Nanoparticle Purity by RP-HPLC Scout20-100 with Imtakt Column (AD)

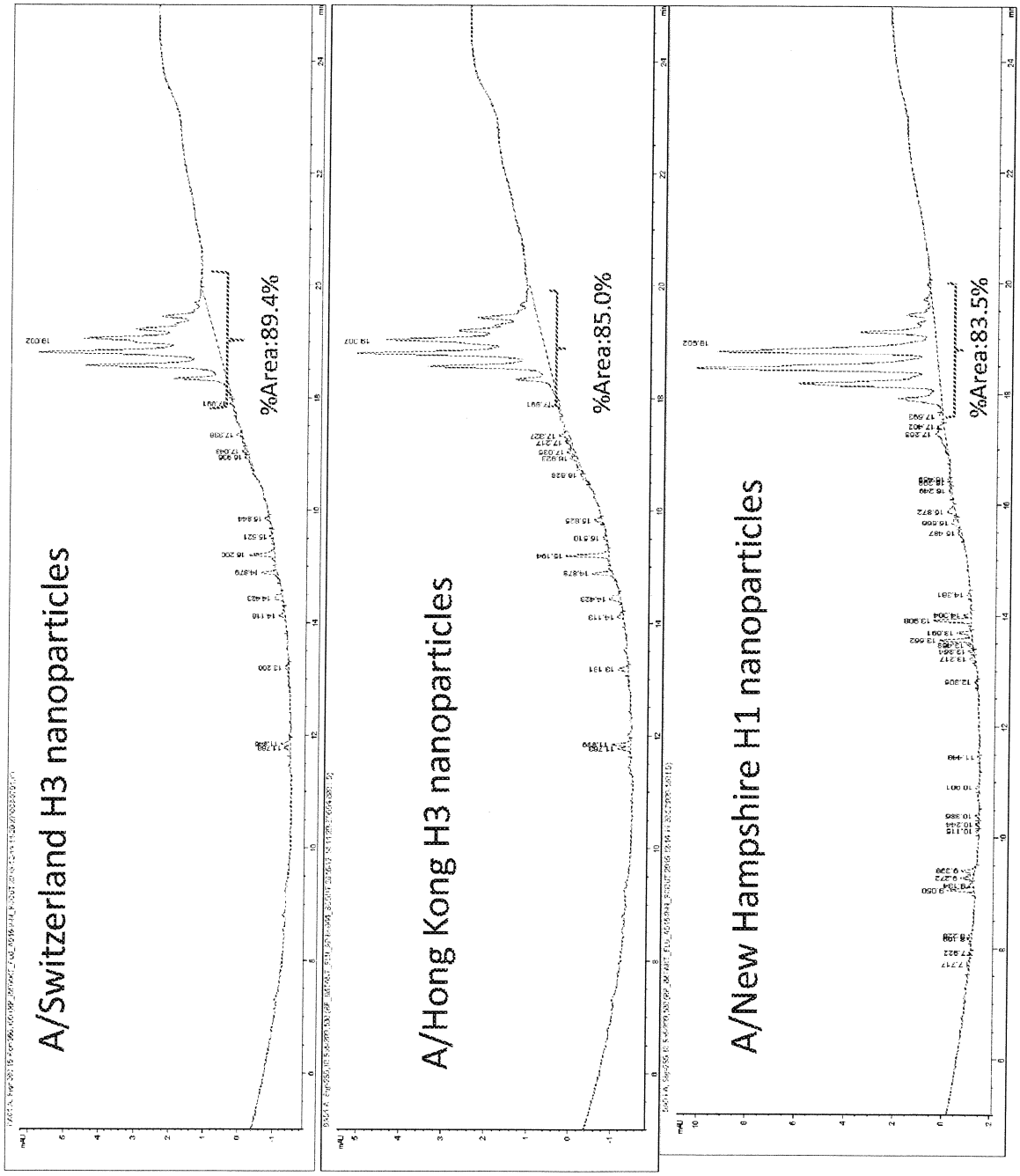
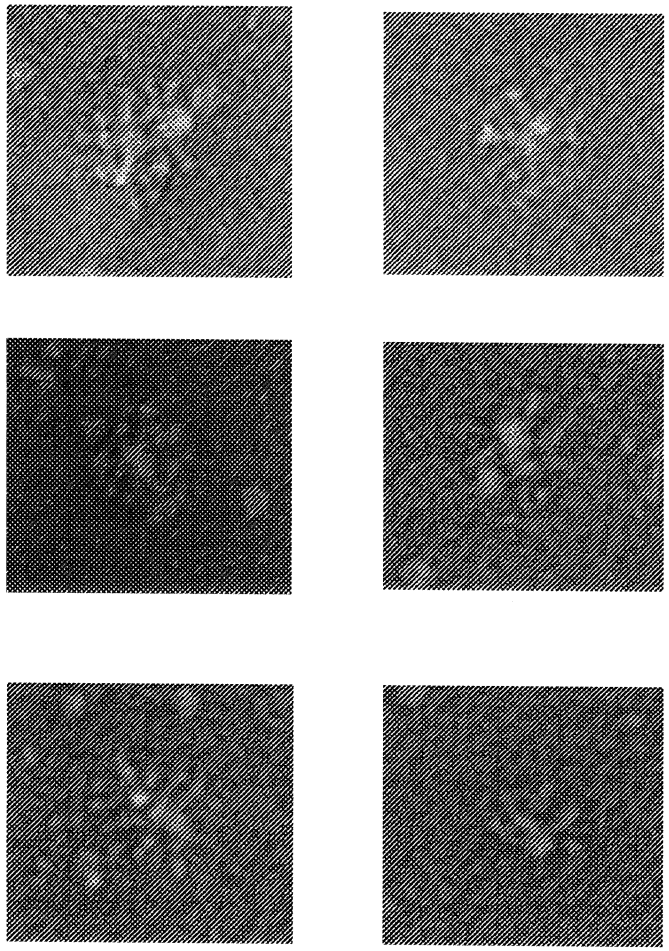
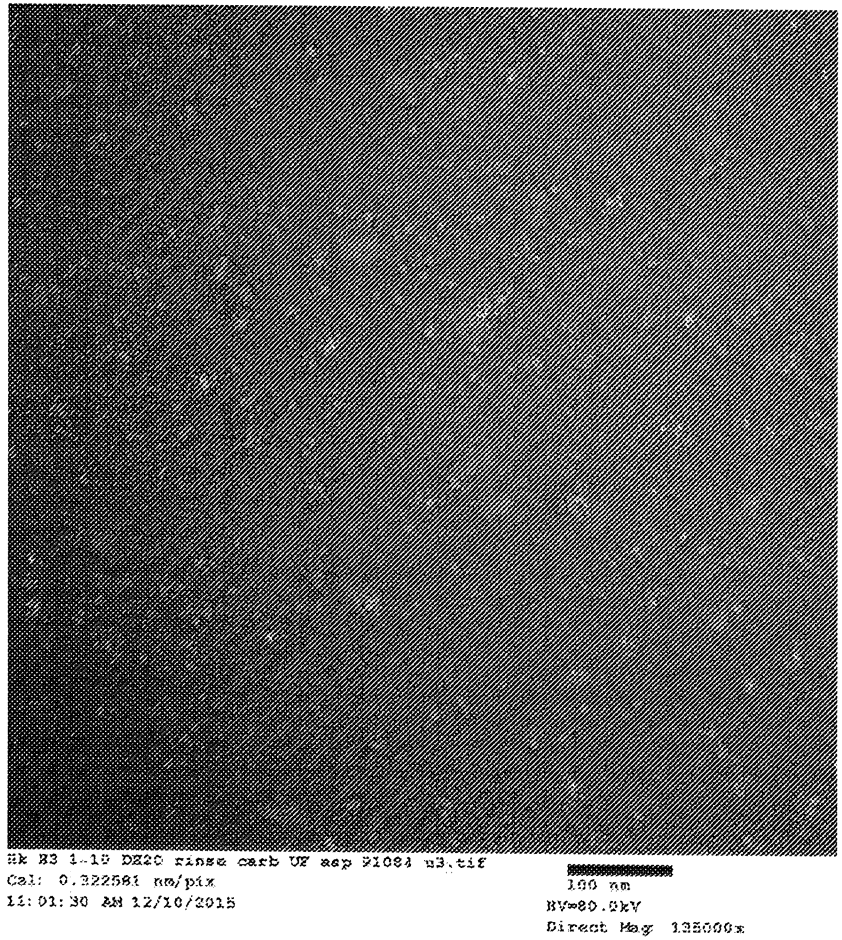


Fig. 19J: HA Nanoparticle Analysis Summary of Production and Analysis

Strains	BCA mg/ml	A280 HA mg/ml	IDMS HA mg/ml	HA% peptide mapping	HA% Densitometry	HA% RP-HPLC	Particle Size d.nm (PDI)	Yield (mg/L A280)
A/New Hampshire/1/2015 H1N1	0.98	0.93	0.772	97.3%	93.4%	83.5%	22.0 (0.126)	19
A/Switzerland/9715293/2013 H3N2	1.06	0.93	0.891	98.1%	94.5%	89.4%	24.7 (0.135)	18
A/Hong Kong/4801/2014 H3N2	0.70	0.55	0.535	95.1%	93.3%	85.0%	24.0 (0.174)	12
B/Phuket/3073/2013	0.60	0.60	0.389	89.6%	95.4%		29.9 (0.118)	20
B/Brisbane/60/2008	1.39	1.44	1.015	90.4%	96.7%		30.5 (0.102)	29

Fig. 20: HA Nanoparticle EM micrograph (negative stain)



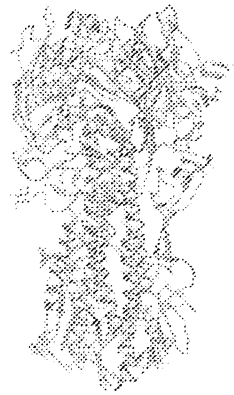


100 nm

Fig. 21A: A/Switzerland/715293/2014 (H3N2) HA Nanoparticles: CryoEM 2D class averaging



Hemagglutinin Trimer



Hemagglutinin Active Form (PDB ID 1GUZ)

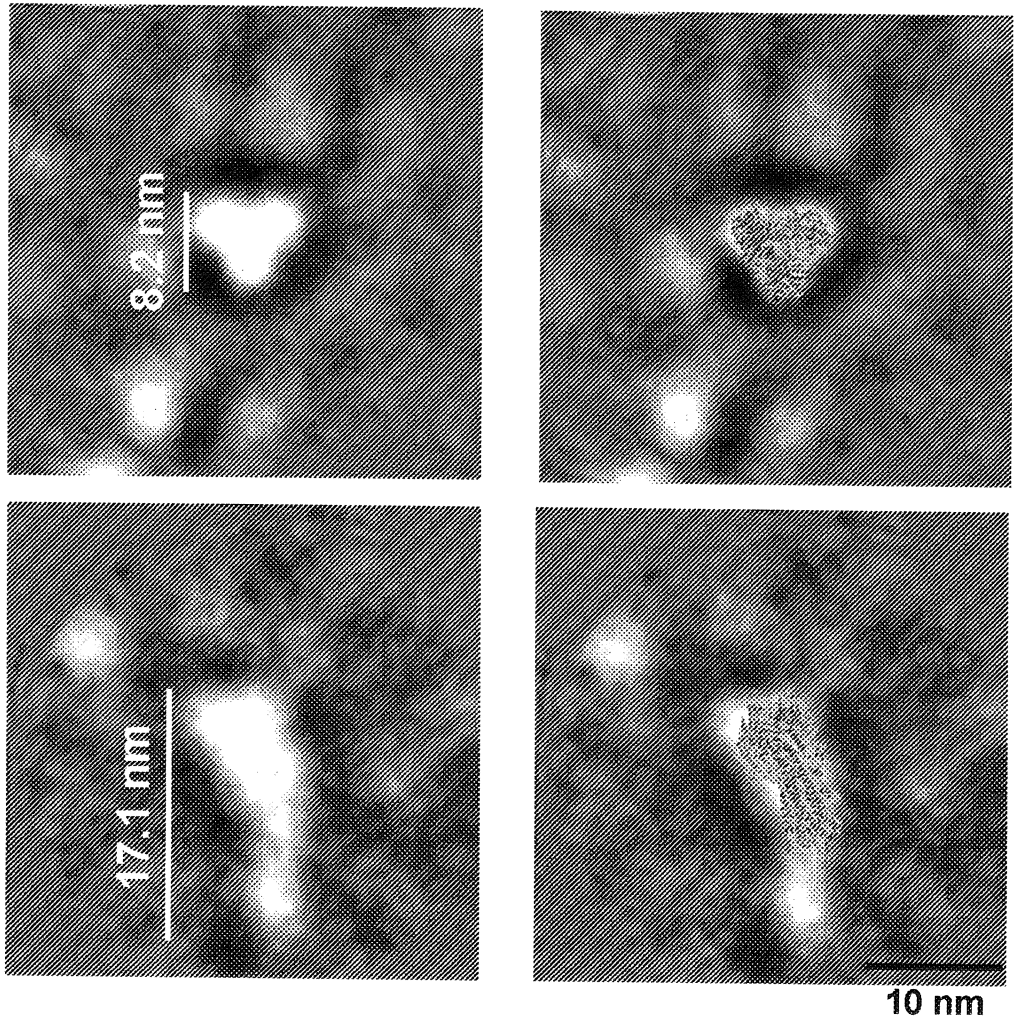


Fig. 21B: A/Switzerland/715293/2014 (H3N2) n15 VLPs: CryoEM 2D class averaging

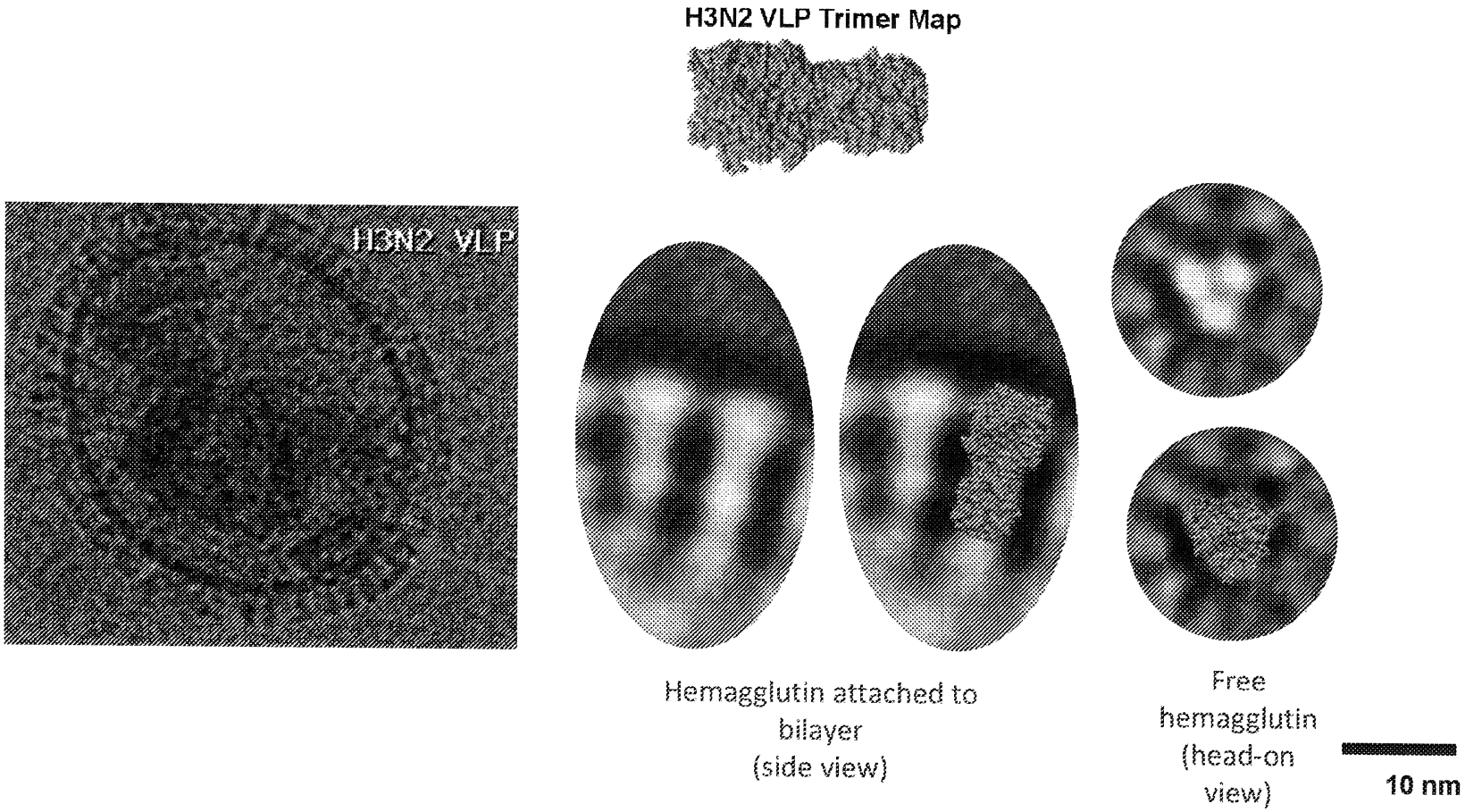


Fig. 22: Immunogenicity : RIC RSV F + A/Switzerland H3 HA Nanoparticle + Matrix M mouse study

Group	N	RSV F 1.5µg	[1]H3N2 nHA 1.5µg	Matrix M 5µg	$AlPO_4$ (60µg)	Immunization (Day)	Blood Collection (Day; n=10)	Spleen Harvest (Day; n=5)
1	15	+	-	-	-	0, 21	-1, 21, [2]28, 35	28
2	15	-	+	-	-	0, 21	-1, 21, [2]28, 35	28
3	15	+	+	-	-	0, 21	-1, 21, [2]28, 35	28
4	15	+	-	+	-	0, 21	-1, 21, [2]28, 35	28
5	15	-	+	+	-	0, 21	-1, 21, [2]28, 35	28
6	15	+	+	+	-	0, 21	-1, 21, [2]28, 35	28
7	15	+	-	-	+	0, 21	-1, 21, [2]28, 35	28
8	15	-	+	-	+	0, 21	-1, 21, [2]28, 35	28
9	15	+	+	-	+	0, 21	-1, 21, [2]28, 35	28
10	5	-	-	-	-	-	-	28

[1]A/Switzerland H3 Nanoparticle (nHA) monovalent

[2]Day 28 blood draw only on mice euthanized for spleen collection

Fig. 23A: Homologous Influenza A/Switzerland/2013 (H3N2) HA Nanoparticle HAI responses

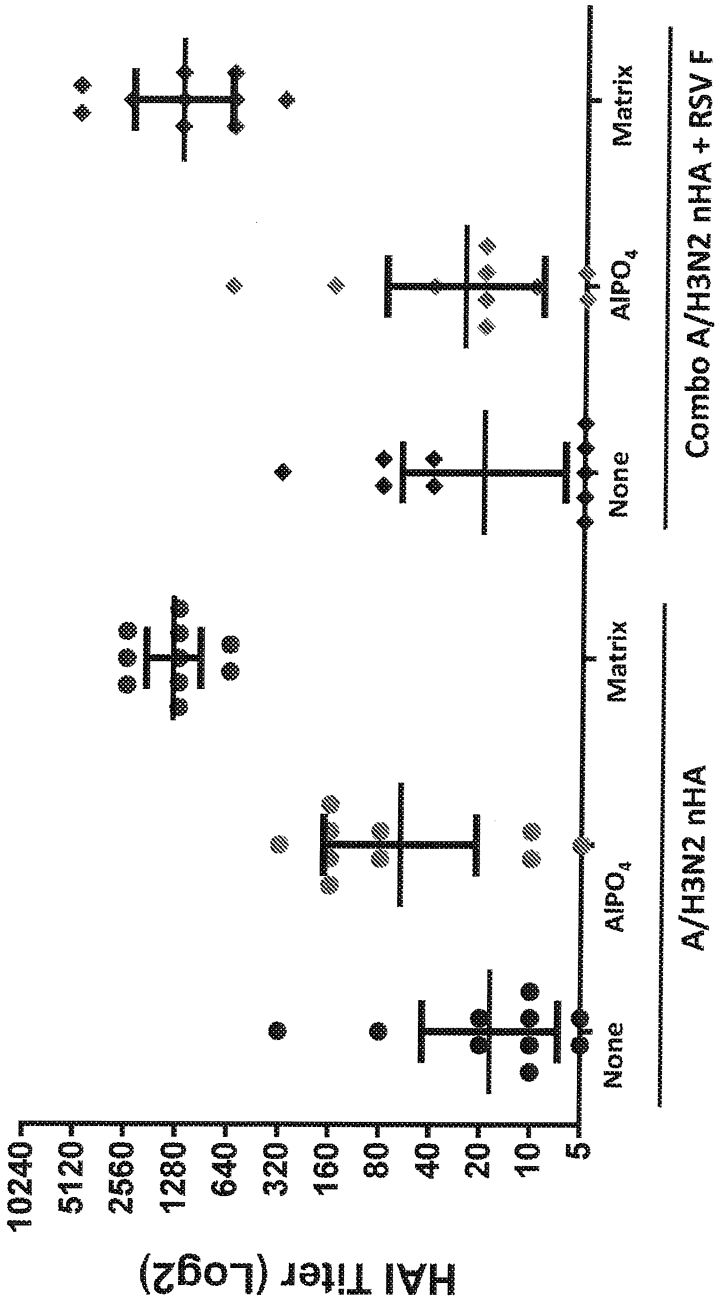


Fig. 23B: Heterologous Influenza A/Texas/50/2012 (H3N2)
HA Nanoparticle HAI responses

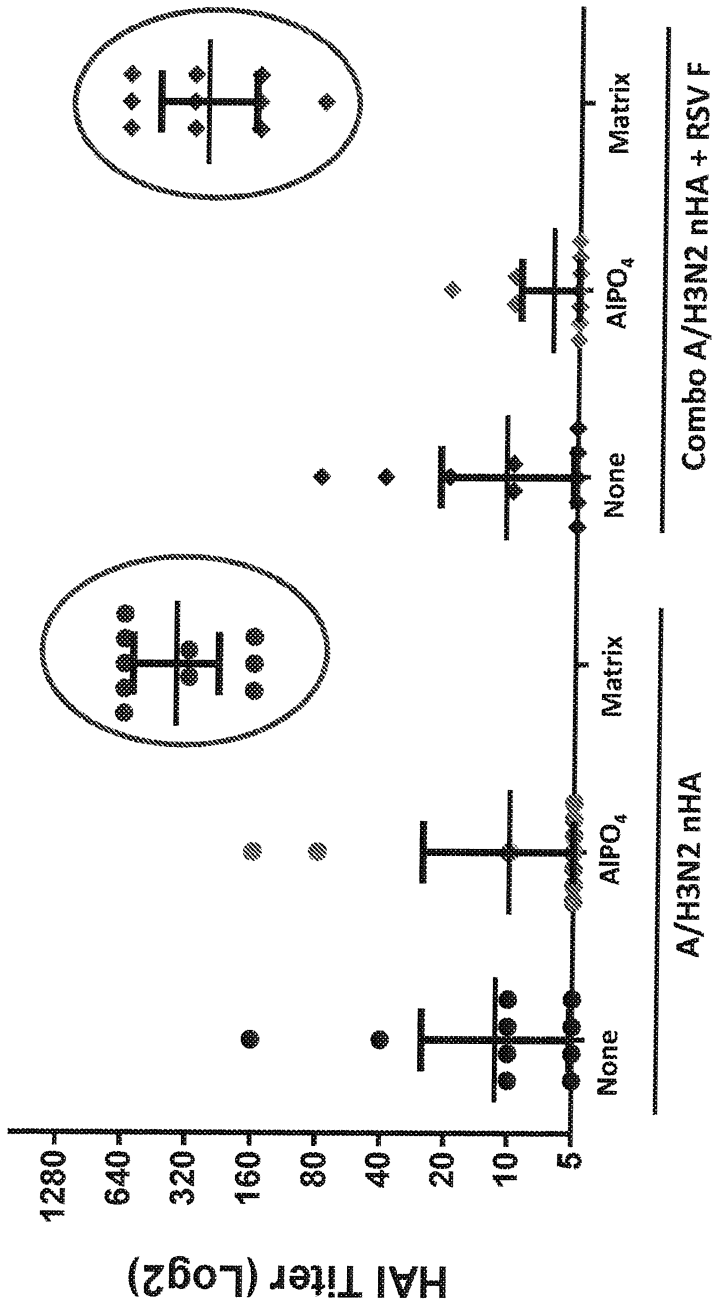


Fig. 23C: RSV F site II Palivizumab Competitive Antibody (PCA) responses

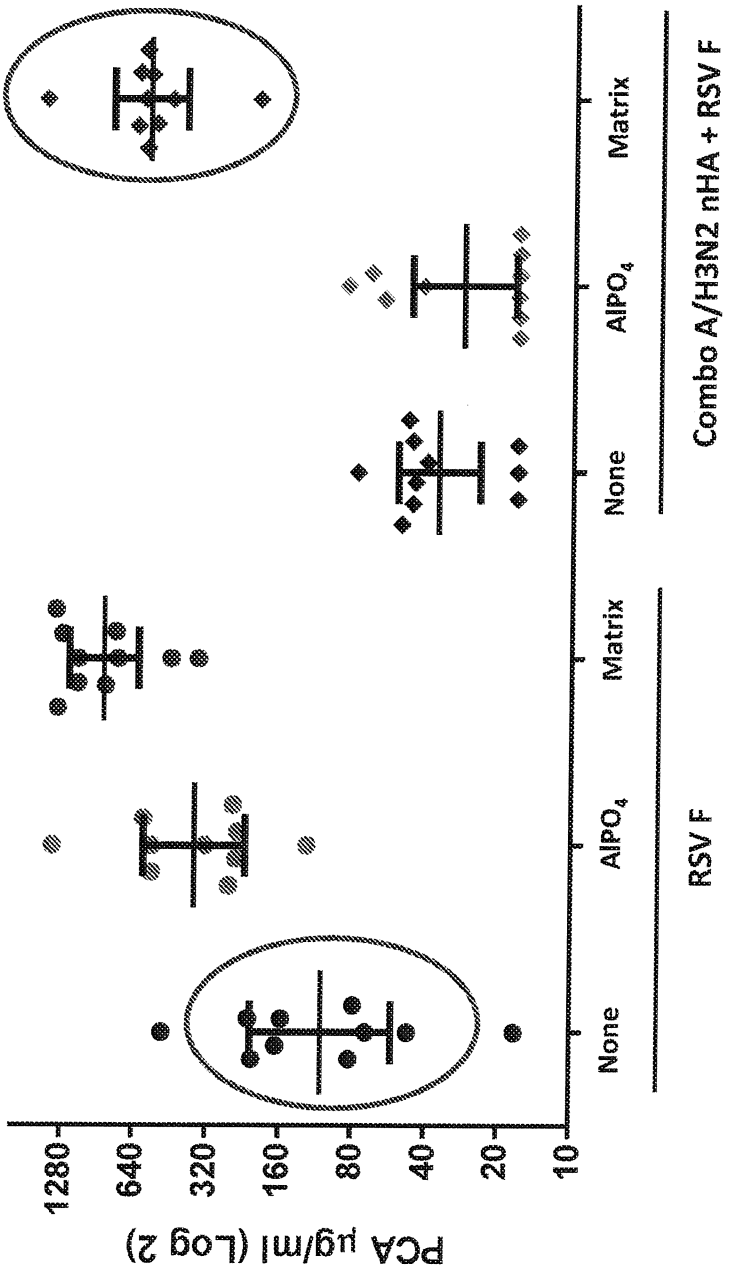


Fig. 23D: RSV A neutralizing antibody responses

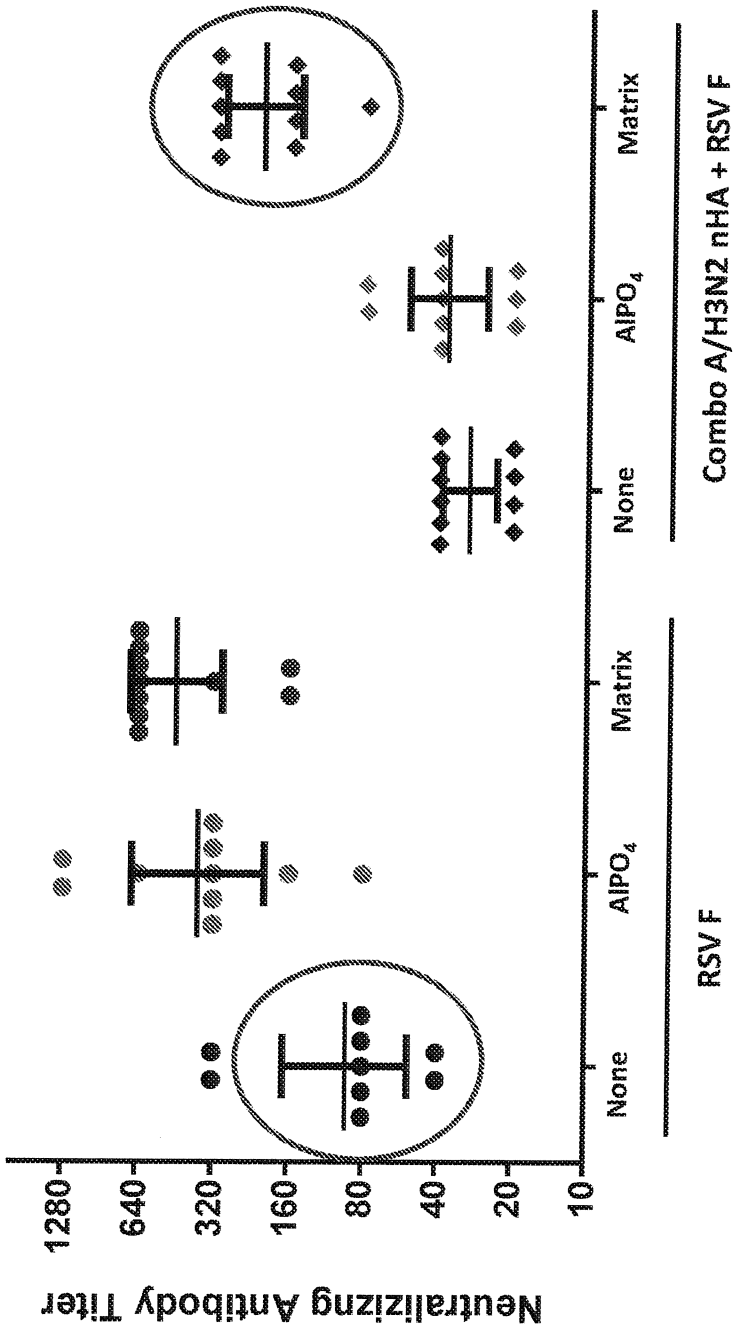
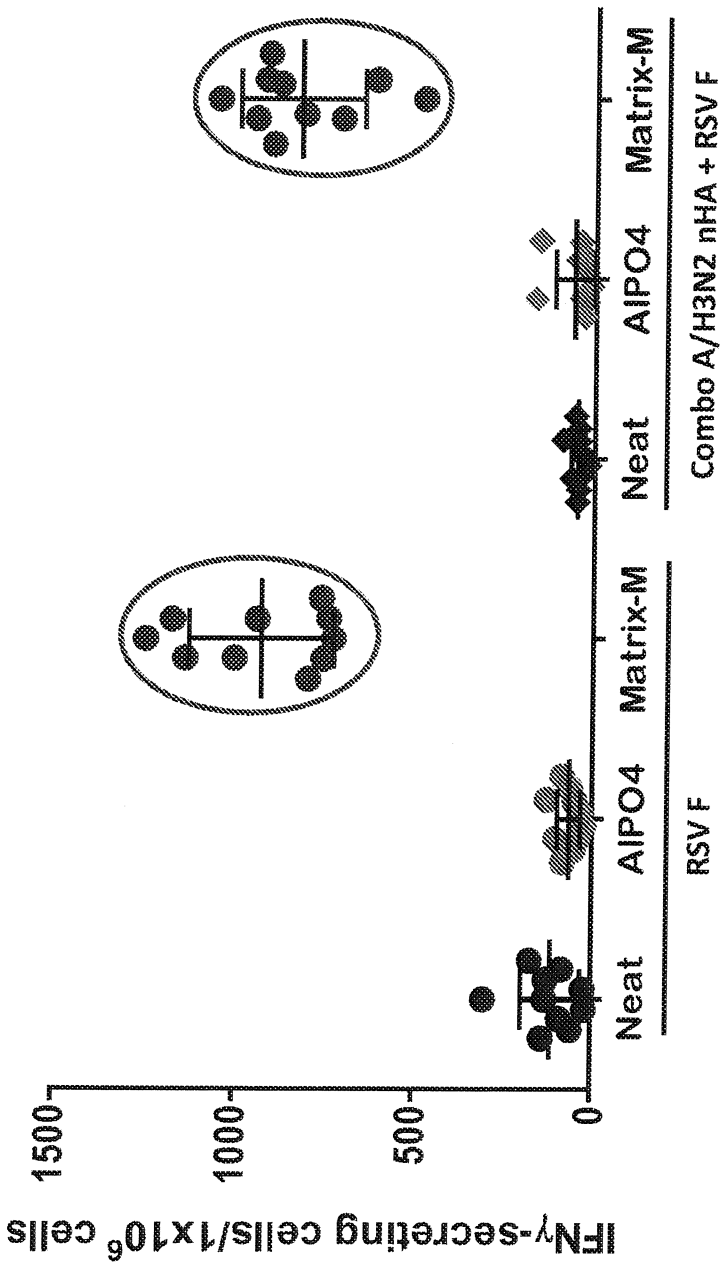


Fig. 23E: RSV F IFNγ T cell ELISPOT responses



Spleen cells (day 35) stimulated with RSV F (aa 1-291) peptide pool

Fig. 23F: A/Switzerland/9715293/2013 (H3N2) IFNγ T cell ELISPOT responses

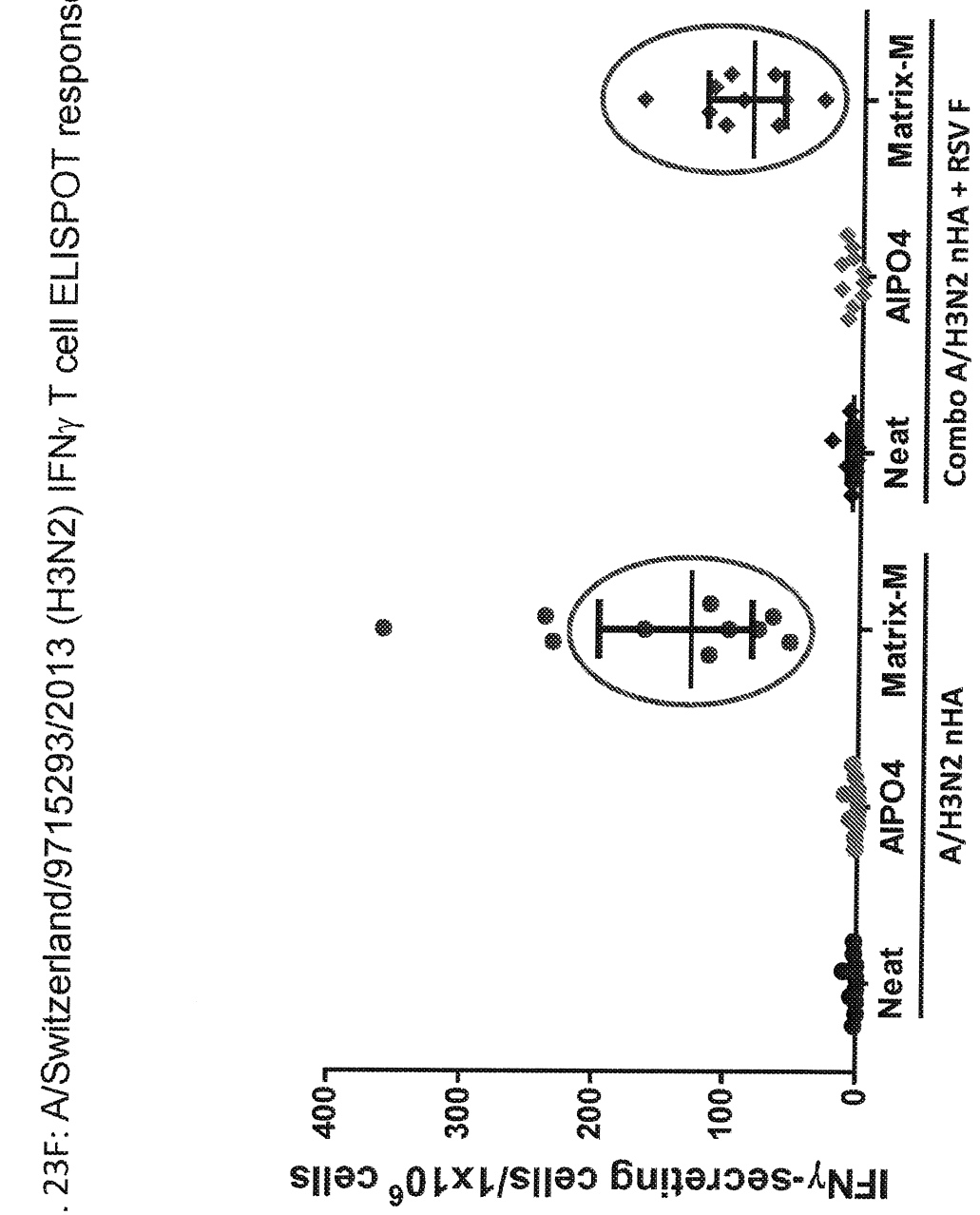


Fig. 24A Enhanced stability HA Nanoparticle purification flow chart neutral pH process

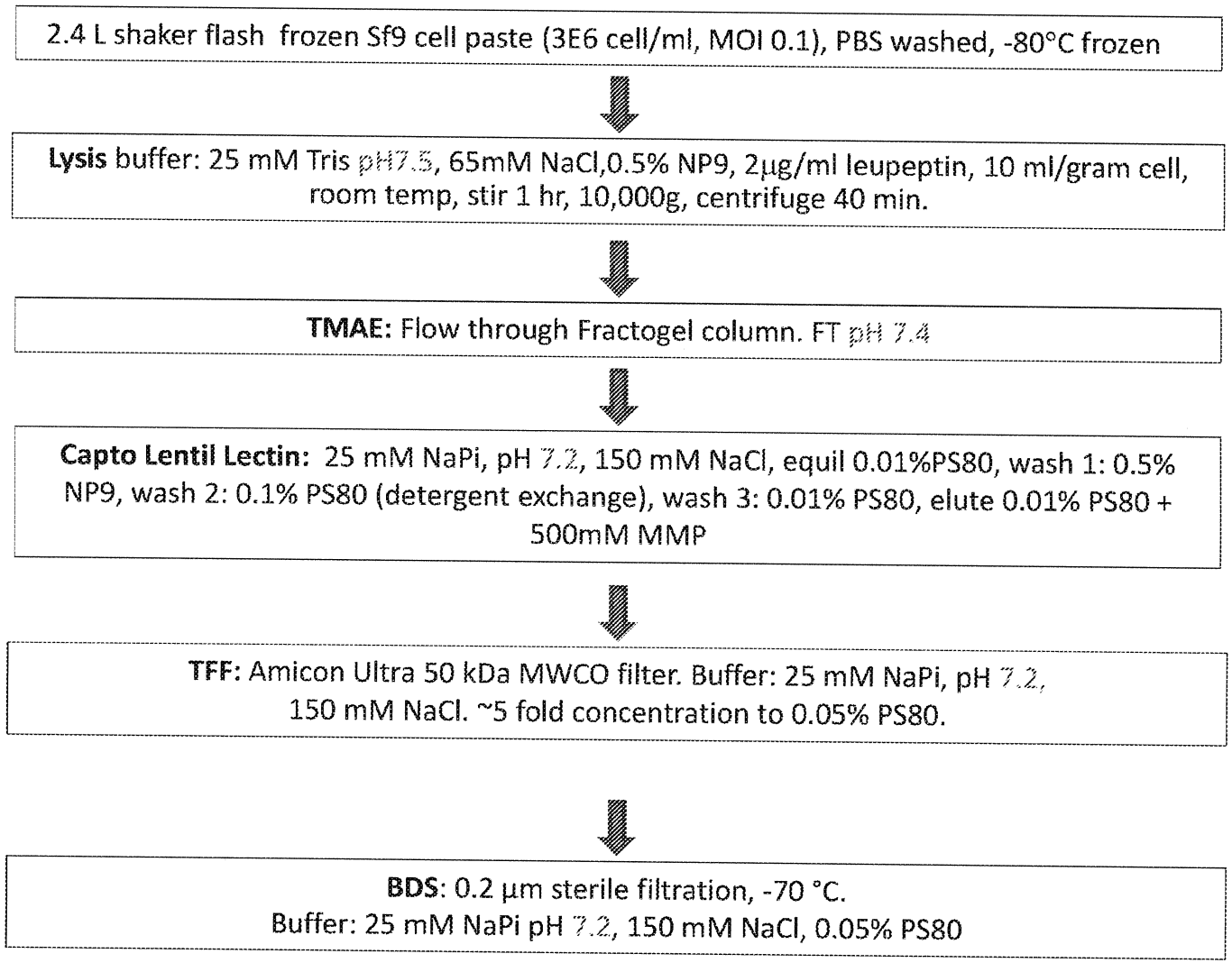
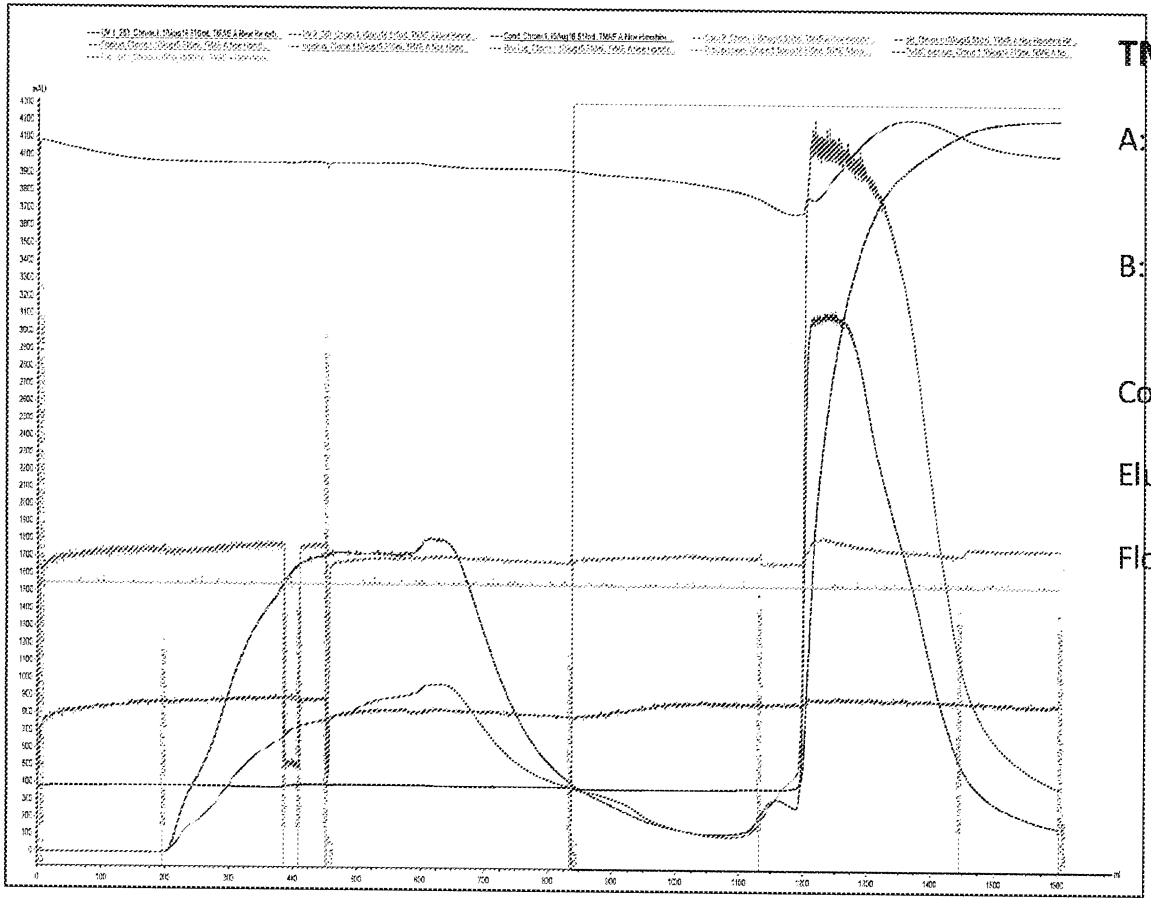


Fig 24B: TMAE Chromatogram of A/New Hampshire/1/2015 (H1N1) HA purification



TMAE XK5x26.5cm, 510 ml

A: 25mM Tris/70mM NaCl/0.02% NP9, pH7.5

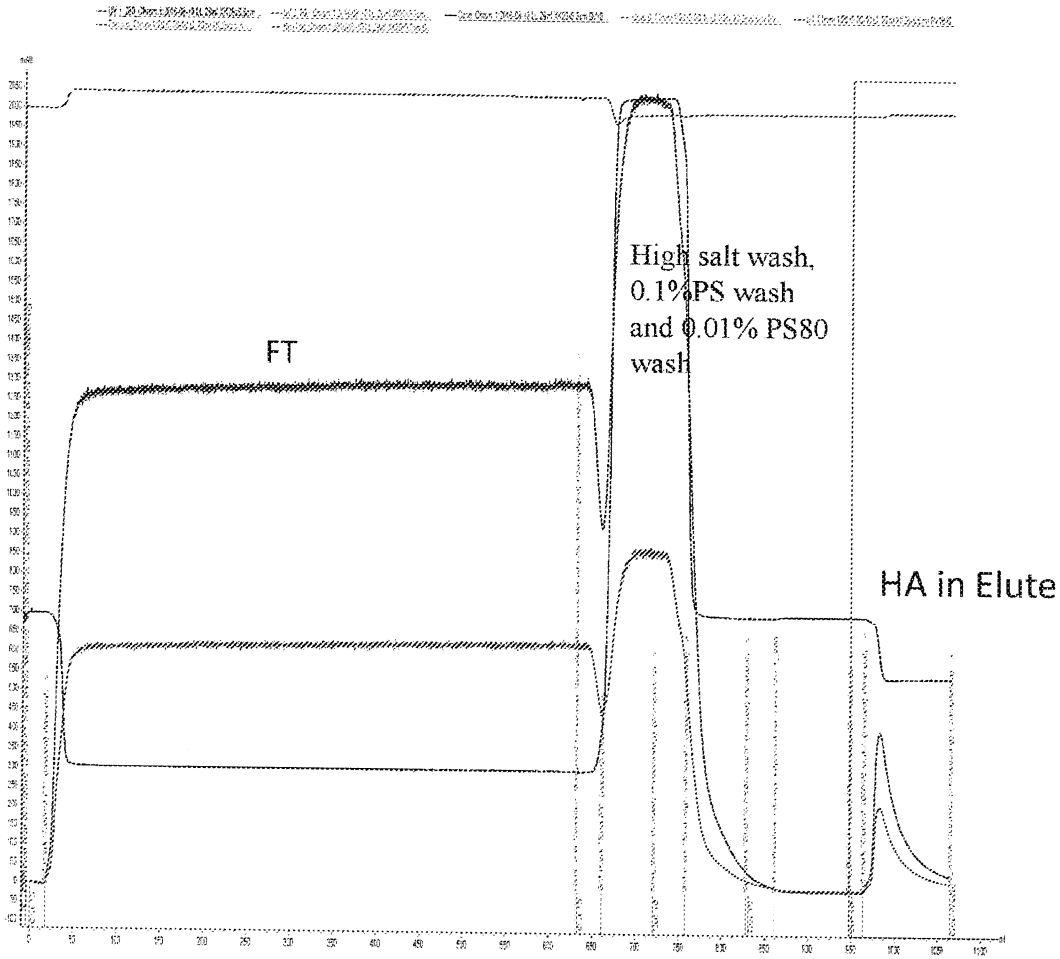
B: 25 mM Tris/1.0M NaCl/0.02% NP9, pH7.5

Collect Flow through: 636ml

Elution: 100%B for 2CV

Flow rate: 20 ml/min = 60 cm/hr

Fig. 24C. Capto Lentil Lectin Chromatogram – BV1849 A/New Hampshire/1/2015 (H1N1) HA



Capto LL XK26x11cm/61 mL

A1: 25mM NaPi, pH7.2, 150mM NaCl, 0.01% PS80

A2: 25mM NaPi, pH7.2, 500mM NaCl, 0.5% NP9

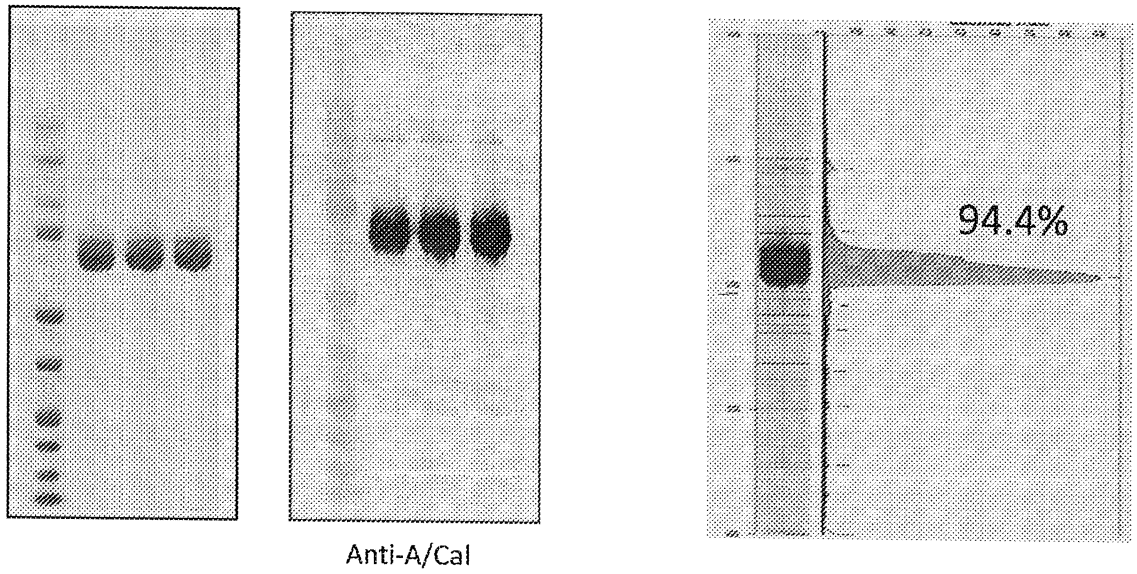
A3: 25mM NaPi, pH7.2, 150mM NaCl, 0.1% PS80

B1: 25mM NaPi, pH7.2, 150mM NaCl, 0.01%PS80, 0.5M MMP

Wash: 3CV A2, 5CV A3. 5CV A1
Elute: 4CV 100%B1, collect 100 ml

Flow rate: 10ml/min = 113cm/hr

Fig 25A. Trypsin-resistant nanoparticles of strain A/New Hampshire/1/2015 (H1N1) HA

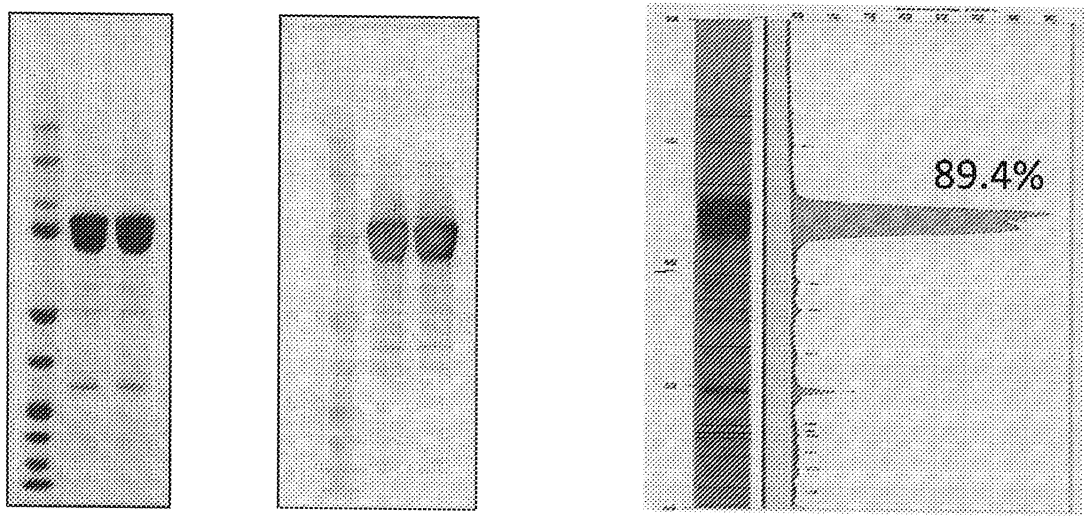


Concentration by A280:
(A280-A340)x5/1.458=(0.679–0.01)x5/1.458 = **2.27mg/ml**
Concentration by SRID with NIBSC reagents = 2.54mg/ml (1.12)

HA Amount =2.27mg/ml×21ml=47.67mg
HA Yield by A280: 47.7mg/2.4L = **19.9mg/L**

Bulk Drug Substance (BDS): 25mM NaPi, 150mM NaCl, ~0.05% PS80, pH7.2

Fig. 25B Trypsin-resistant nanoparticles of strain BV1834 B/Brisbane/60/08 HA

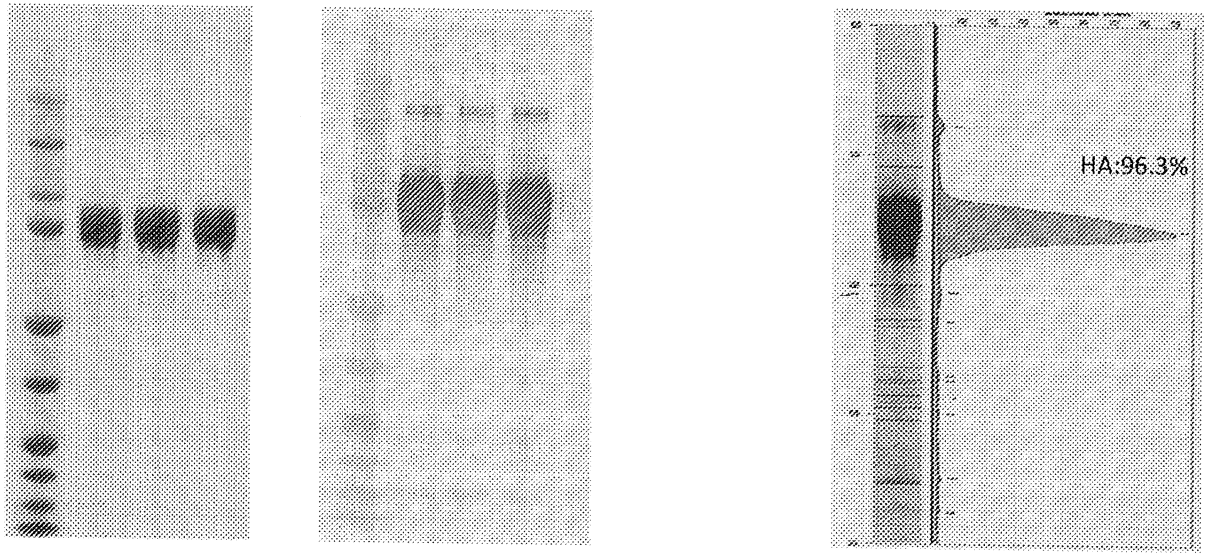


Concentration by A280:
(A280-A340)x5/0.8583=(0.611−0.024)x5/0.8583 = **3.42mg/ml**
Concentration by SRID with NIBSC reagents = 3.26 mg/ml (0.95)

Total HA Amount by A280=3.42mg/ml×21ml=71.8mg
HA Yield by A280: 71.8mg/2.4L= **29.9mg/L**

BDS: 25mM NaPi, 150mM NaCl, ~0.05% PS80, pH7.2

Fig. 25C Trypsin-resistant nanoparticles of strain A/Hong Kong/4801/2014 (H3N2)



Anti-A/Switz

Concentration by A280:
(A280-A340)x5/1.4025=(0.696–0.011)x5/1.402=2.44mg/ml
Concentration by SRID with NIBSC reagents =

Total HA Amount by A280=2.44mg/ml×20ml=48.8mg
HA Yield by A280: 48.8mg/2.4L=**20.3mg/L**

BDS: 25mM NaPi, 150mM NaCl, ~0.05% PS80, pH7.2

Fig. 25D: Enhanced stability trypsin-resistant HA nanoparticles by nano differential scanning calorimetry (nDSC)

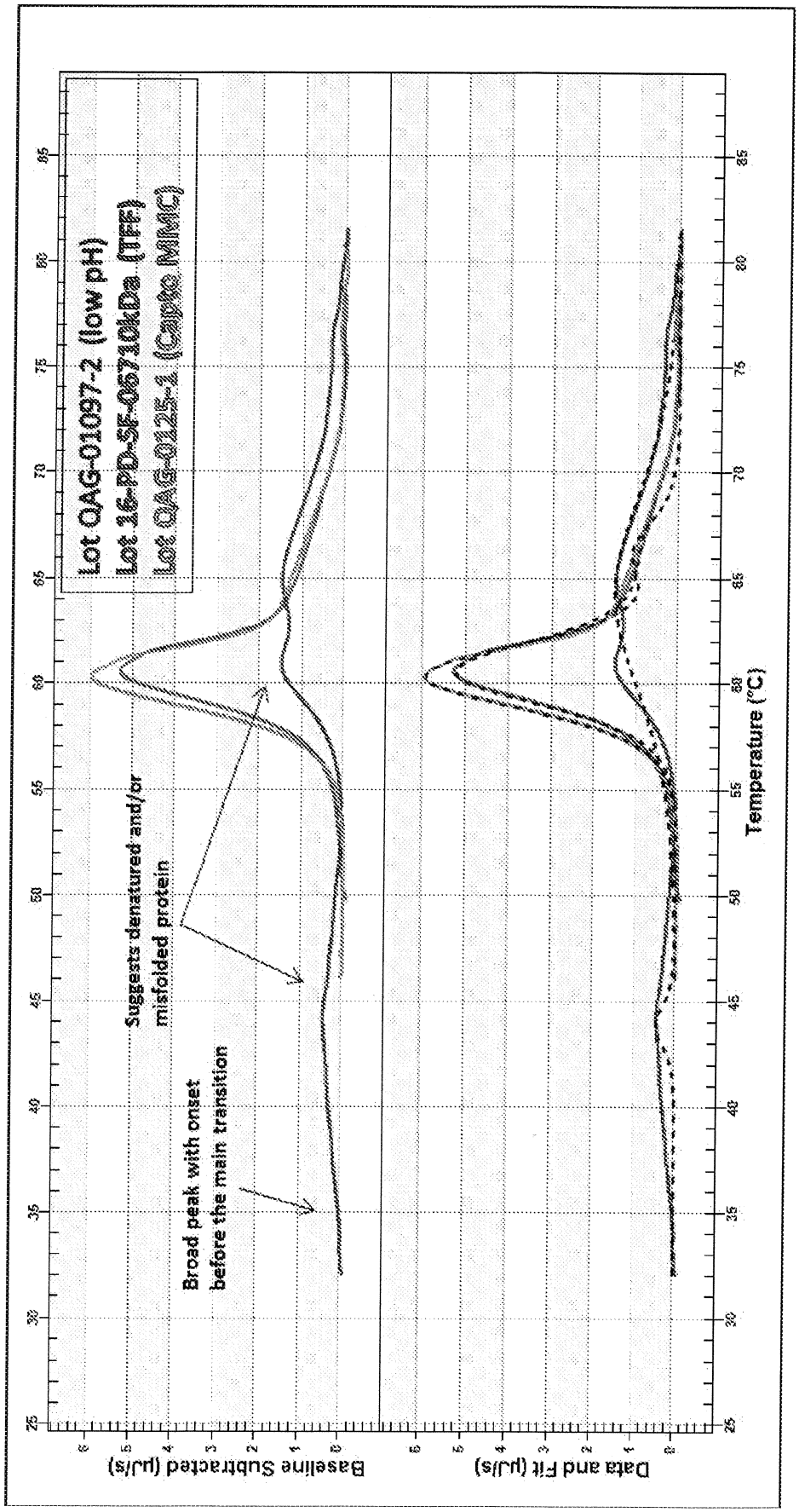
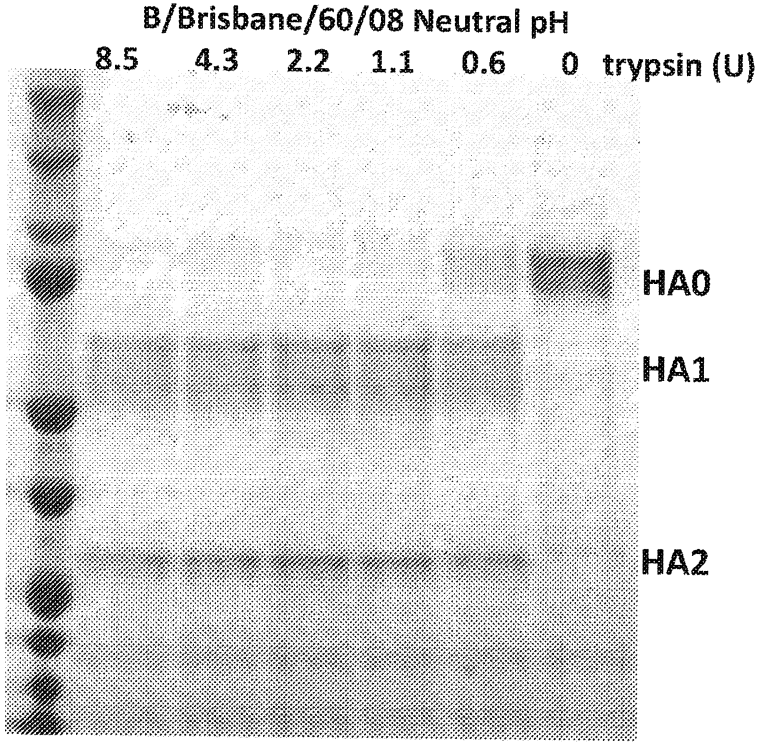


Fig 26A: Comparison of low pH and neutral pH synthesized B strain HA nanoparticles



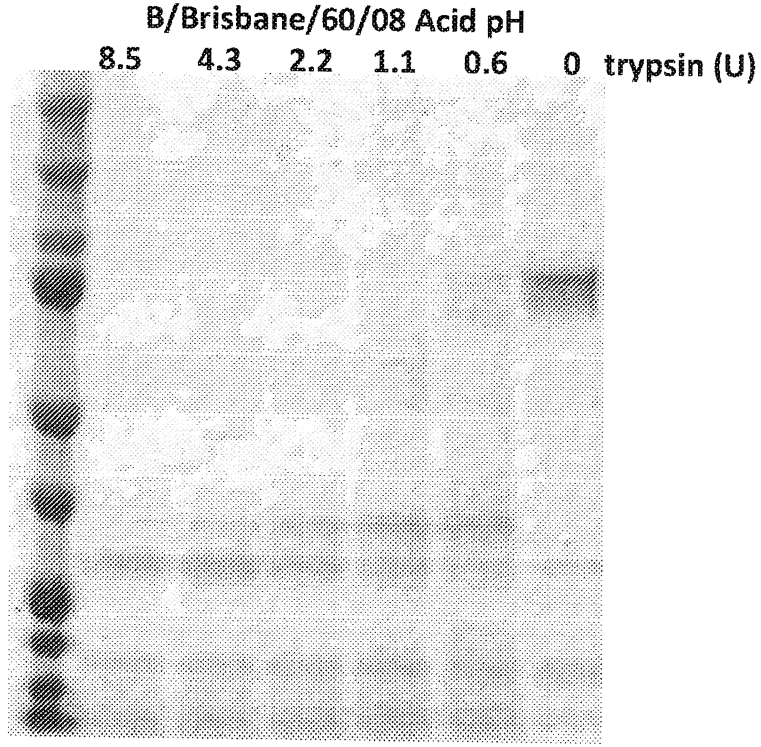


Fig 26B: Comparison of low pH and neutral pH synthesized A/Hong Kong/4801/2014 HA nanoparticles

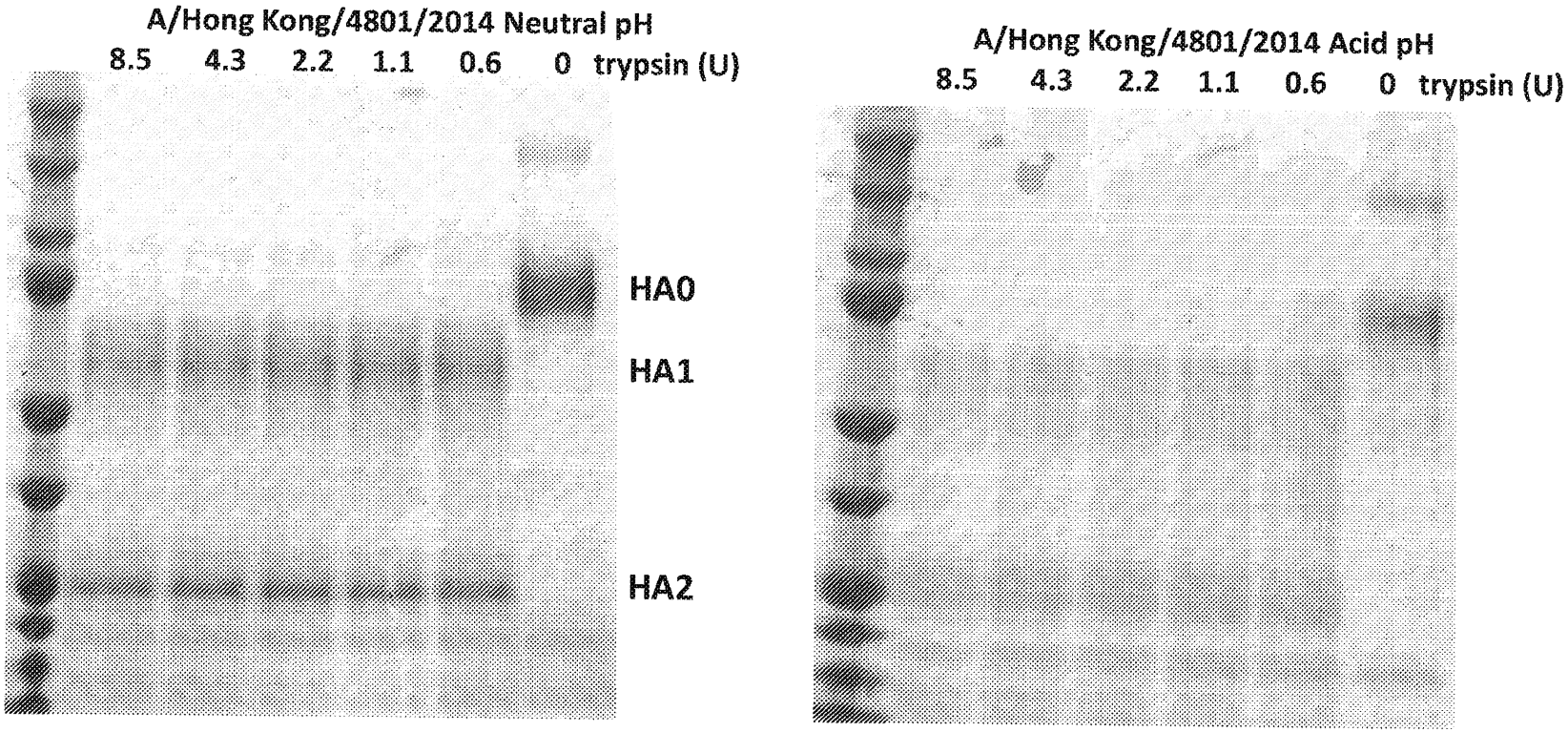


Fig 26C: Trypsin resistance of neutral pH A/New Hampshire/1/2015 (H1N) HA nanoparticles

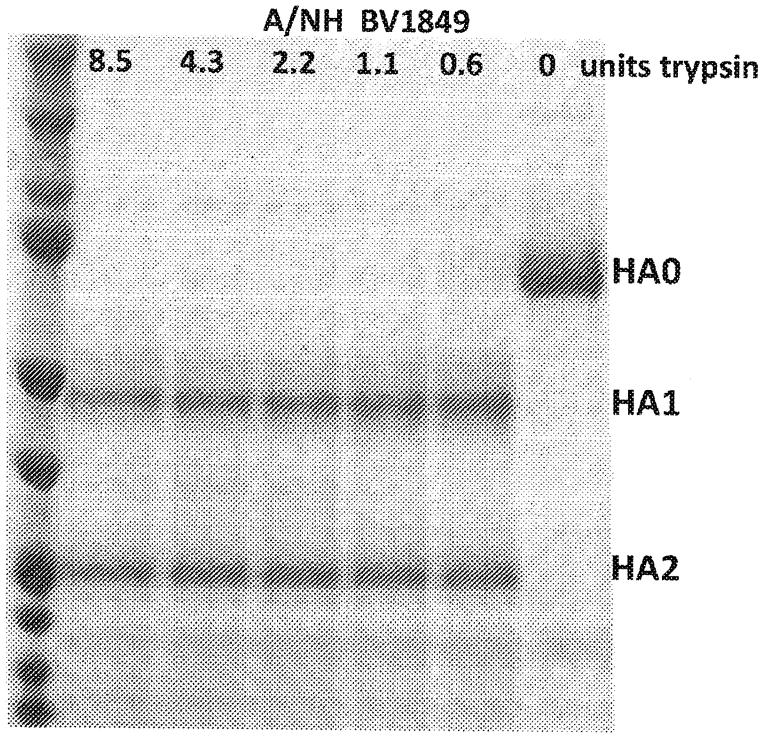


Fig. 27 Trypsin sensitivity of high dose Fluzone and Flublok

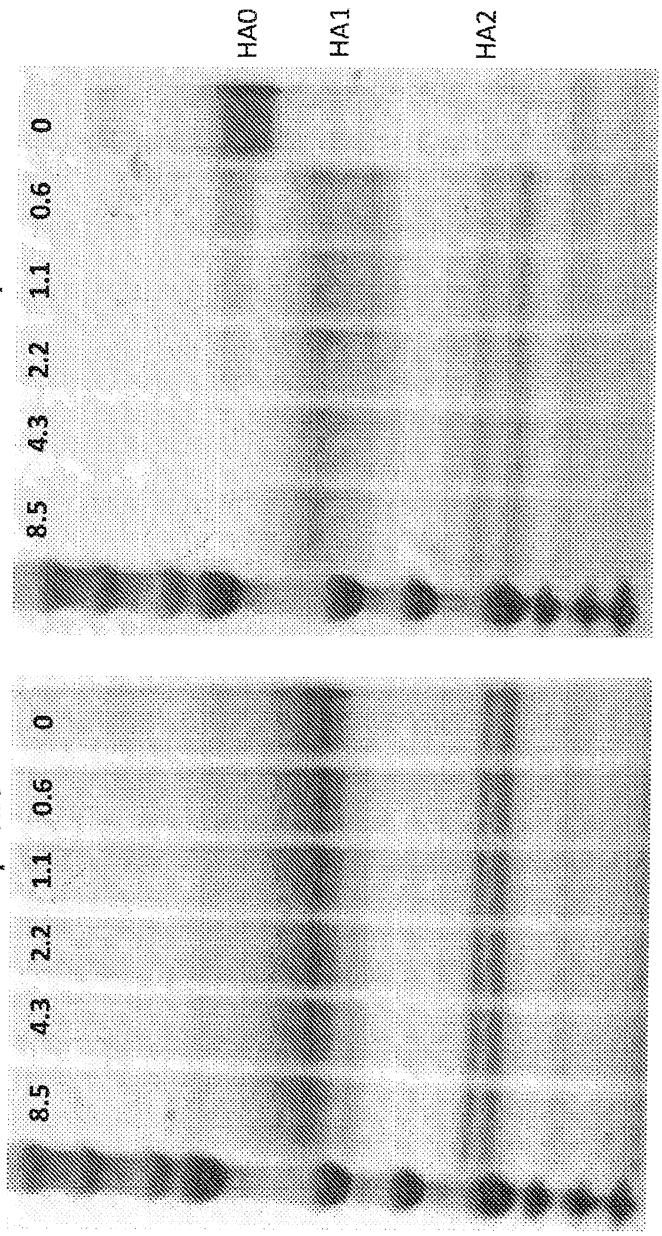


Fig. 28: Ebola nanoparticles induce antibodies and protect against infection

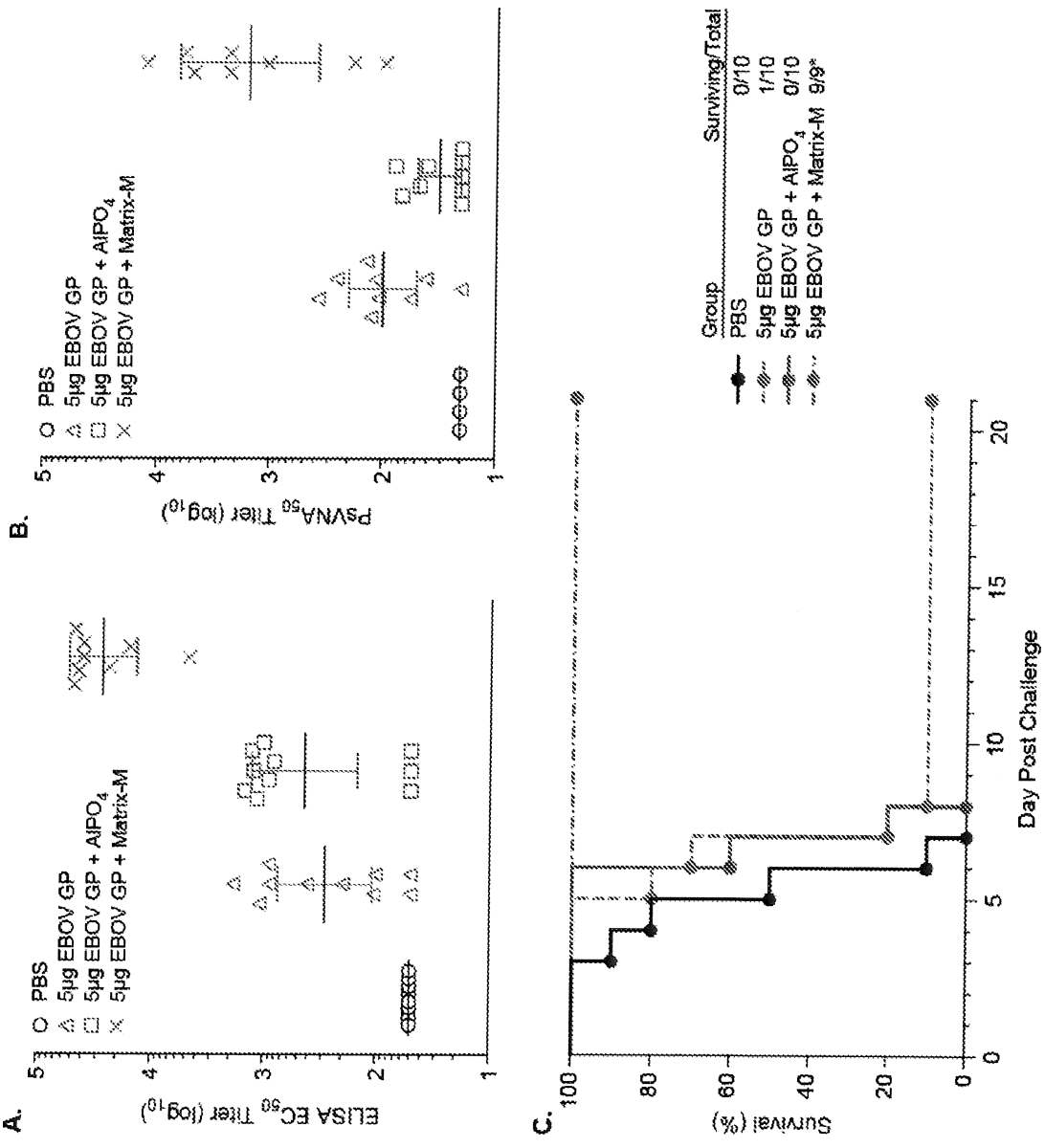


Fig. 29

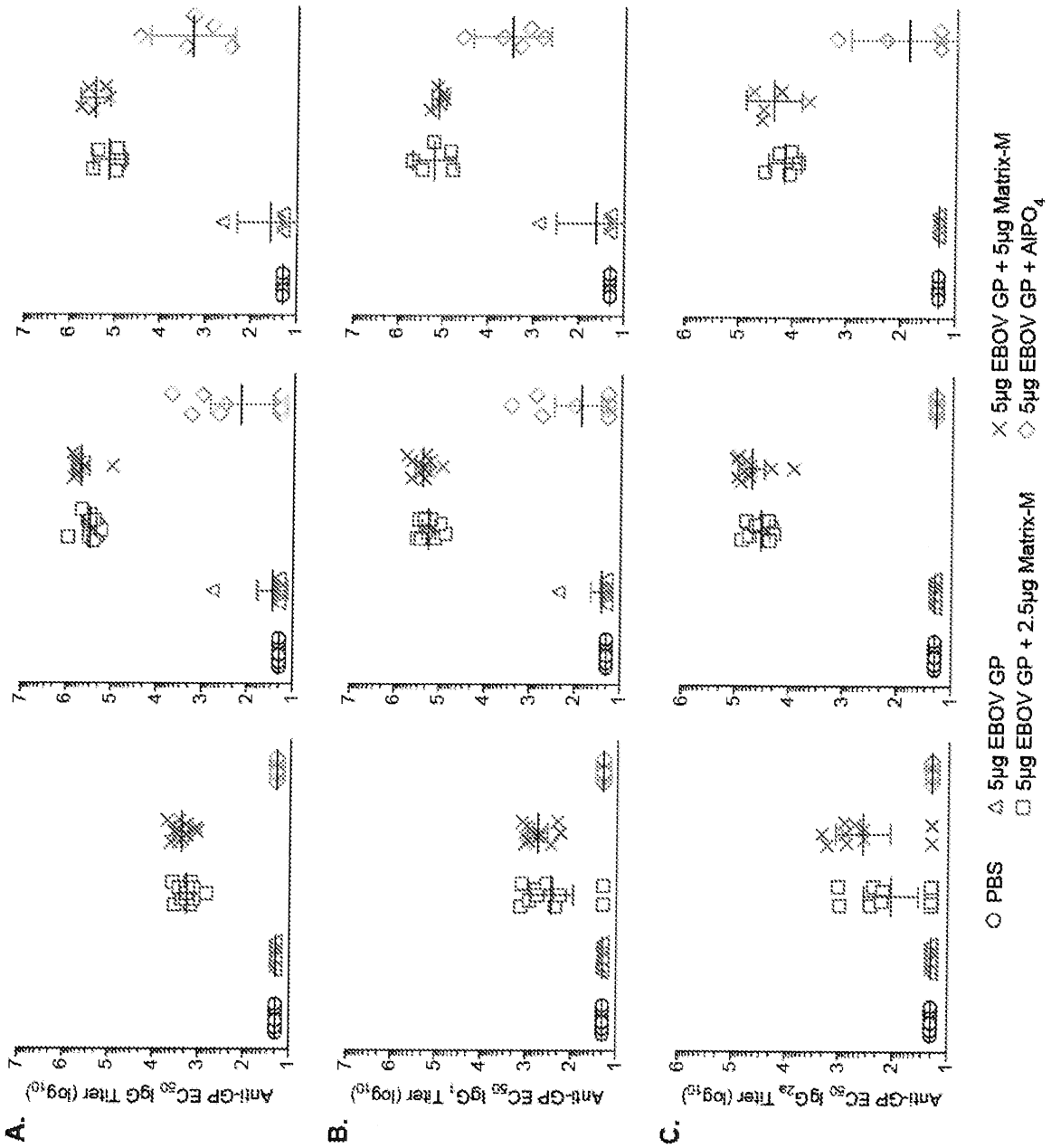

Day 21
Day 28
Day 60
A.
B.
C.
Anti-GP EC50 IgG Titer (log10)
Anti-GP EC50 IgG1 Titer (log10)
Anti-GP EC50 IgG2a Titer (log10)
PBS
5µg EBOV GP
5µg EBOV GP + 2.5µg Matrix-M
5µg EBOV GP + 5µg Matrix-M
5µg EBOV GP + AlPO4

Fig. 30: T cell responses induced by adjuvanted Ebola vaccine

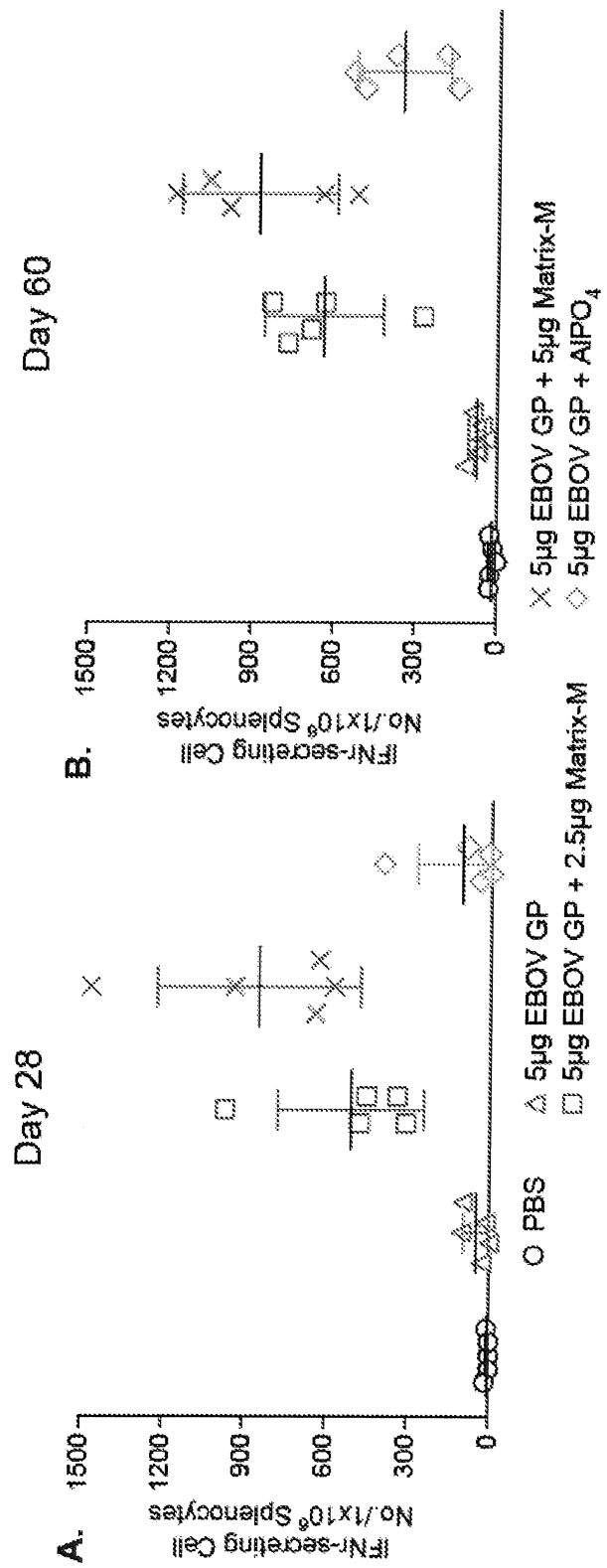


Fig. 30 (cont'd)

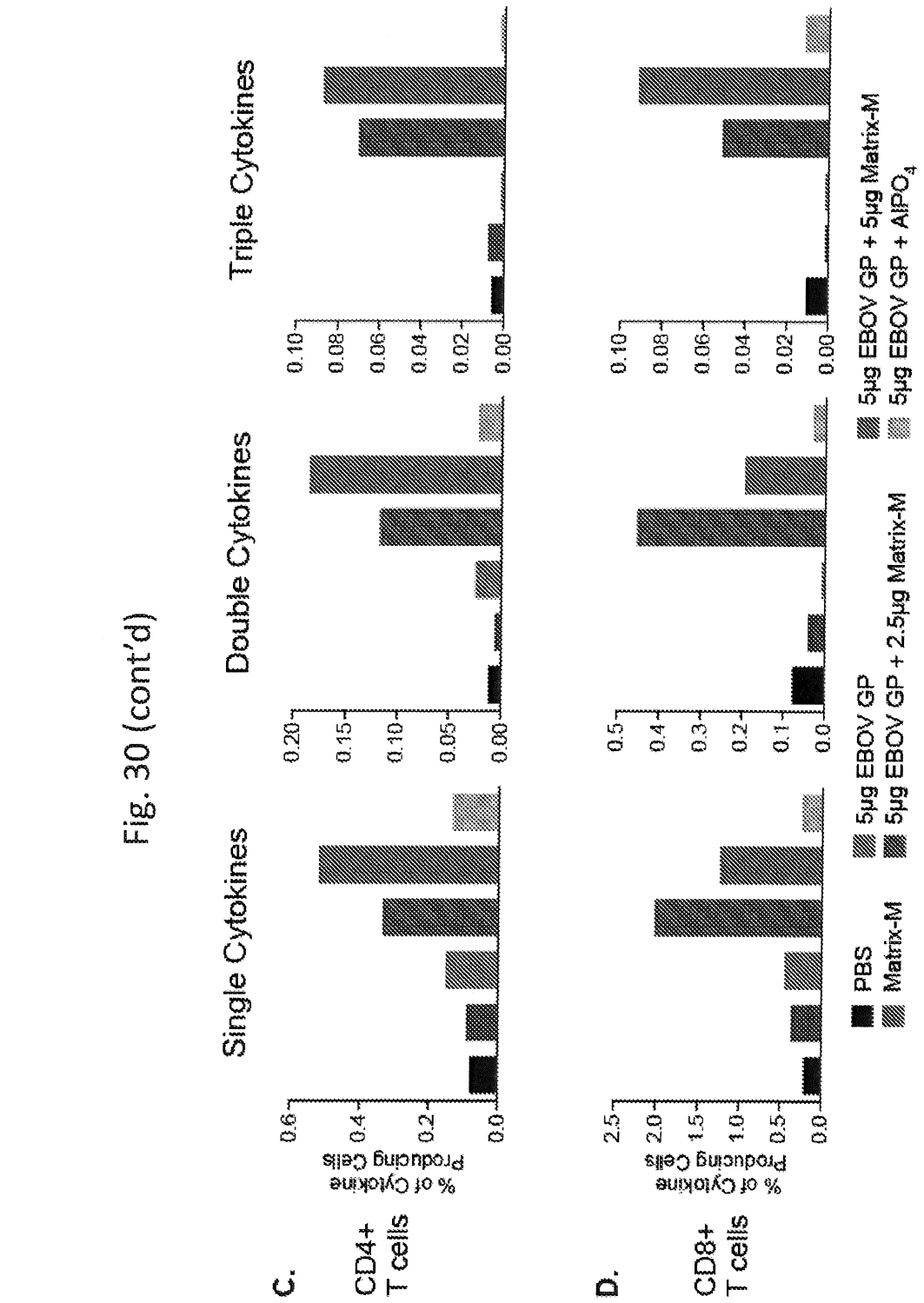

C.
CD4+ T cells
Single Cytokines
Double Cytokines
Triple Cytokines
% of Cytokine Producing Cells
0.6
0.4
0.2
0.0
0.20
0.15
0.10
0.05
0.00
0.10
0.08
0.06
0.04
0.02
0.00
D.
CD8+ T cells
% of Cytokine Producing Cells
2.5
2.0
1.5
1.0
0.5
0.0
0.5
0.4
0.3
0.2
0.1
0.0
0.10
0.08
0.06
0.04
0.02
0.00
PBS
Matrix-M
5µg EBOV GP
5µg EBOV GP + 2.5µg Matrix-M
5µg EBOV GP + 5µg Matrix-M
5µg EBOV GP + AlPO4

Fig. 31

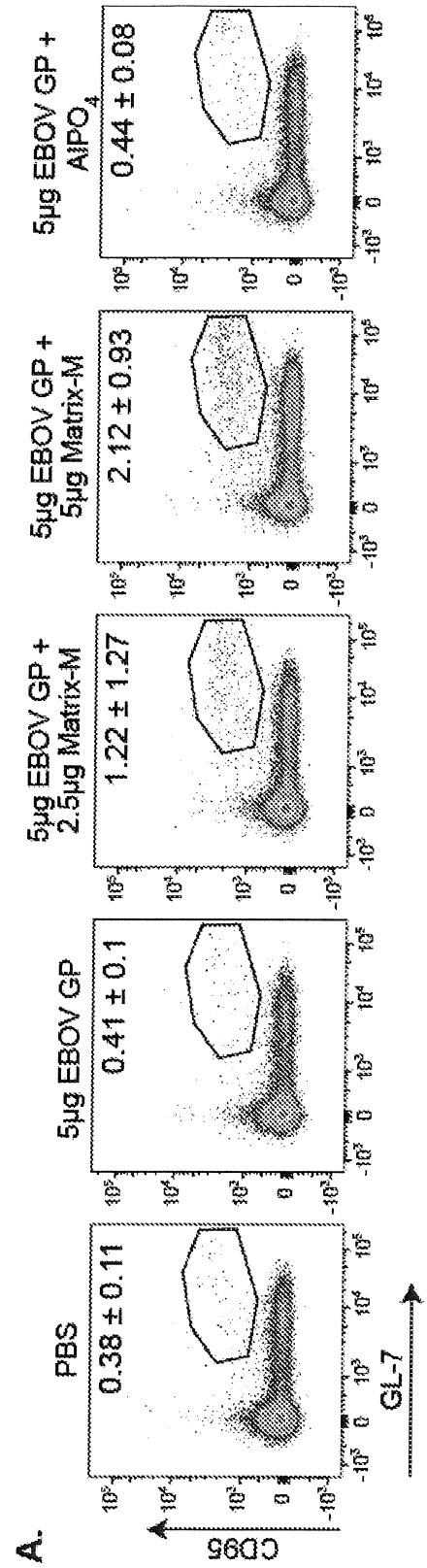

A.
PBS
0.38 ± 0.11
5µg EBOV GP
0.41 ± 0.1
5µg EBOV GP + 2.5µg Matrix-M
1.22 ± 1.27
5µg EBOV GP + 5µg Matrix-M
2.12 ± 0.93
5µg EBOV GP + AlPO4
0.44 ± 0.08
CD95
GL-7

Figure 31 (cont'd)

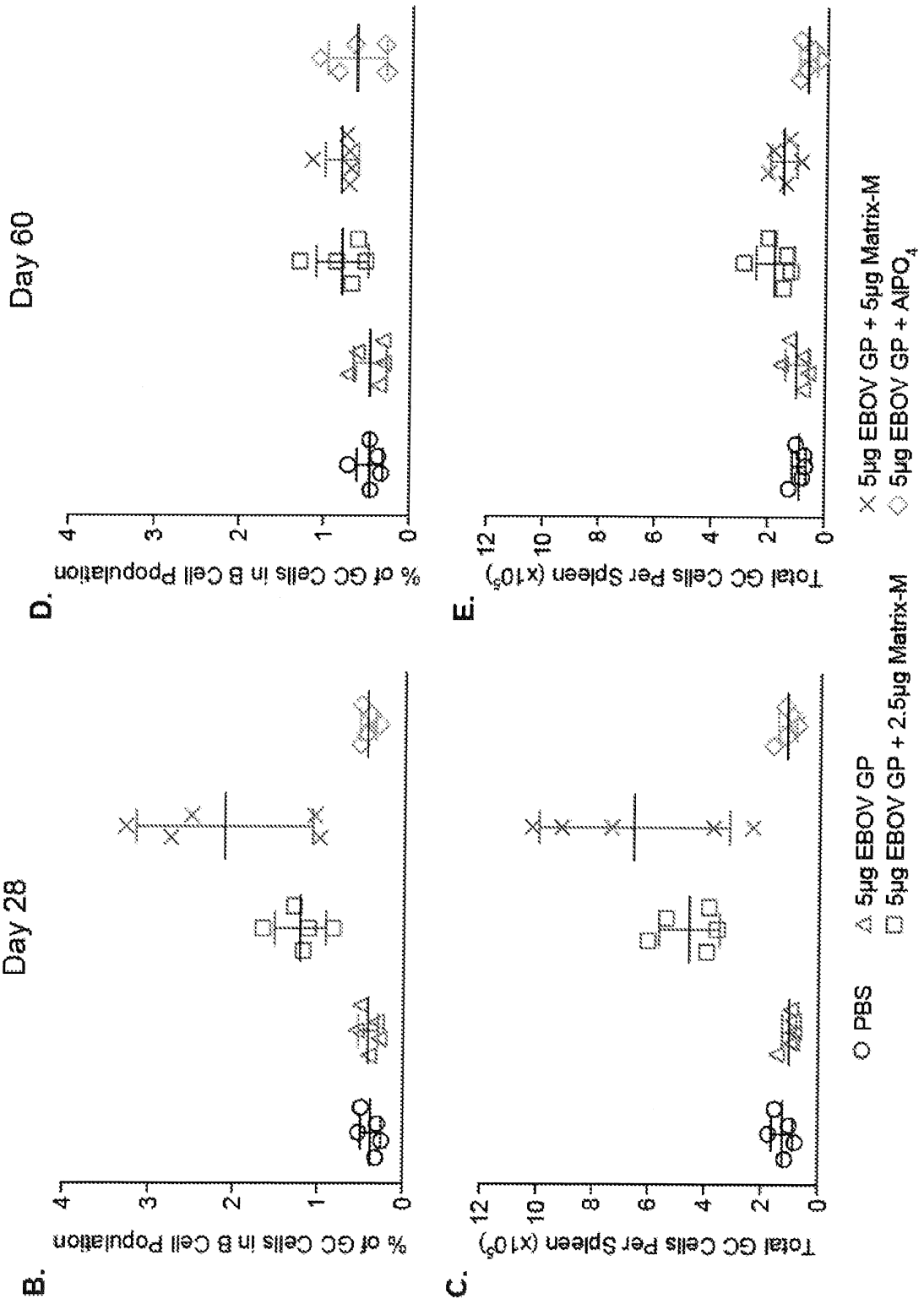


Figure 32

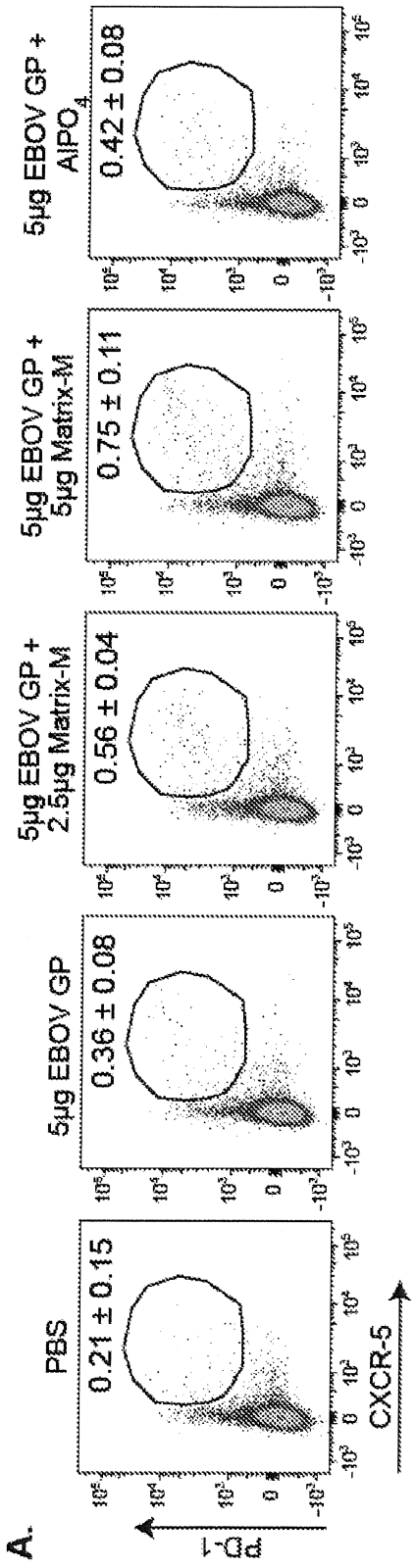

A.
PBS
0.21 ± 0.15
5µg EBOV GP
0.36 ± 0.08
5µg EBOV GP + 2.5µg Matrix-M
0.56 ± 0.04
5µg EBOV GP + 5µg Matrix-M
0.75 ± 0.11
5µg EBOV GP + AlPO4
0.42 ± 0.08
PD-1
CXCR-5

Figure 32 (cont'd)

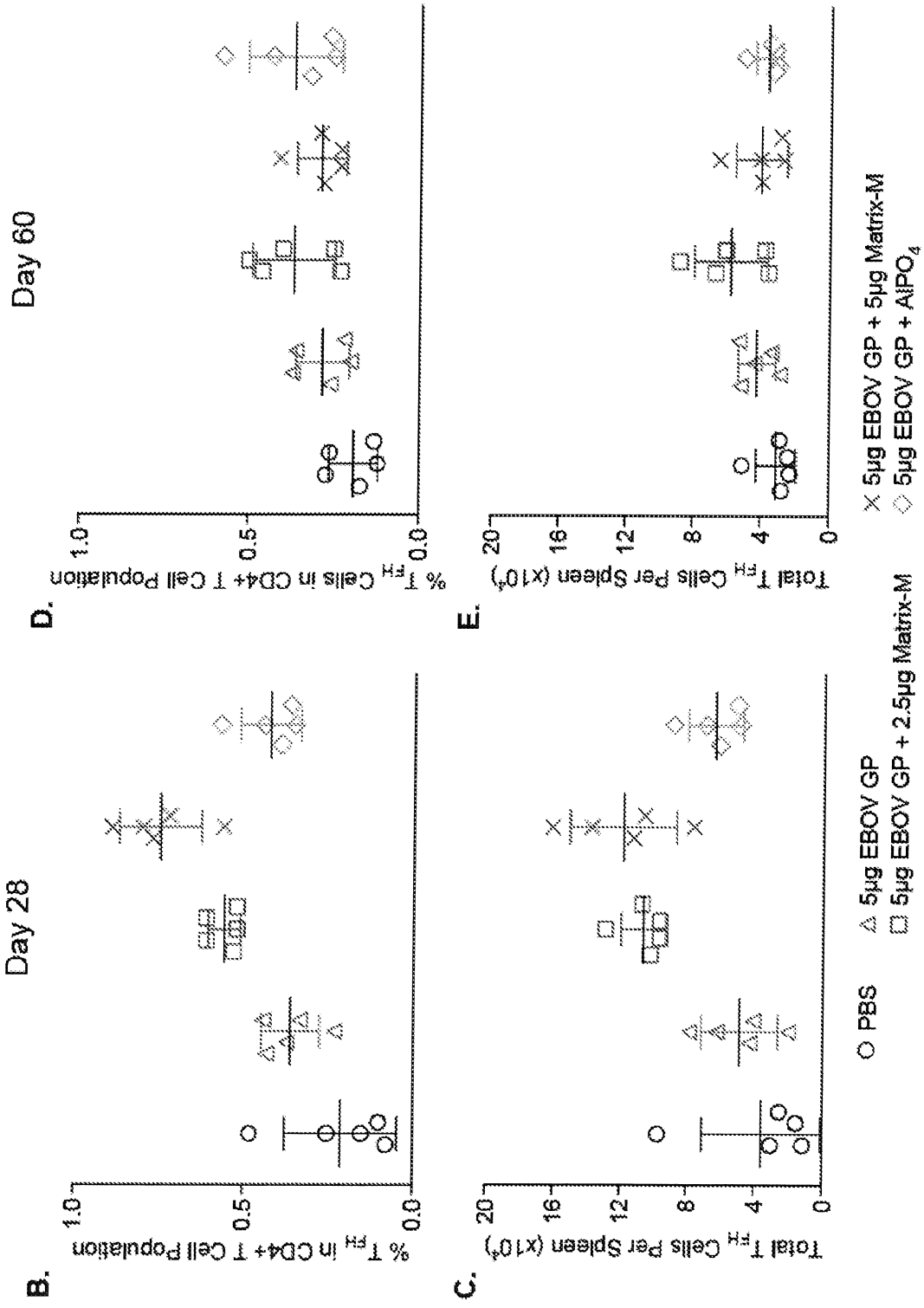


Figure 33

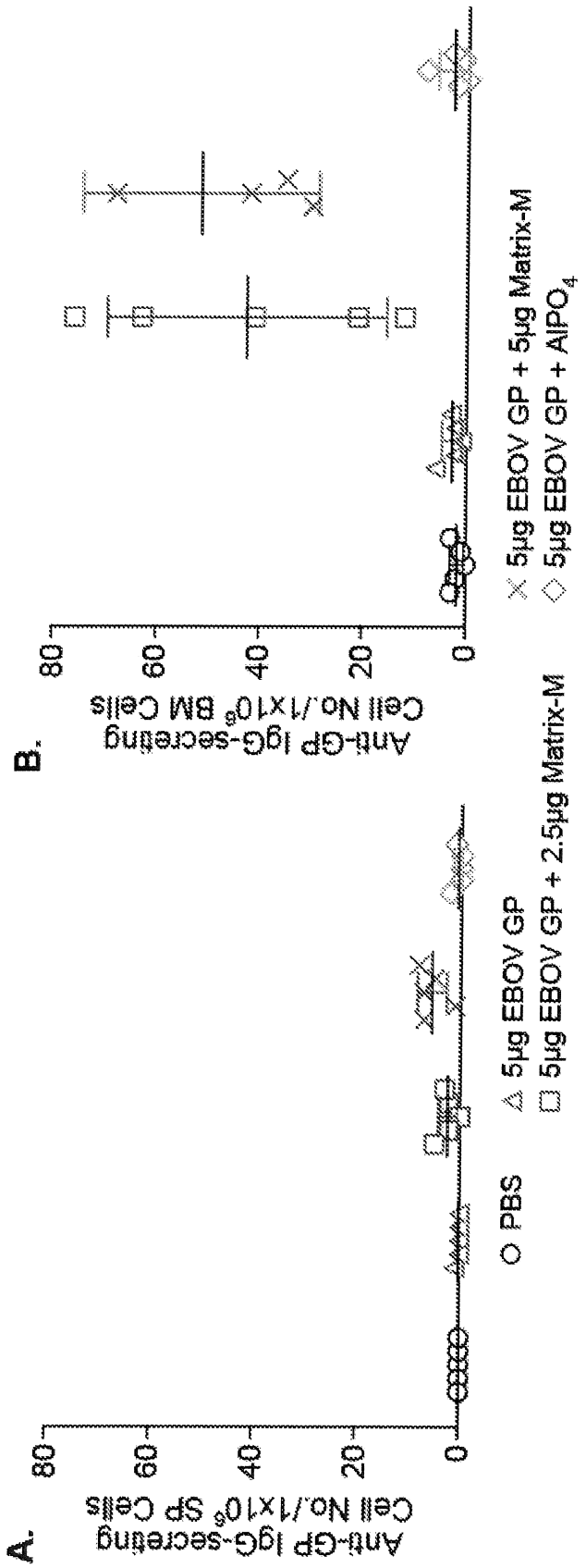

A.
Anti-GP IgG-secreting Cell No./1x10^6 SP Cells
80
60
40
20
0
B.
Anti-GP IgG-secreting Cell No./1x10^6 BM Cells
80
60
40
20
0
O PBS
△ 5µg EBOV GP
□ 5µg EBOV GP + 2.5µg Matrix-M
× 5µg EBOV GP + 5µg Matrix-M
◇ 5µg EBOV GP + AlPO4

Fig. 34A: Ebola Glycoprotein Structure

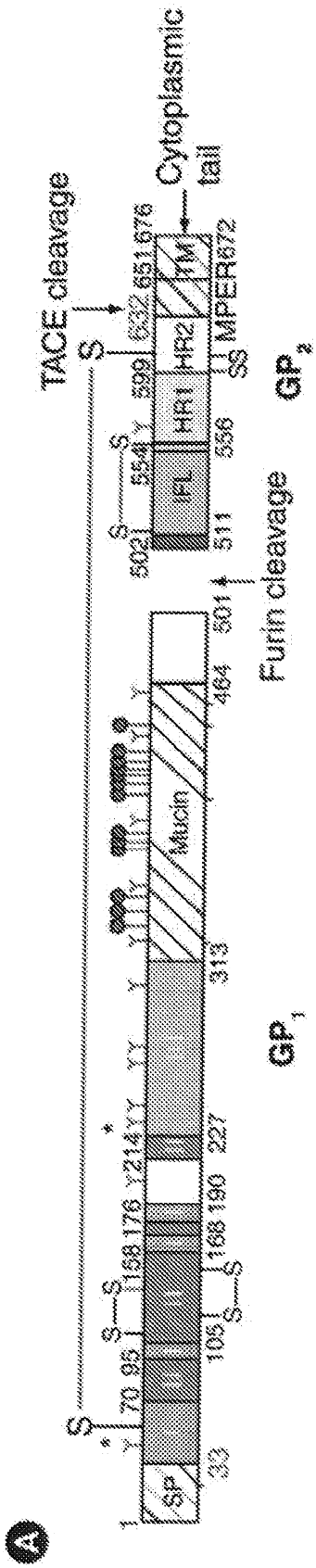


Fig. 34B: Ebola Glycoprotein Sequence

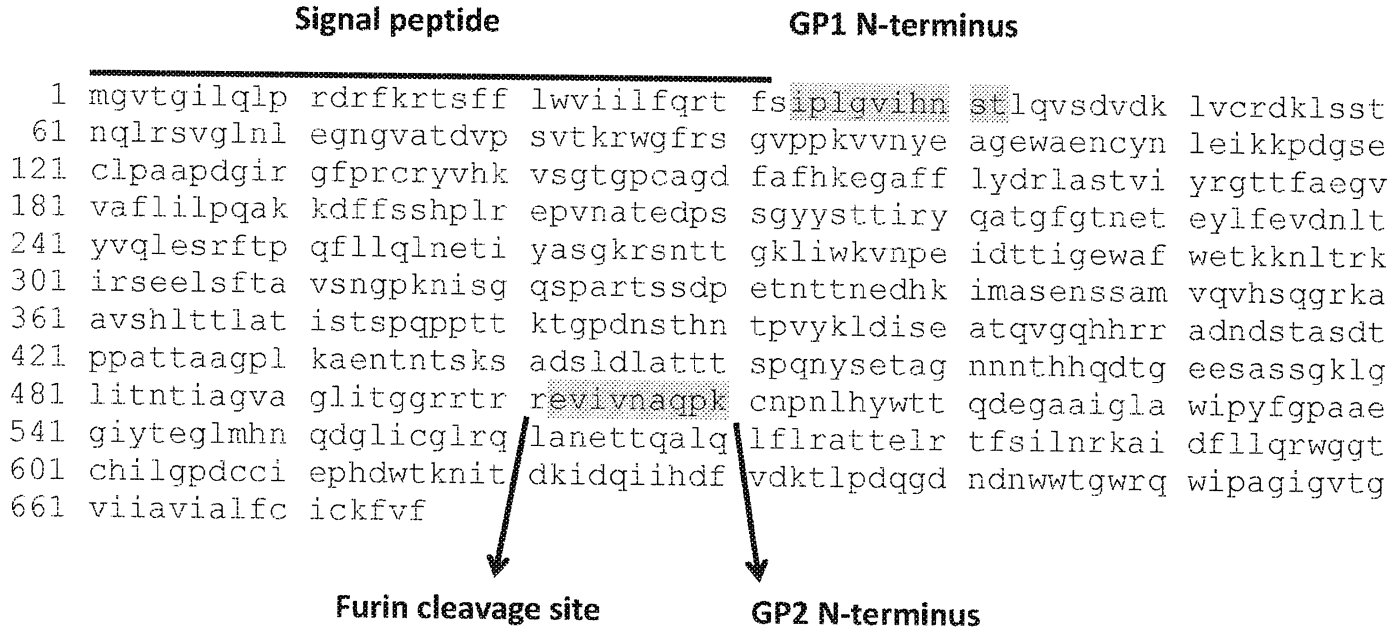
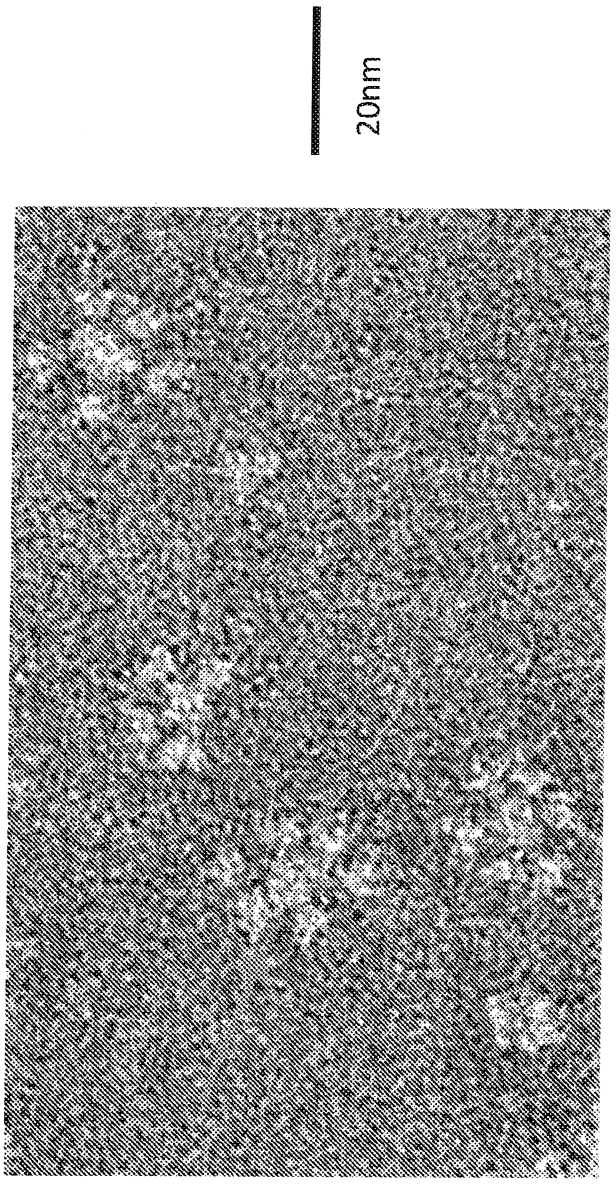


Figure 35A: Electron Microscopy Analysis of Nanoparticles



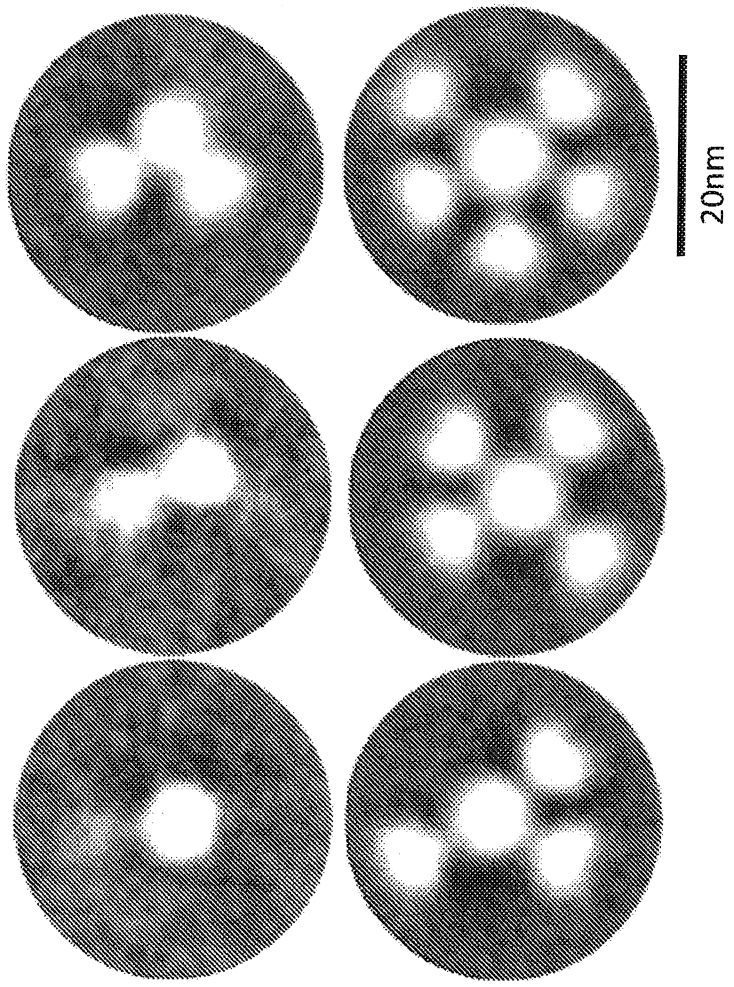


Fig. 35B: Electron Image Showing Exemplary nanoparticles

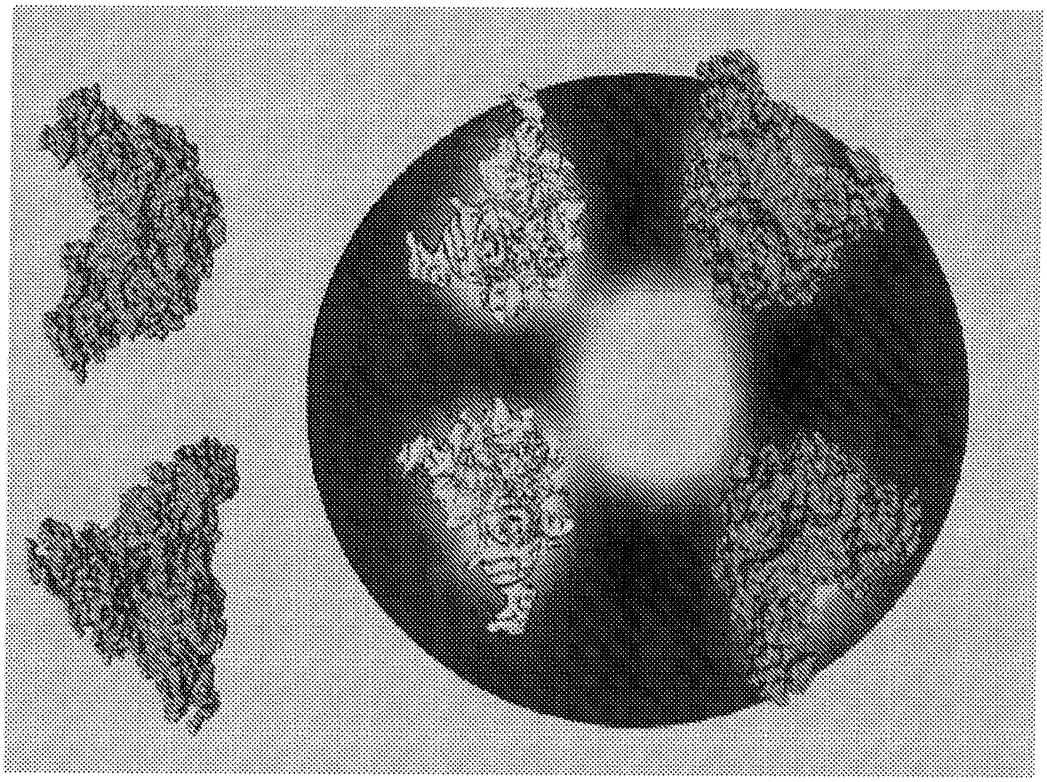
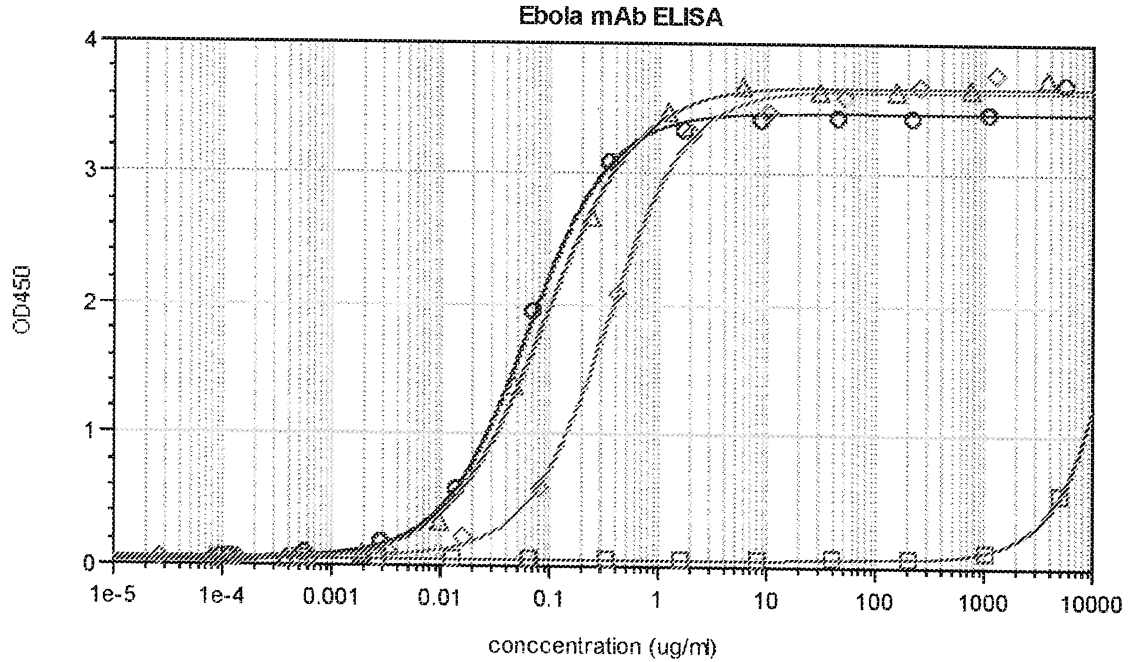


Fig. 35C: Nanoparticle with Docked Trimers

Figure 36: Anti-Ebola ELISA EC50 of mAb 13C6, 6D8 and KZ52



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
c13c6 (Group01: Dilution vs Values)	0.0539	1.14	0.0623	3.46	0.998
H13F6 (Group02: Dilution vs Values)	0.0485	1.26	1.18e+08	1.53e+05	0.999
c6D8 (Group03: Dilution vs Values)	0.0243	1.02	0.0882	3.67	0.999
KZ52 (Group04: Dilution vs Values)	0.0645	1.21	0.34	3.64	0.999

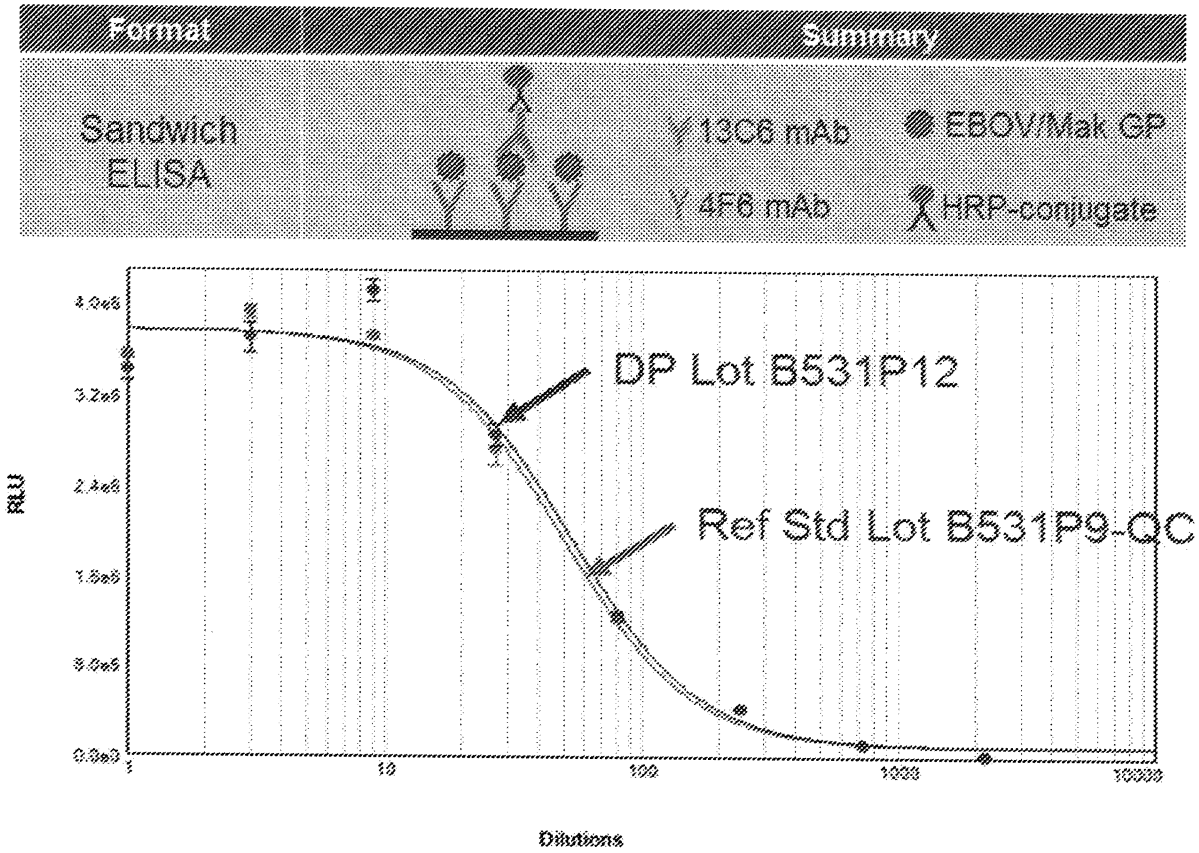
Weighting: Fixed

mAb	EC_{50} (µg/mL)
13C6	0.0623
13F6 *	-
6D8	0.0882
KZ52	0.34

Figure 37: Binding kinetics purified recombinant EBOV/Mak GP to functional EBOV mAb

mAb	EBOV GP Epitope		SPR K_D (nM)
KZ52	aa 42-43, 513, 550-553, 556 GP1/GP2	Conformational Pre-fusion GP2	2.36
13C6	aa 1-295 GP1	Conformational In ZMapp	8.35
6D8	aa 389-405 GP1 HNTPVYKLDISEATQVE	Linear	3.67
13F6	aa 401-417 GP1 ATQVEQHHRRTDNDSTA ATQVGQHHRRADNDSTA[1]	Linear Neutralizing	No binding

Figure 38: EBOV GP potency assay: Sandwich ELISA based on binding 13C6 mAb



Sample	13C6 ELISA (µg/mL)	A280 O.D. (µg/mL)
EBPV/Mak DP Lot B531P12	135.0	133.9

Figure 39: Baboon immunogenicity: Nanoparticle vaccine study design

Group	N	Ebola GP Dose	Adjuvant	Day of Immunization (IM)	Day for Blood Draw
1	3	60µg	No Adjuvant	0, 21	0, 21, 31, 42, 60, 90, 120, 150, 180
2	3	60µg	800µg $AlPO_4$		
3	3	60µg	50µg Matrix-M		
4	3	5µg	50µg Matrix-M		

Figure 40A: Anti-EBOV/Makona GP ELISA EC90 responses in baboons
(Response at Day 21)

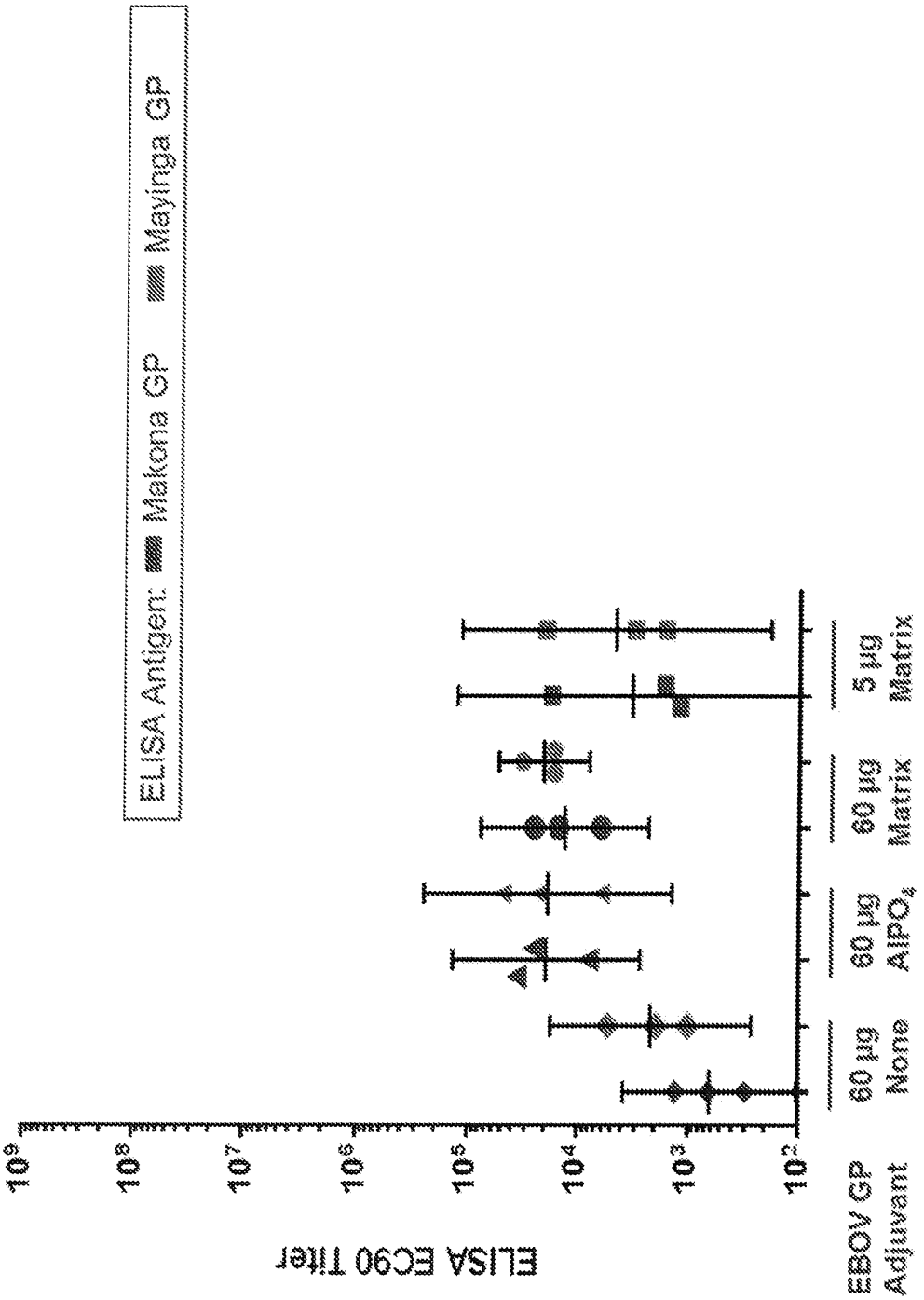


Figure 40B: Anti-EBOV/Makona GP ELISA EC90 responses in baboons
(Response at Day 31)

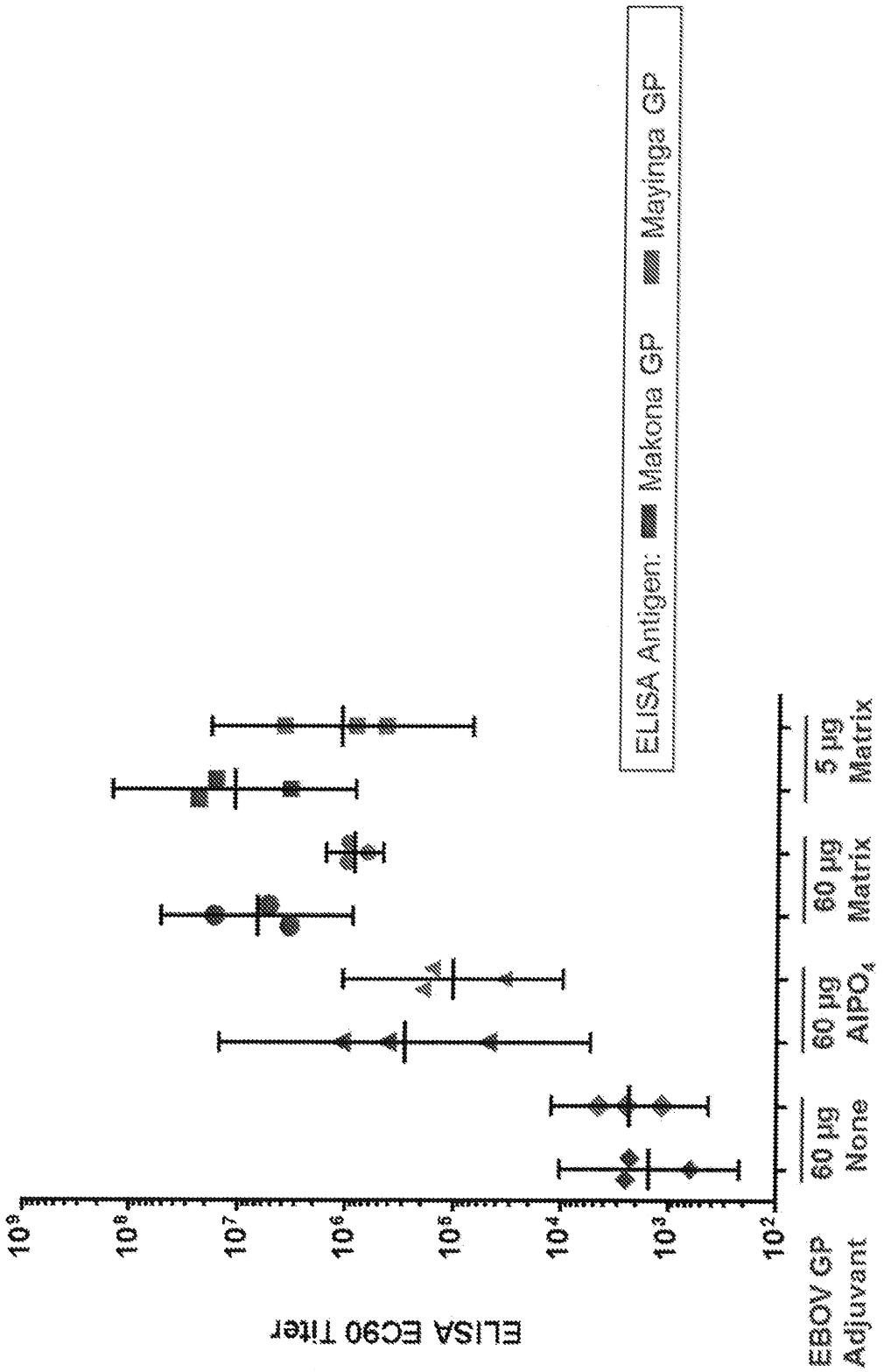


Fig. 41: Durability of EBOV GP ELISA (EC50) IgG Response

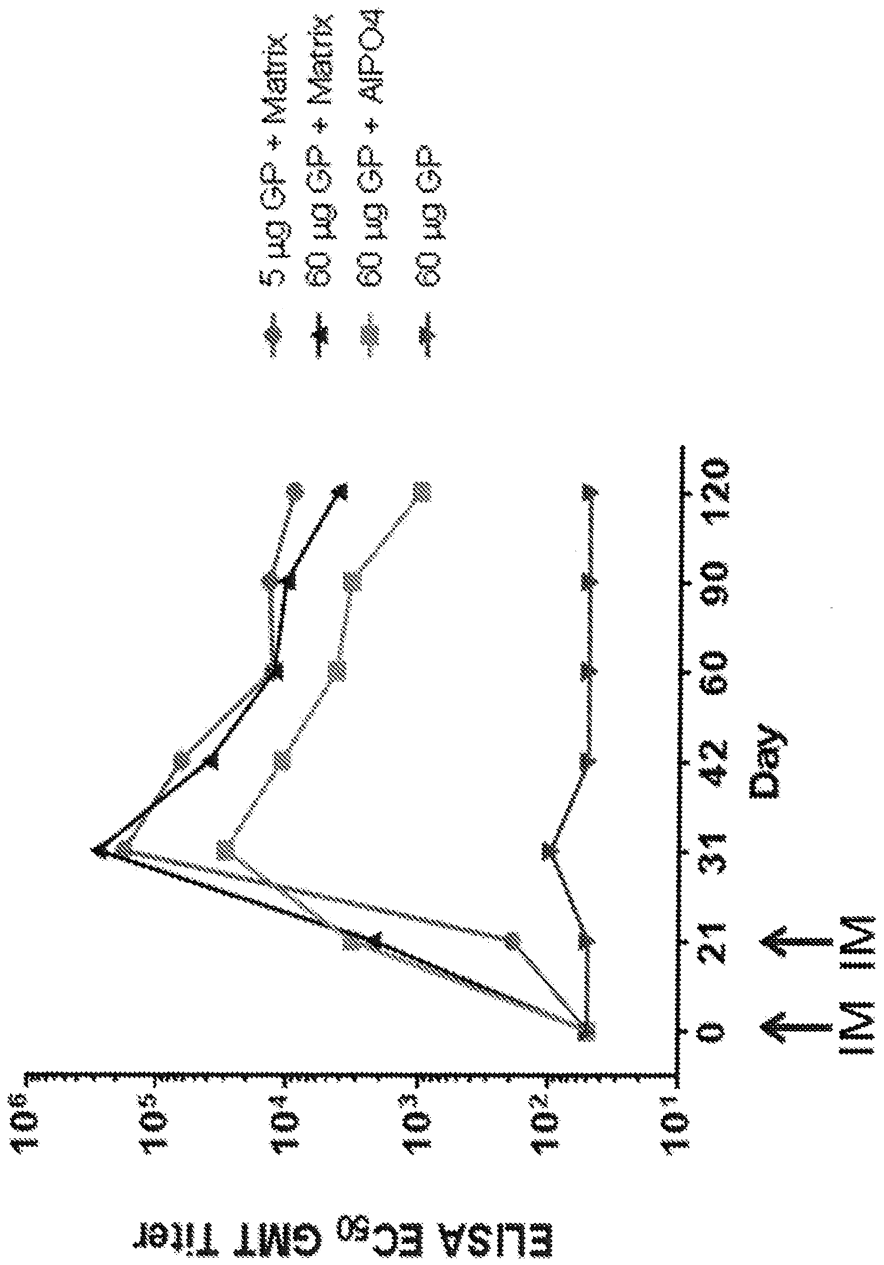


Figure 42: EBOV/Makona GP vaccine IFNγ-Elispot response in baboons day 31

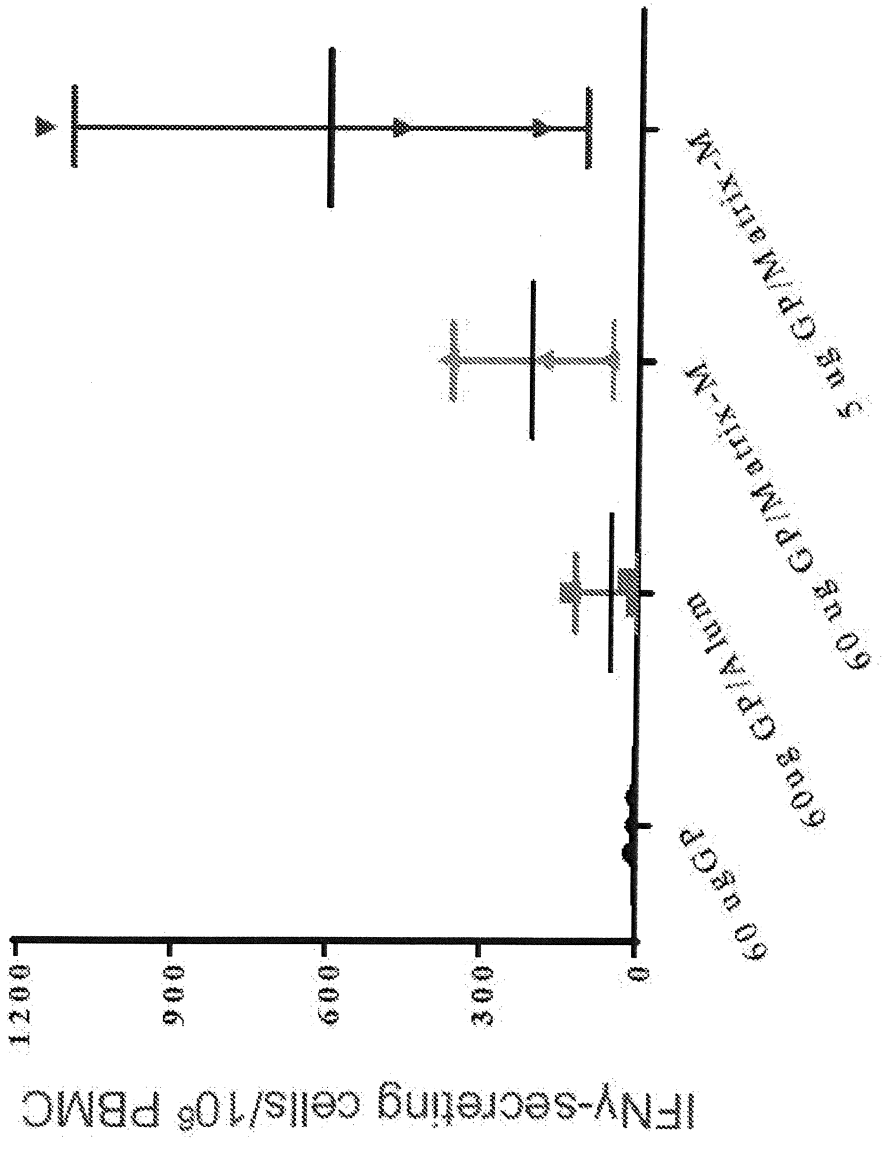


Fig. 43: Ebola-GP/Matrix-M multifunctional IFNγ TNFα T-cells

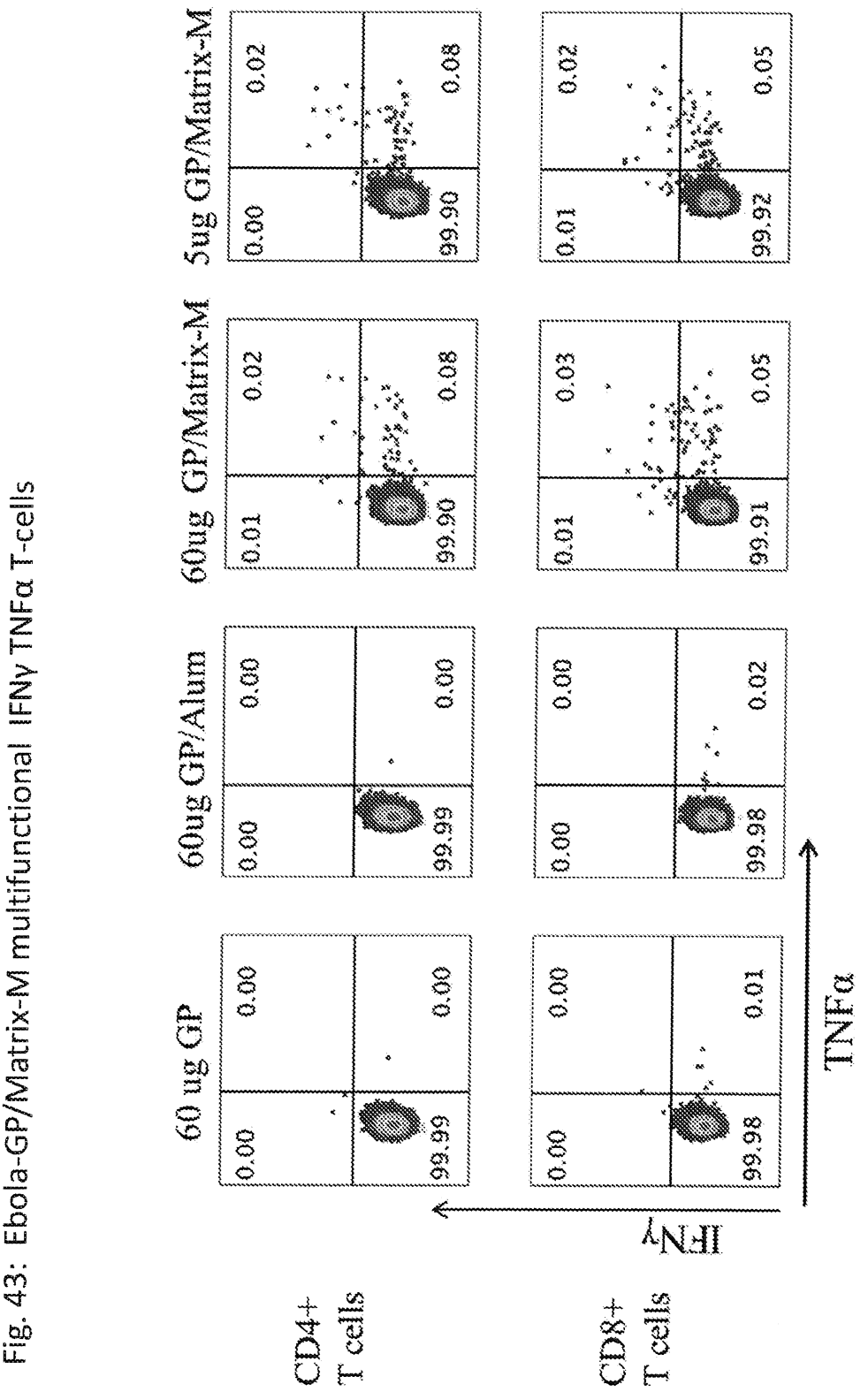


Fig. 44 Ebola-GP/Matrix-M multifunctional T-cells and Cytokines

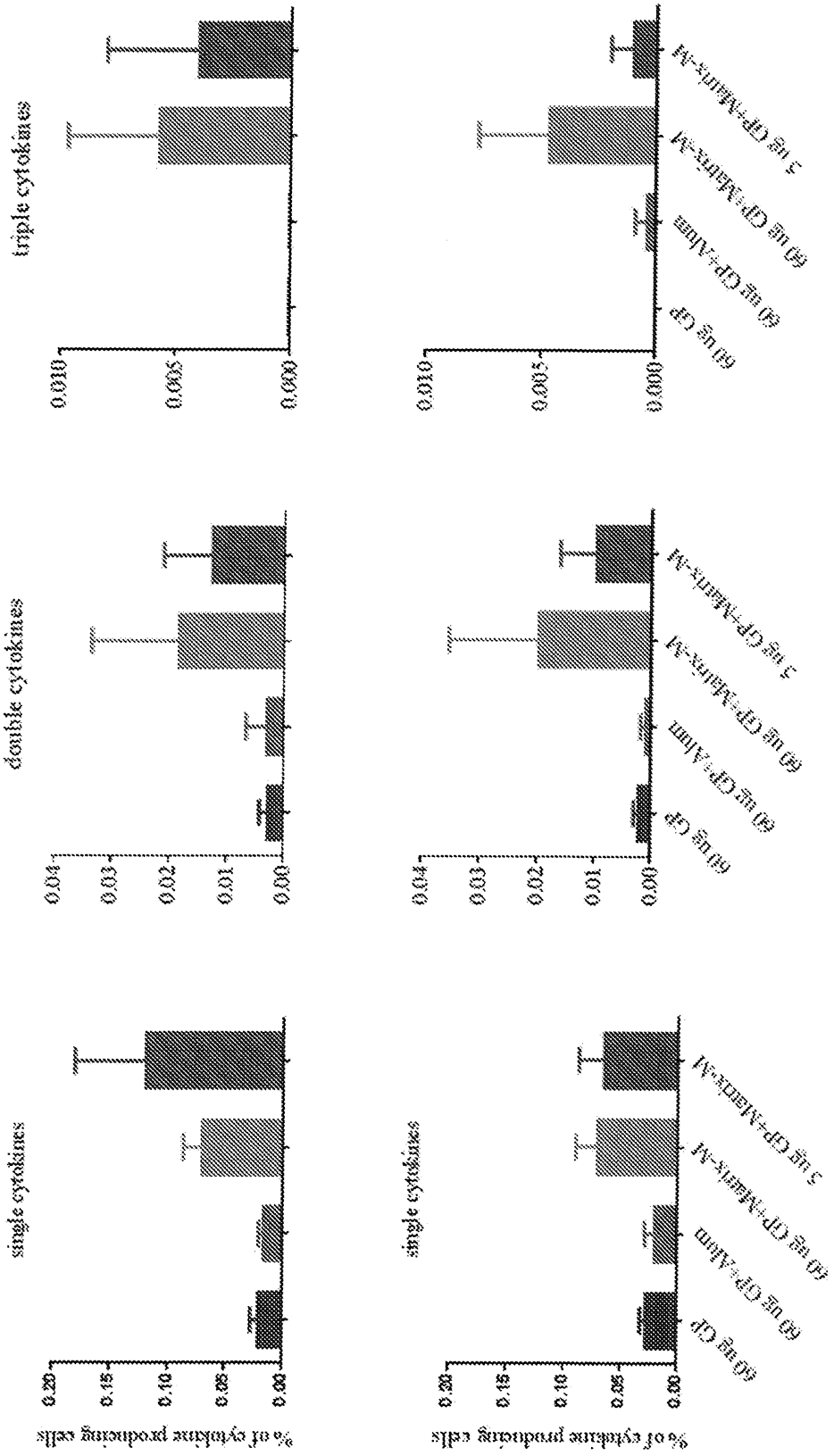


Fig. 45 Ebola-GP/Matrix-M vaccine trial design performed in Cynomolgus macaques

Group	Vaccine	N	Immunization Day	Challenge [1] Day	End of Study Day
1	5 µg GP + 50 µg Matrix-M	3	0, 21	42	60
2	control	1	--	42	60

1 Challenge virus: 100 pfu wild-type EBOV/Kikwit 9510621

Fig. 46: EBOV/Makona vaccine anti-EBOV/Makona GP IgG ELISA (EC50) responses in macaques

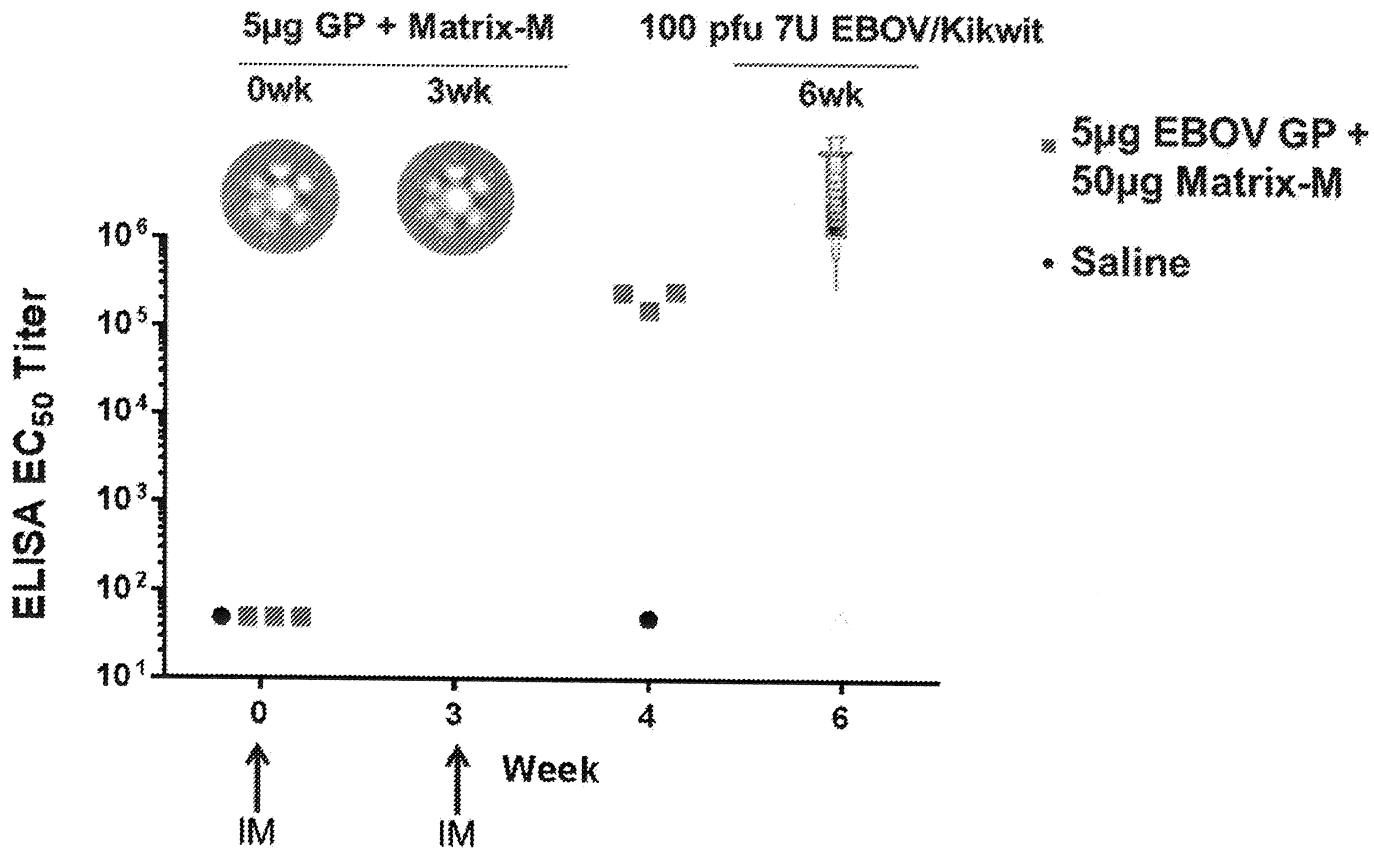


Figure 47: Anti-EBOV/Makona GP immune response

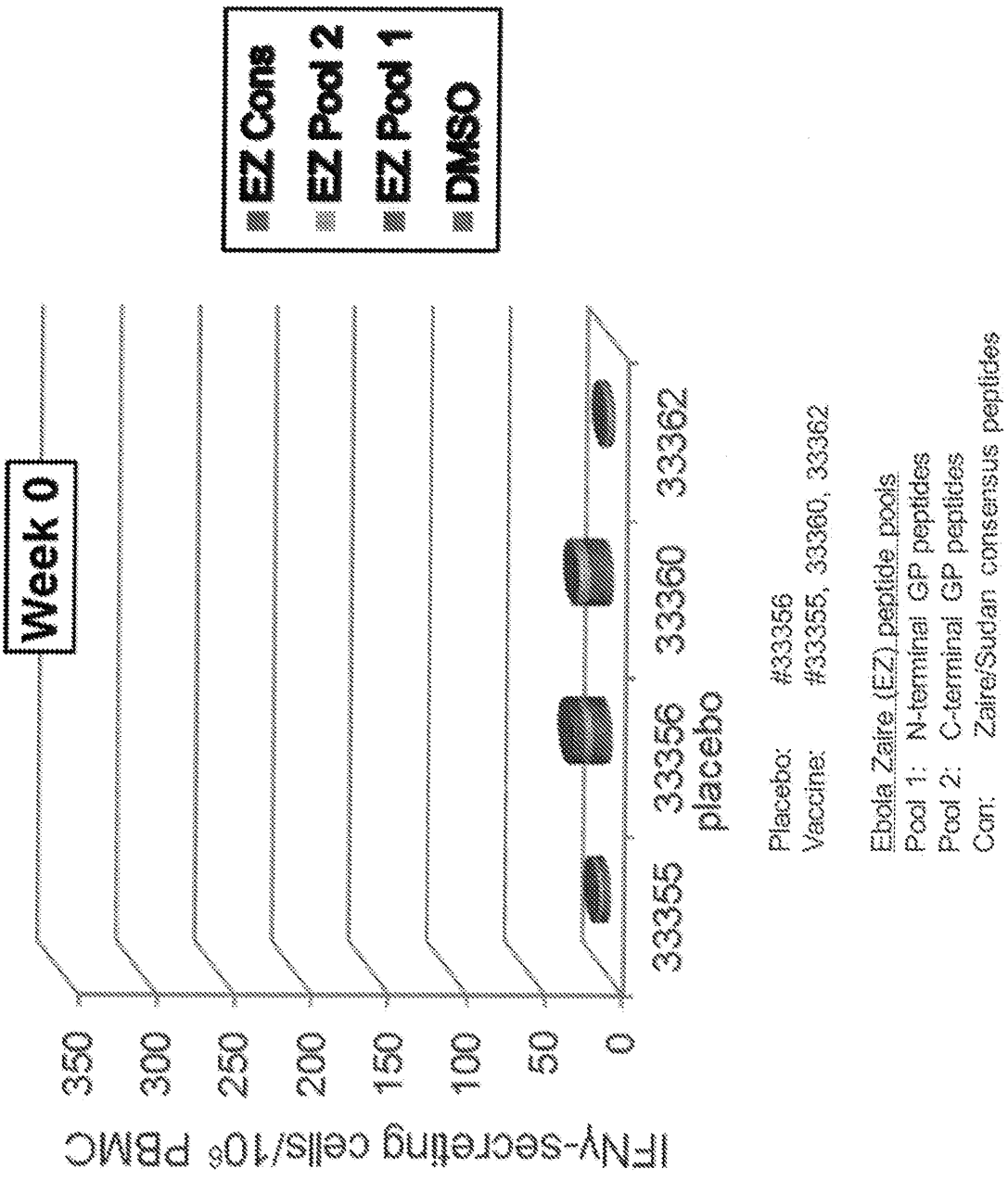
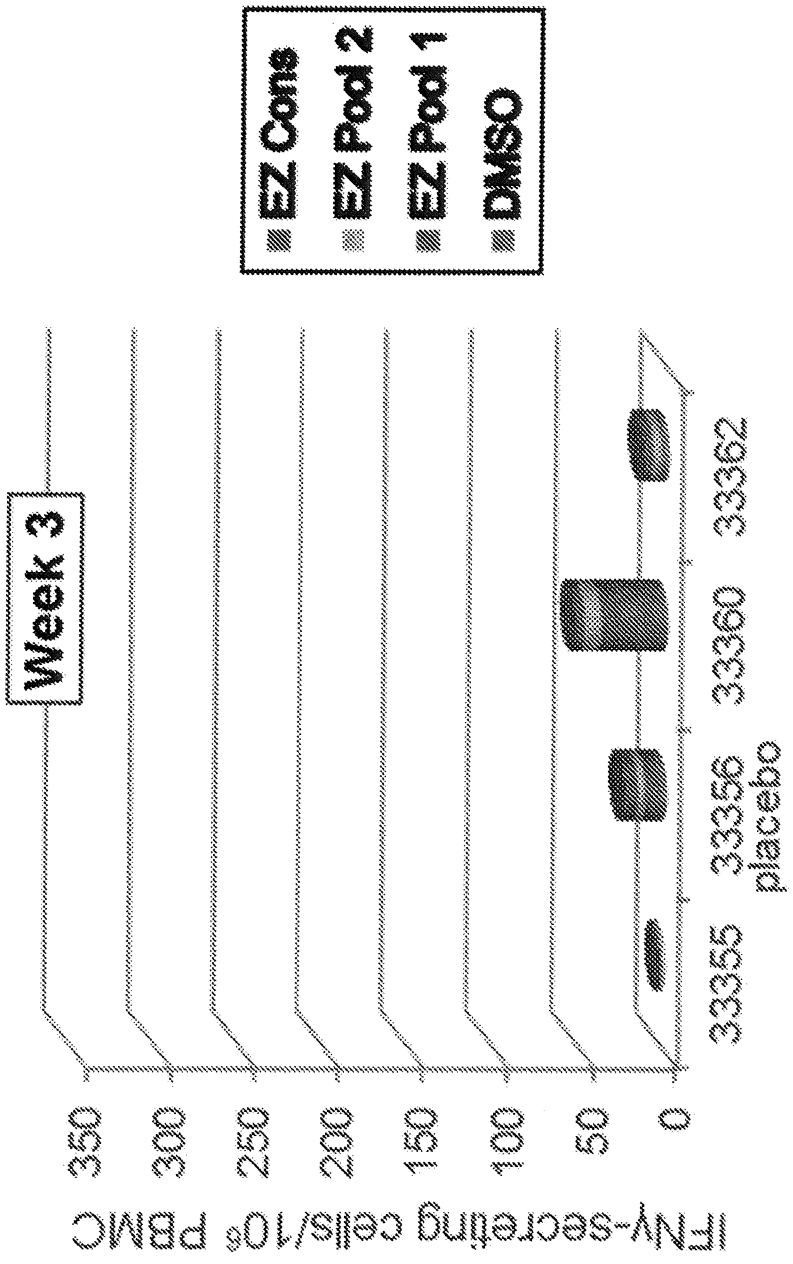


Figure 47(cont'd): Anti-EBOV/Makona GP immune response



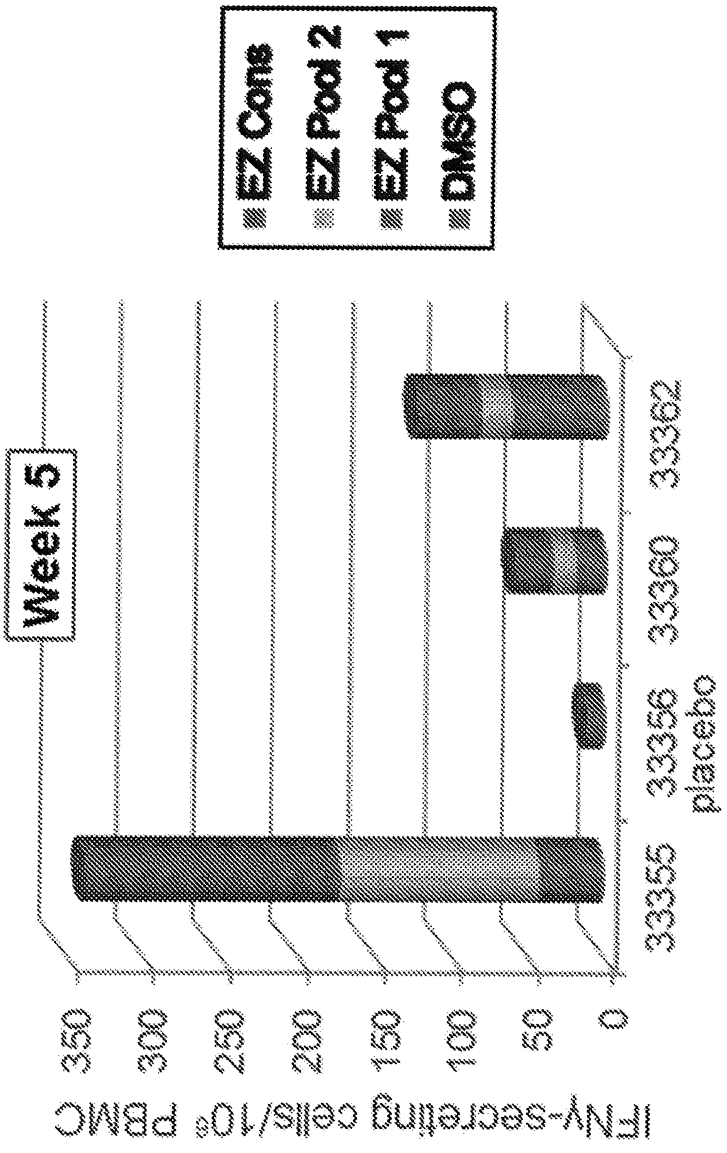
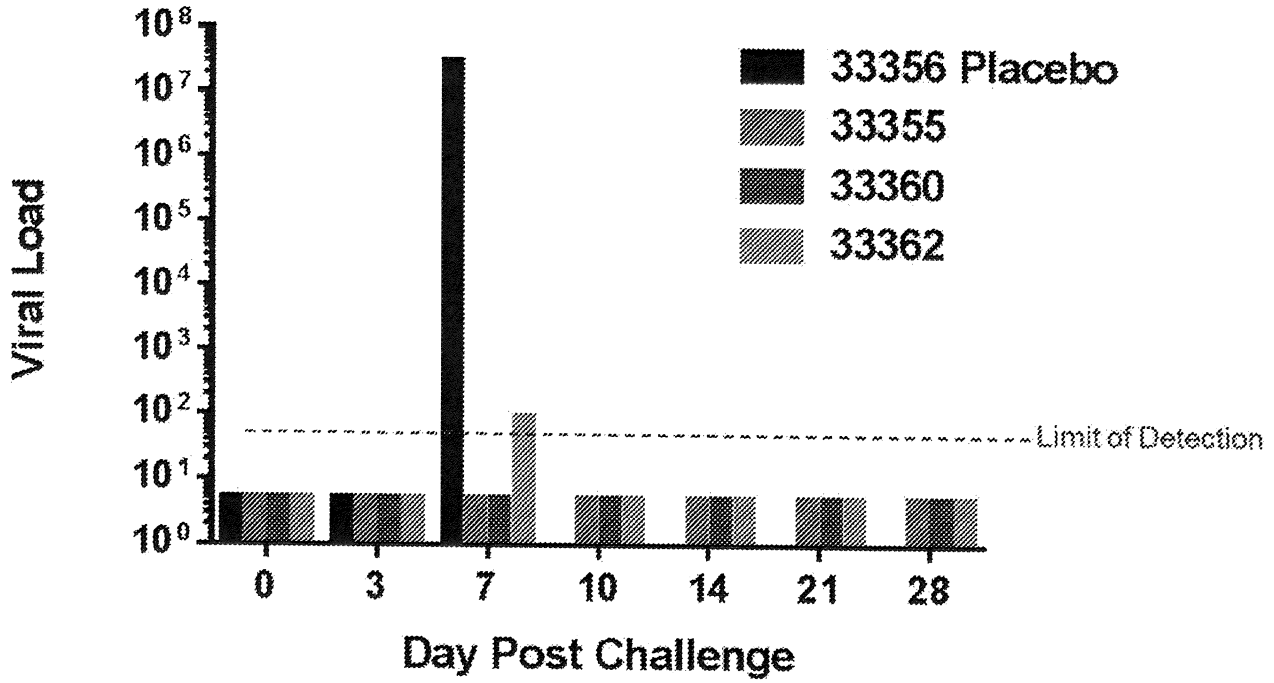


Figure 47(cont'd): Anti-EBOV/Makona GP immune response

Figure 48: Viral load rtPCR and survival in nanoparticle-vaccinated macaques



Vaccinated
3/3 survived
Clinical scores ≤ 2

Day 9 placebo euthanized (Clinical score 22)

Figure 49: Second Study of Ebola virus challenge of *Cynomolgus macaques*: Study Design

Group	Vaccine	N	Immunization wks	Challenge[1] wks
F	5 µg GP + 50 µg Matrix-M	2	0, 6	12
G	5 µg GP + 50 µg Matrix-M	2	0, 3	9
A	Control - PBS	2	0, 3, 6	12

[1]Challenge virus: 100 pfu wild-type 7U EBOV/Kikwit

Figure 50: Second Study of Ebola virus challenge of *Cynomolgus macaques*:EBOV/Makona vaccine anti-EBOV/Makona GP IgG ELISA (EC50) and NHP survival

