Figure 1

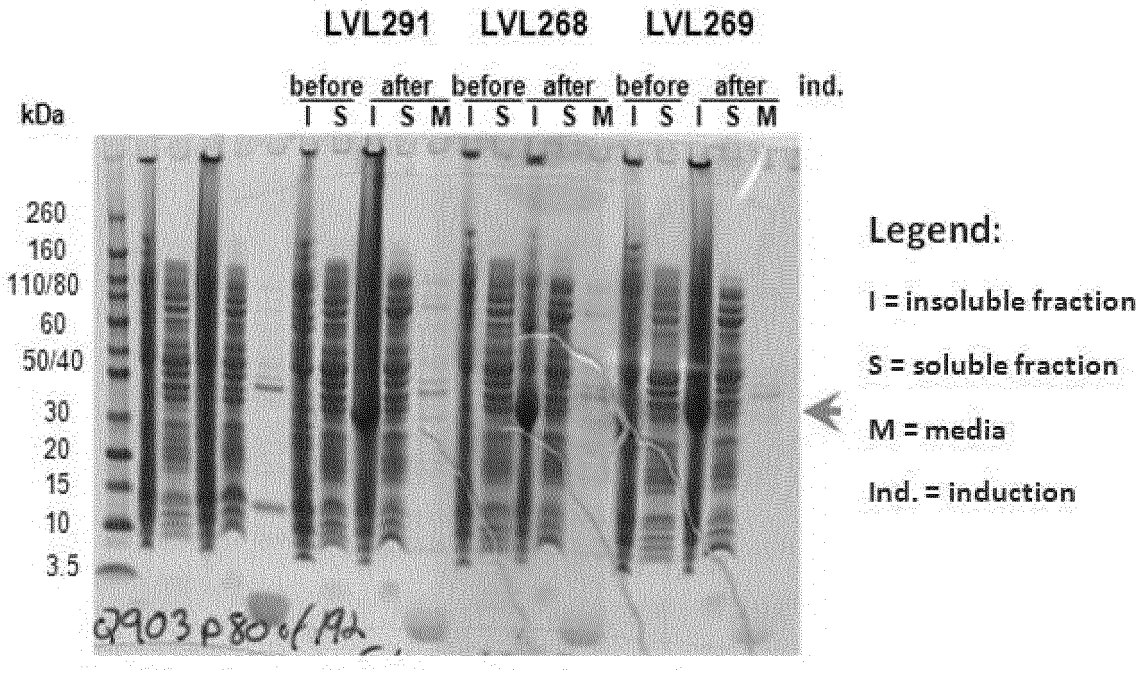


Figure 2

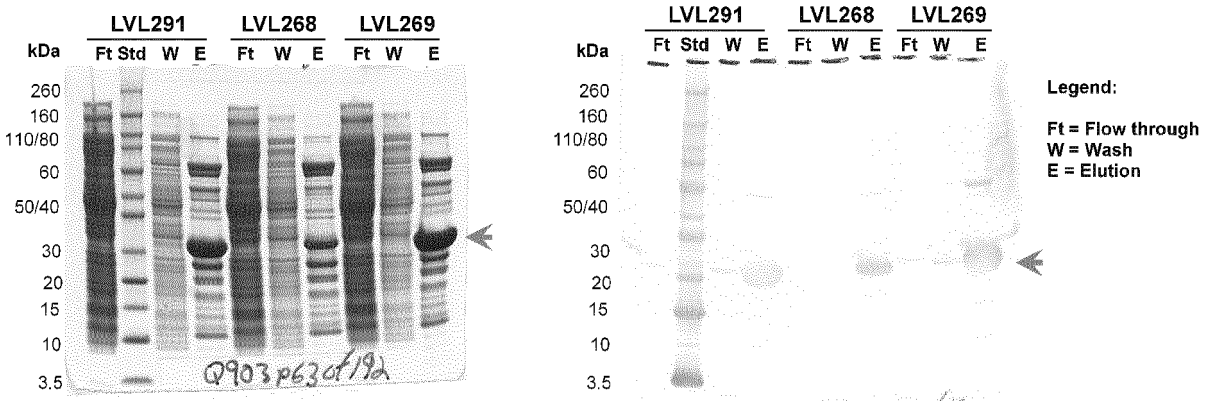


Figure 3

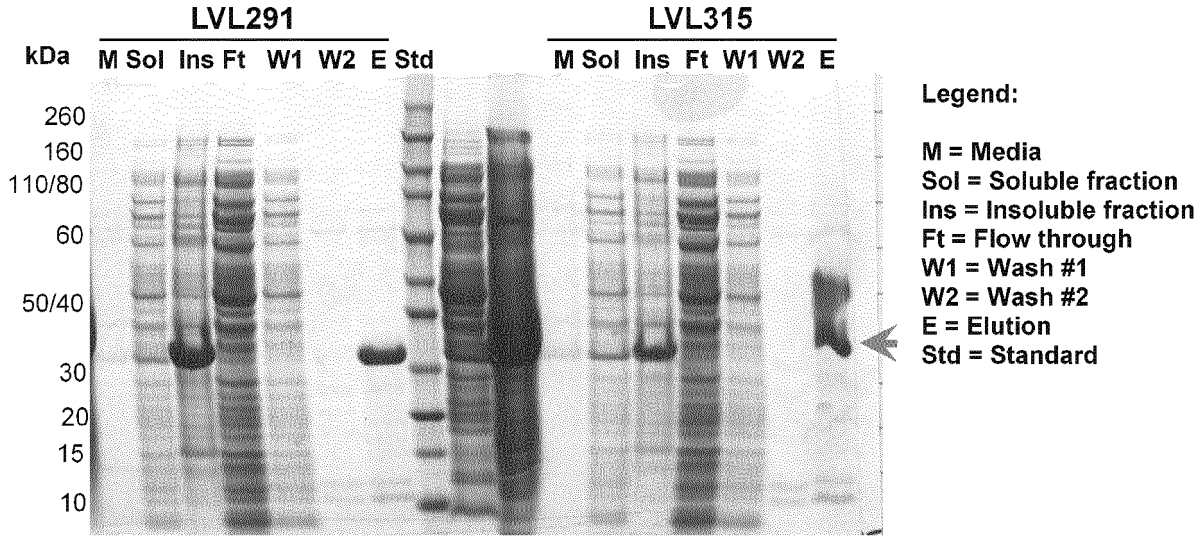


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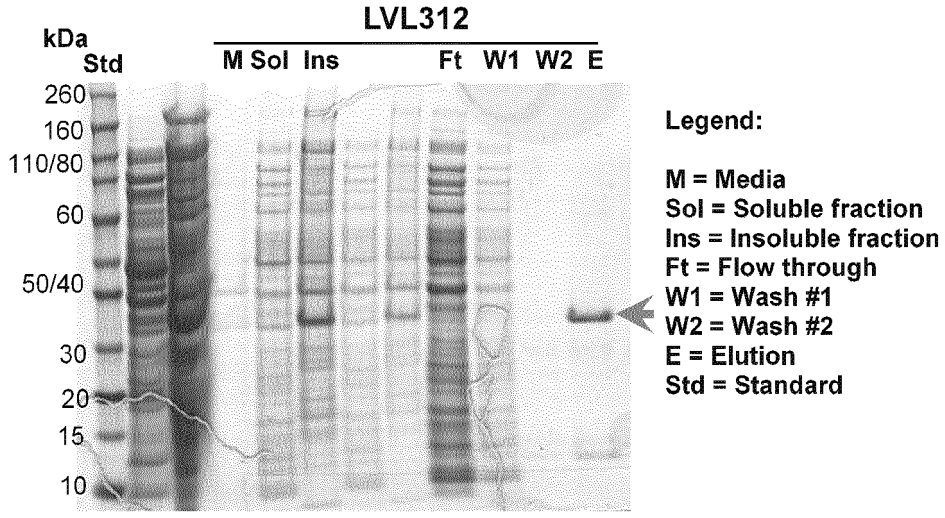


Figure 5

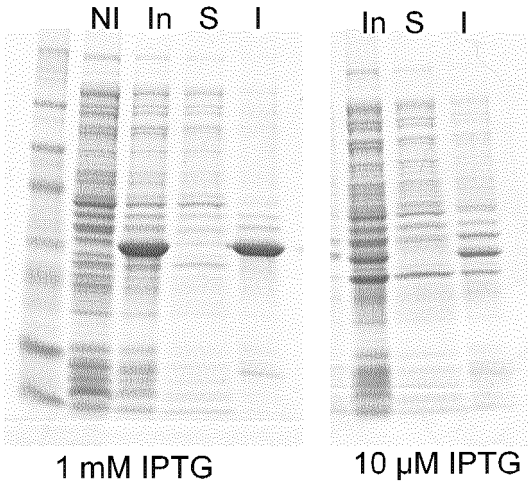


Figure 6

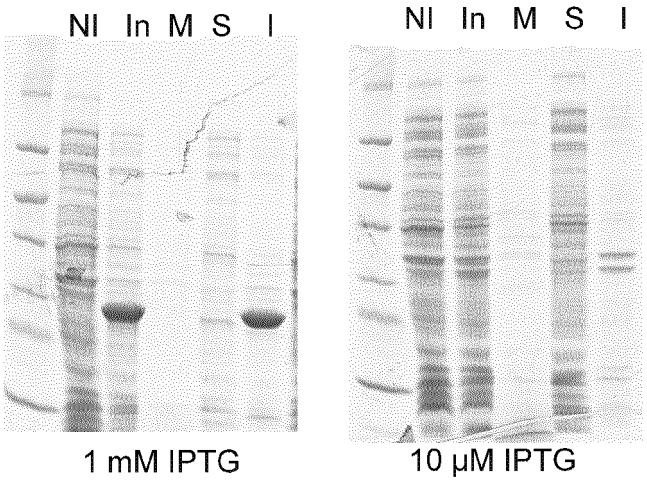


Figure 7

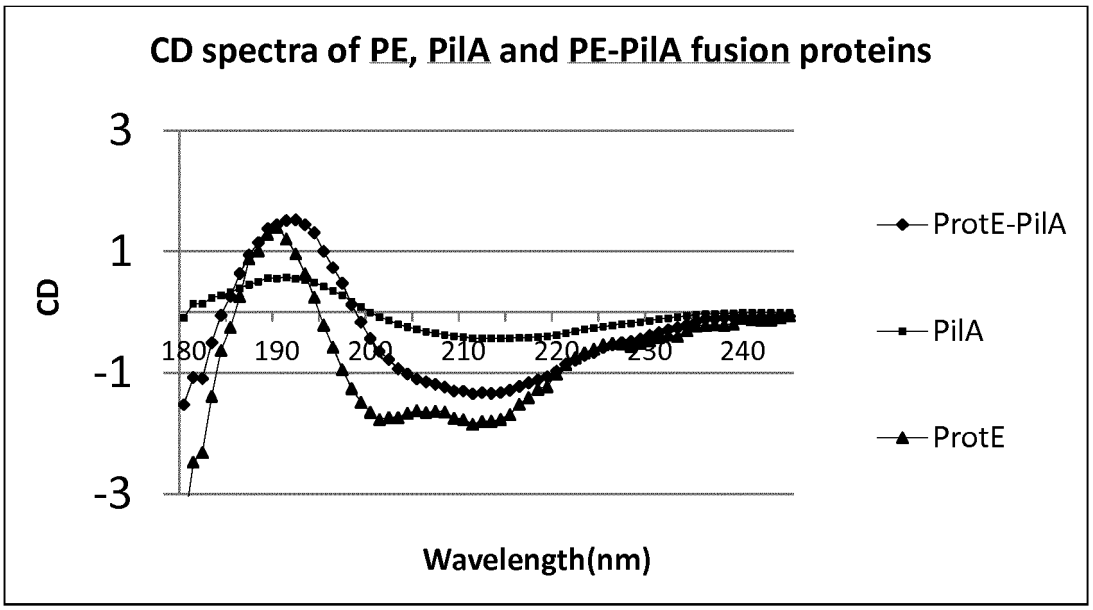


Figure 8

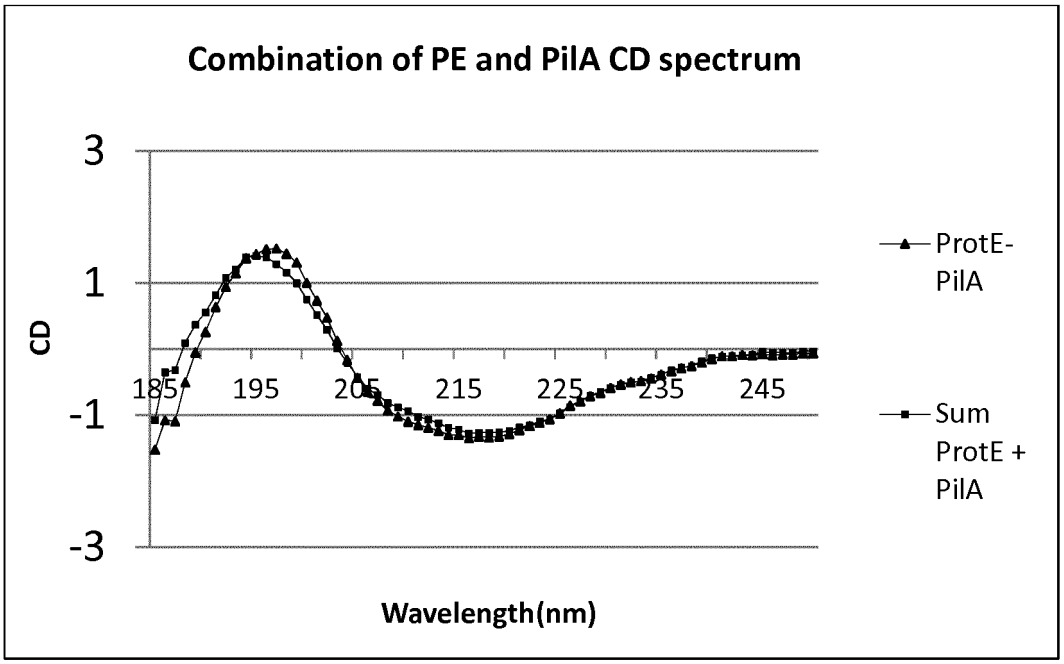


Figure 9

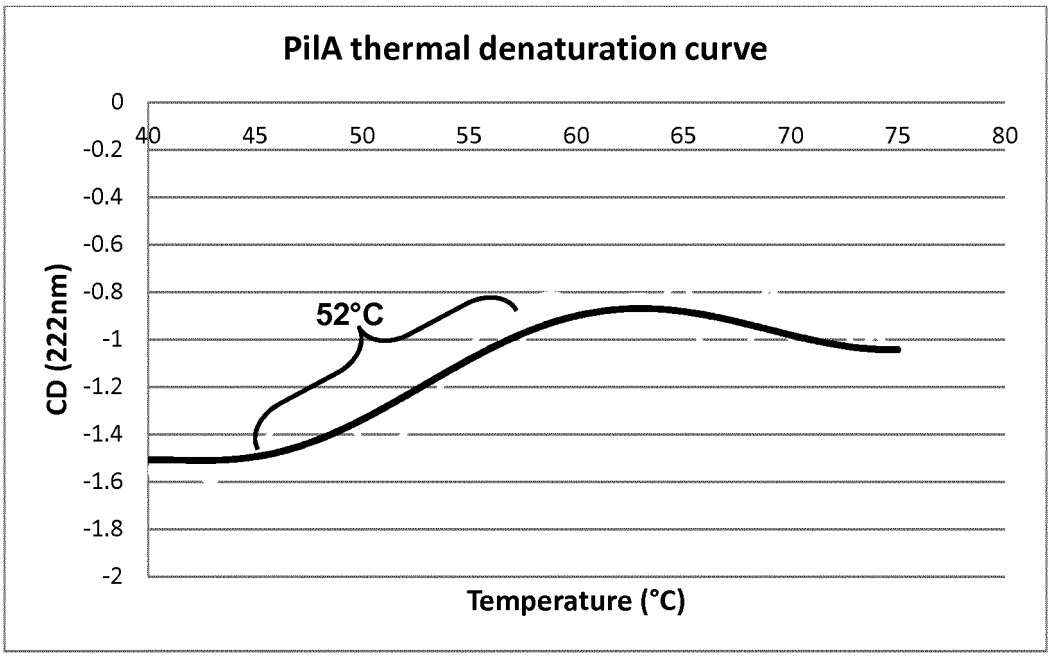


Figure 10

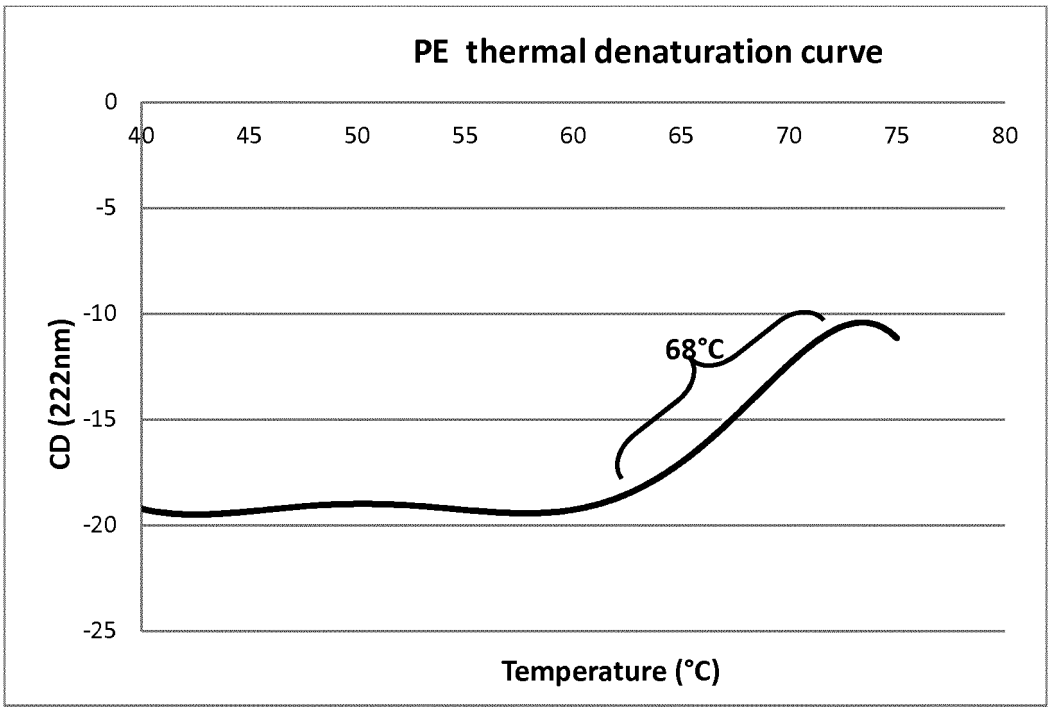


Figure 11

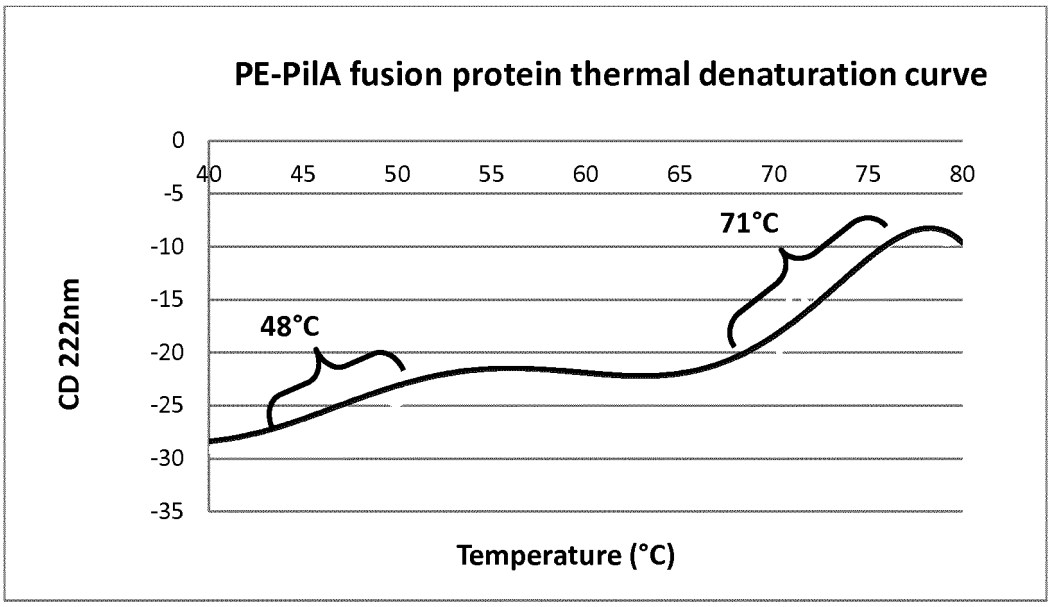


Figure 12

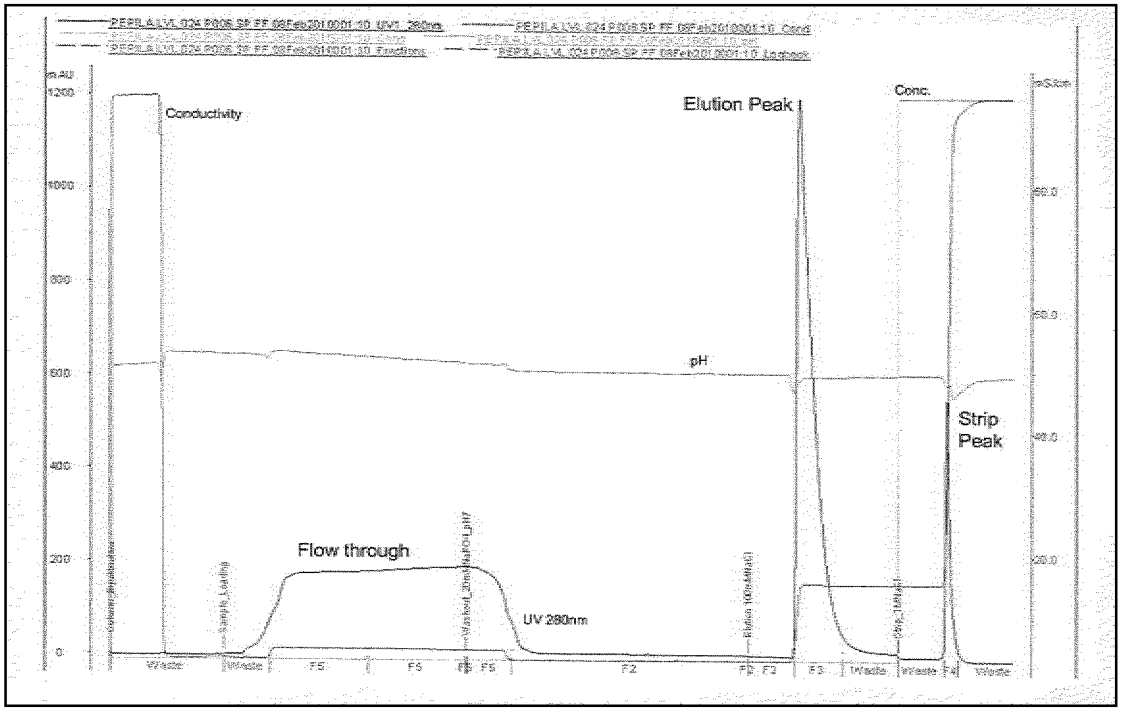


Figure 13

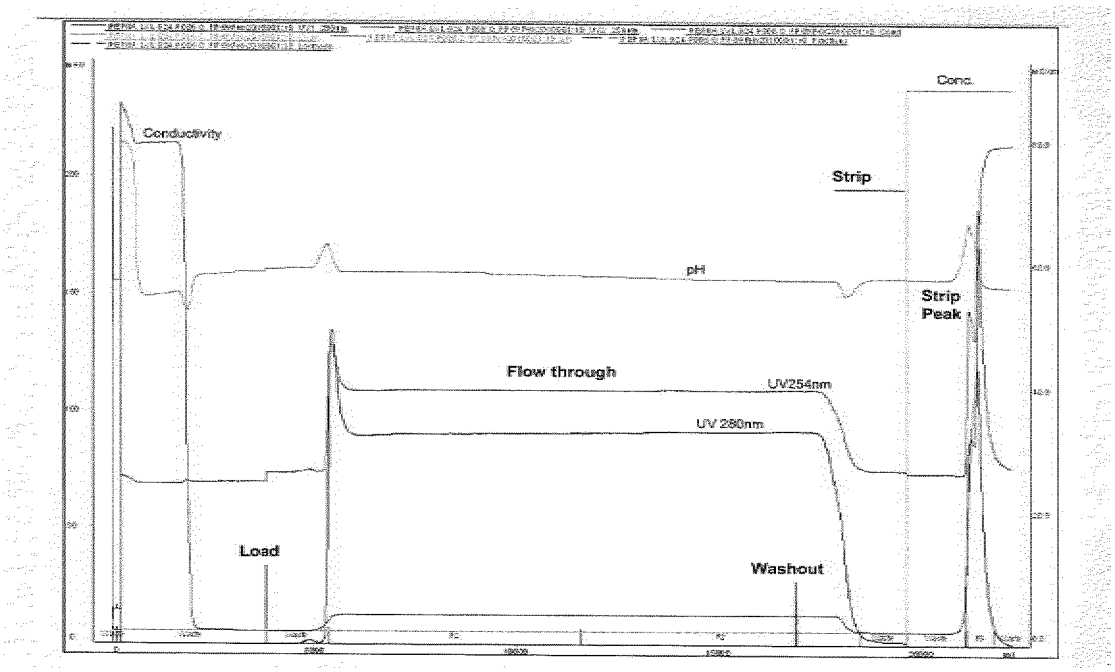


Figure 14

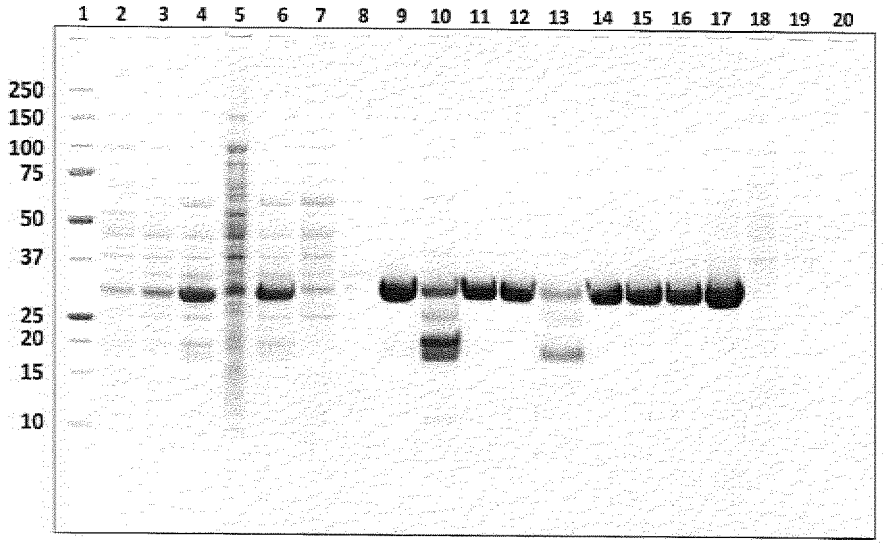


Figure 15

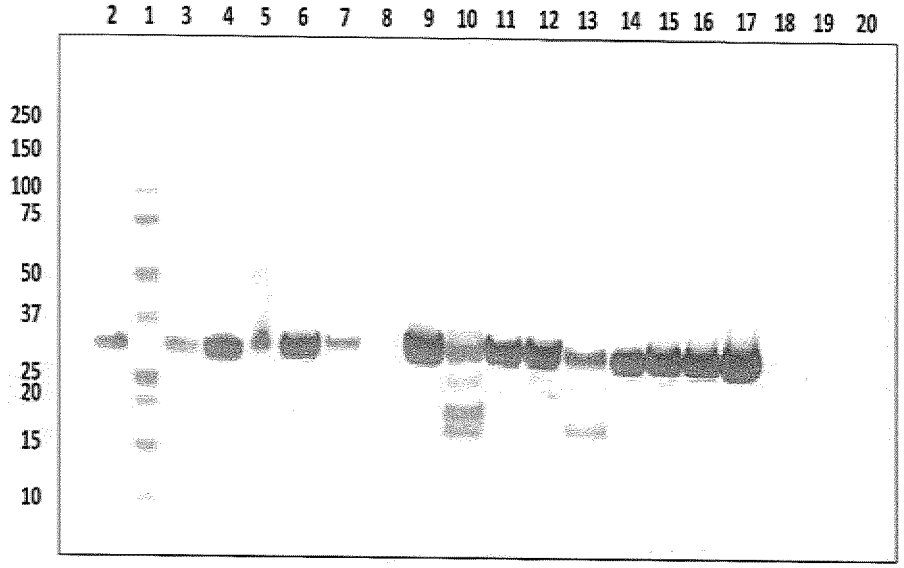


Figure 16

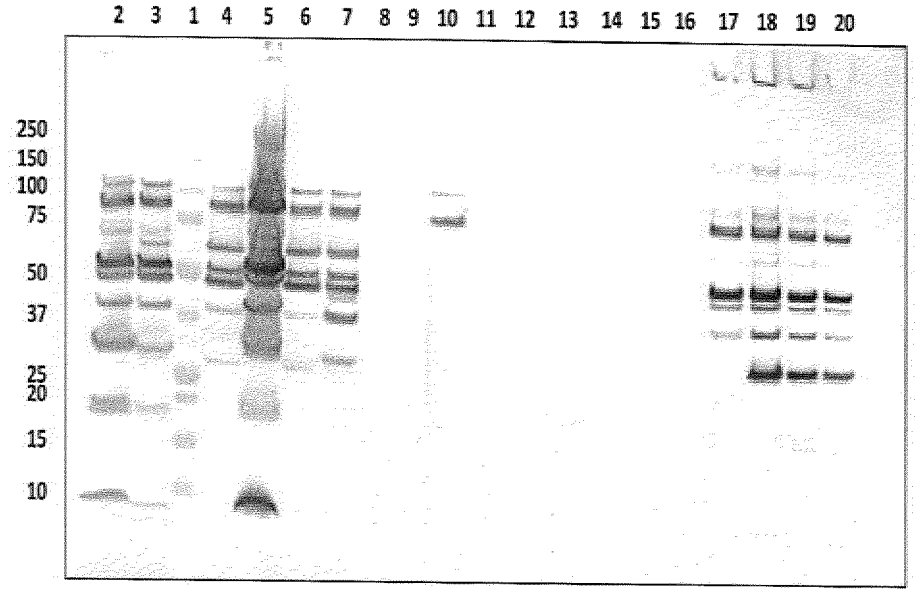


Figure 17

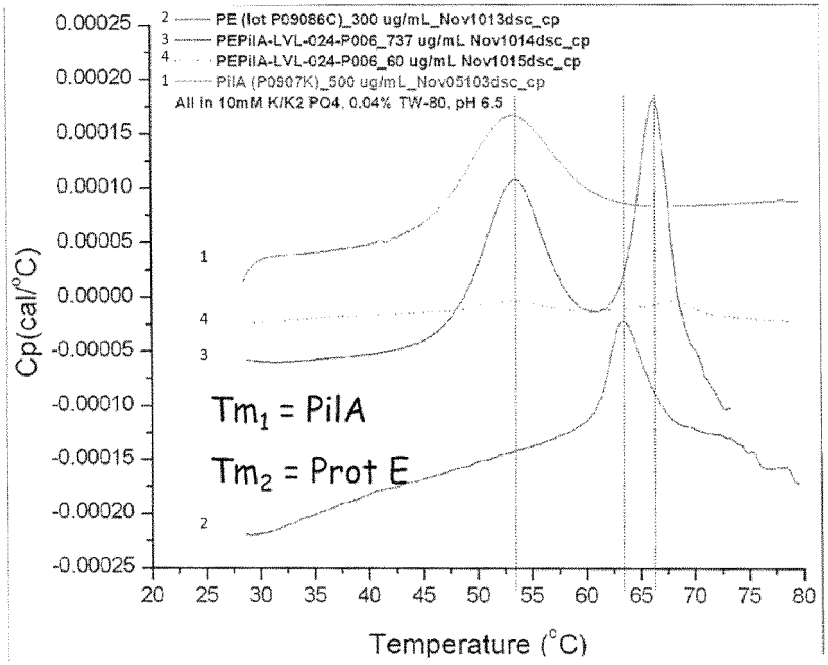


Figure 18

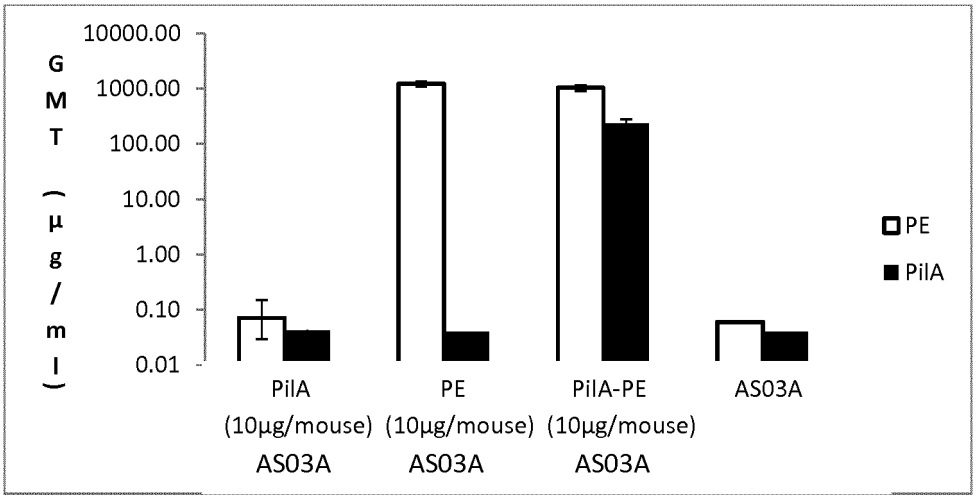


Figure 19

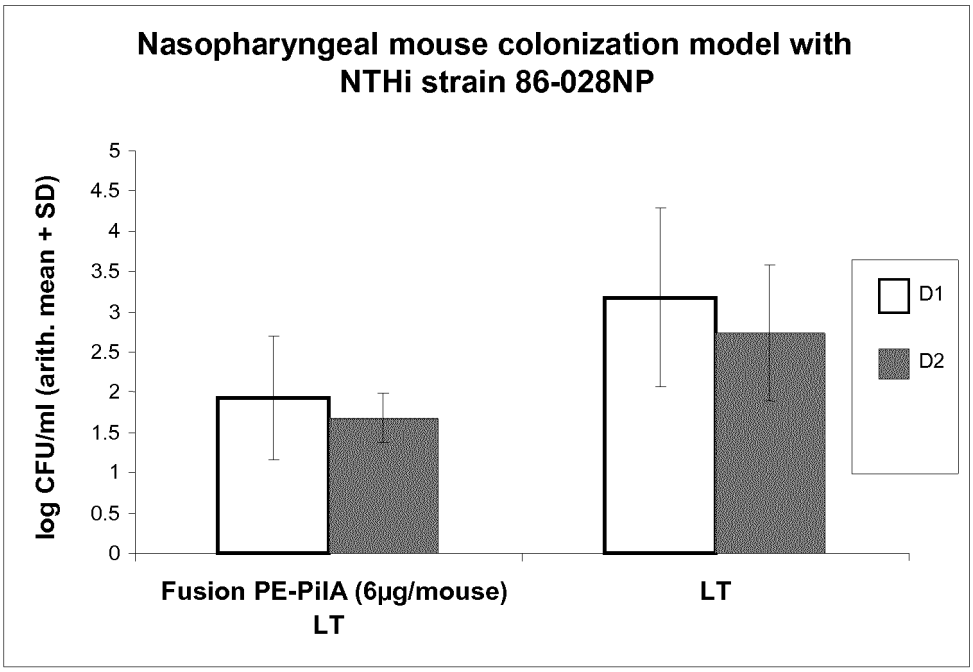

Nasopharyngeal mouse colonization model with NTHi strain 86-028NP
log CFU/ml (arith. mean + SD)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
D1
D2
Fusion PE-PilA (6µg/mouse) LT
LT

Figure 20

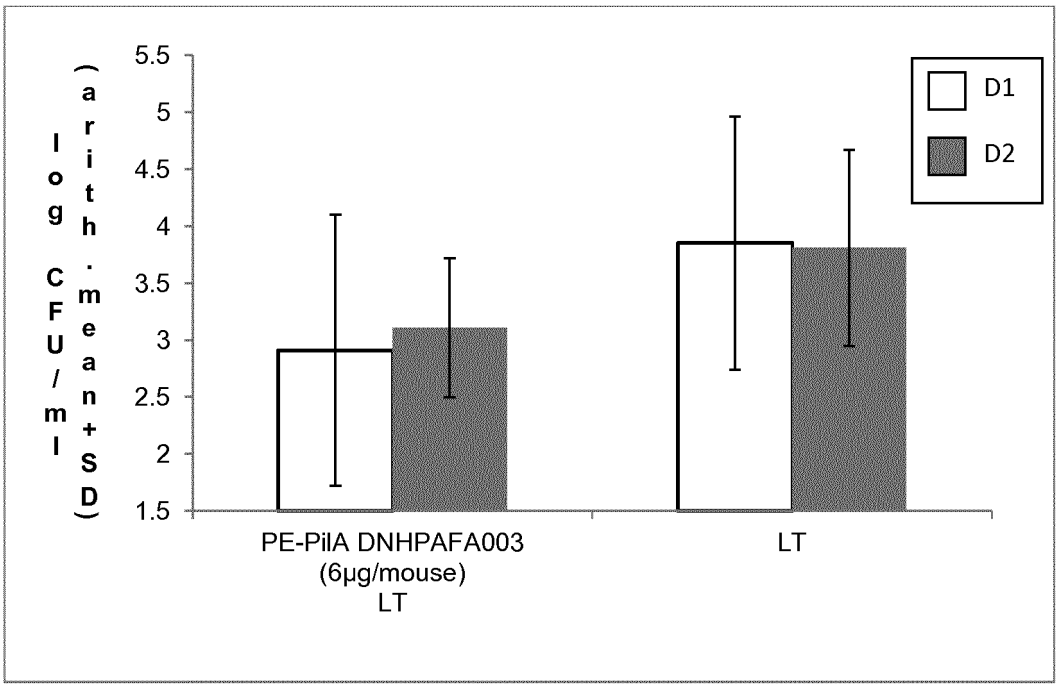

log CFU/ml (arith. mean+SD)
5.5
5
4.5
4
3.5
3
2.5
2
1.5
D1
D2
PE-PilA DNHPAFA003 (6µg/mouse) LT
LT

Figure 21

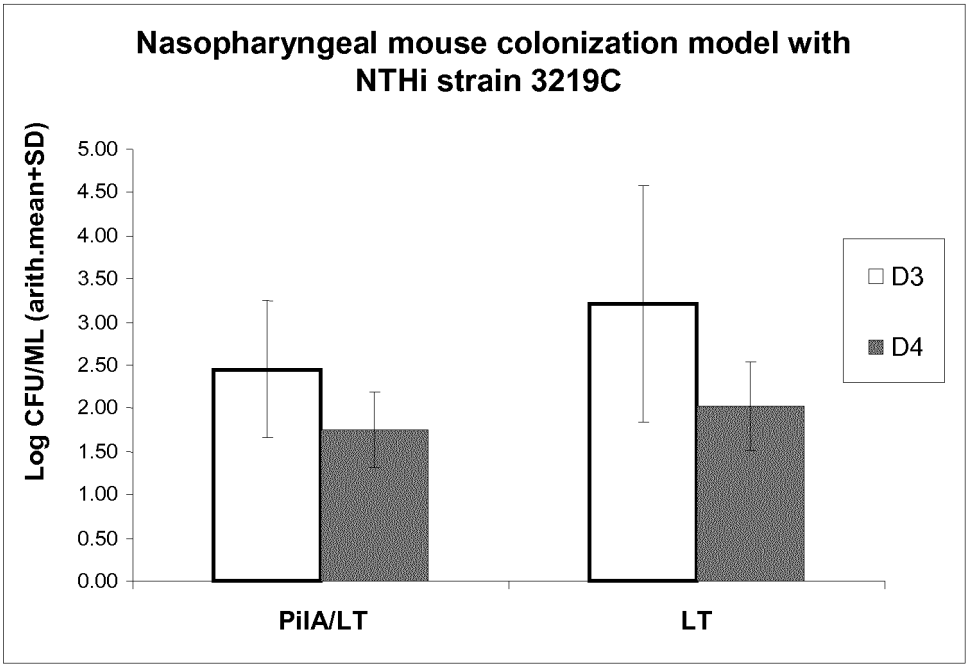
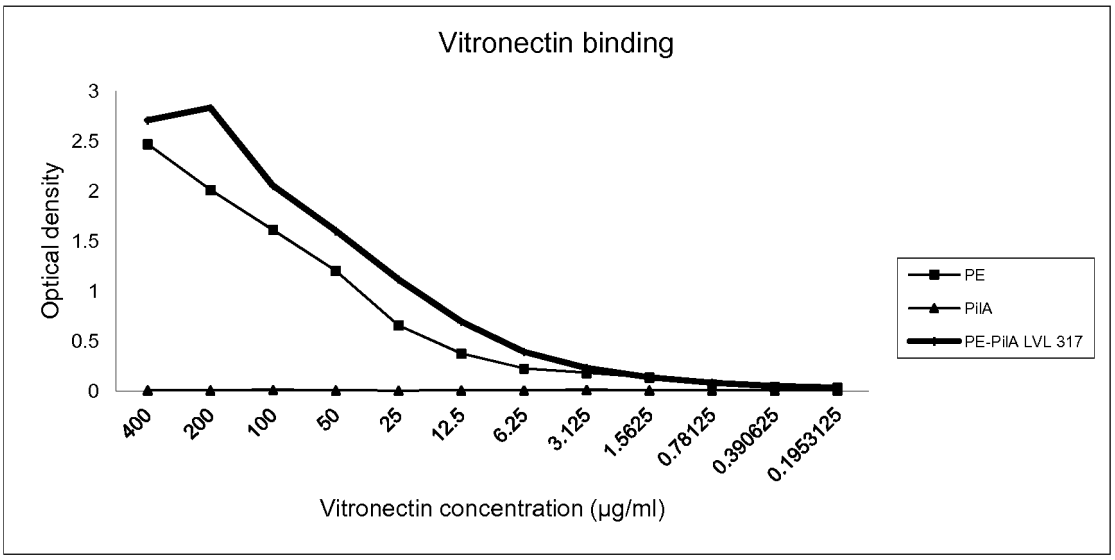

Nasopharyngeal mouse colonization model with NTHi strain 3219C
Log CFU/ML (arith.mean+SD)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
D3
D4
PilA/LT
LT

Figure 22



Figure 23

(a)


Vitronectin binding
Optical density
3
2.5
2
1.5
1
0.5
0
400
200
100
50
25
12.5
6.25
3.125
1.5625
0.78125
0.390625
0.1953125
Vitronectin concentration (µg/ml)
PE
PilA
PE-PilA LVL 317

(b)

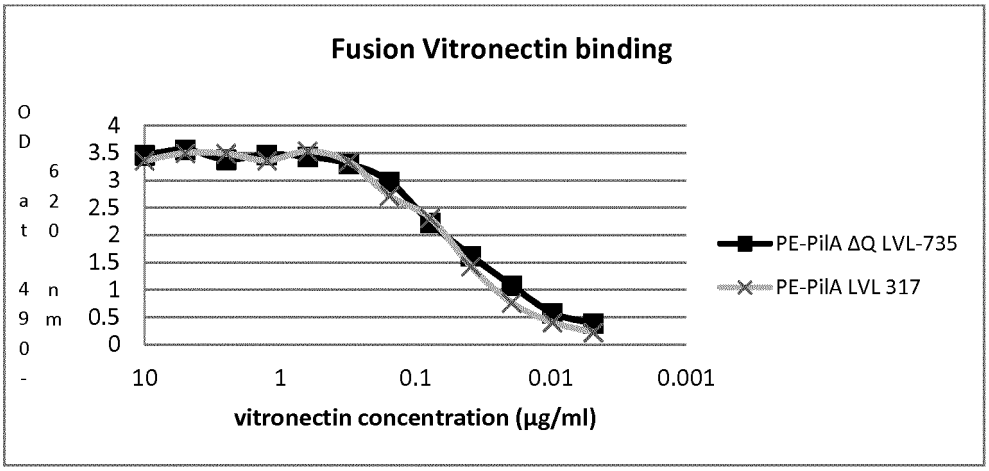

Fusion Vitronectin binding
OD 620 nm at 490 -
4
3.5
3
2.5
2
1.5
1
0.5
0
10
1
0.1
0.01
0.001
vitronectin concentration (µg/ml)
PE-PilA ΔQ LVL-735
PE-PilA LVL 317

Figure 24

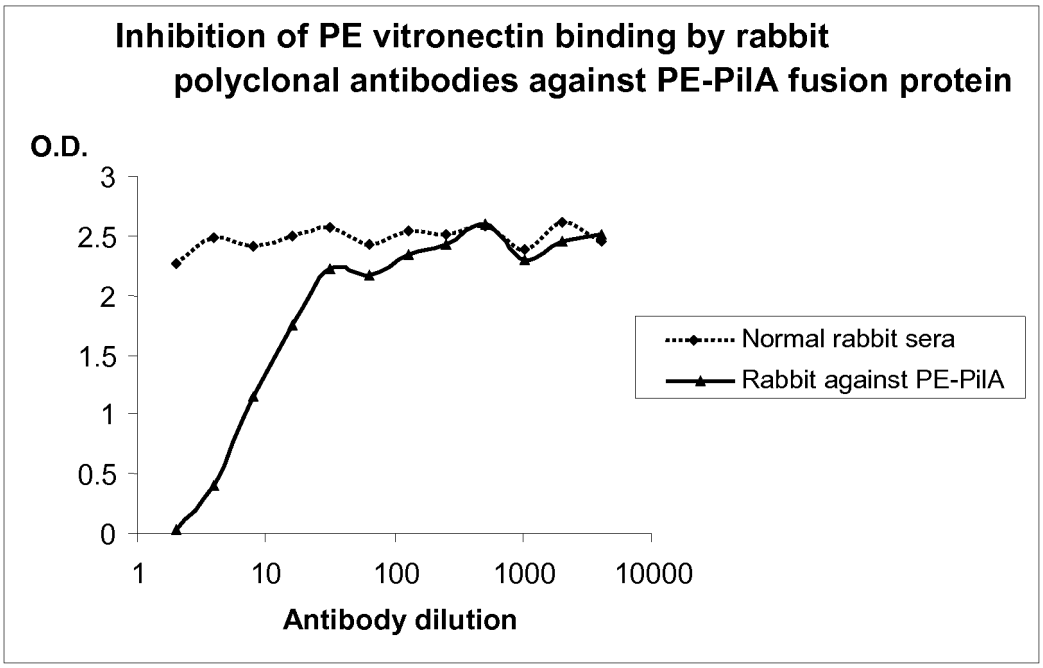
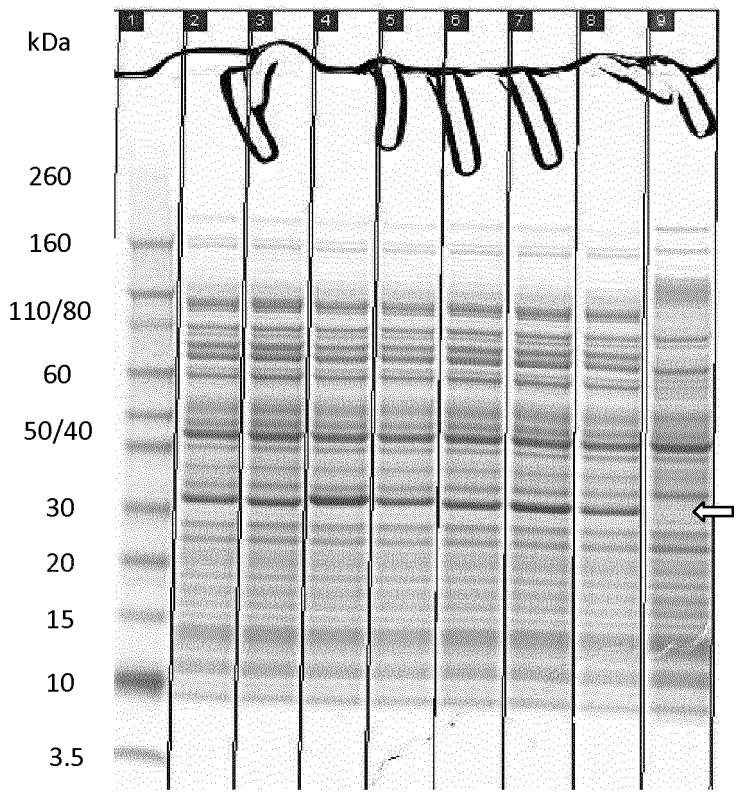

Inhibition of PE vitronectin binding by rabbit polyclonal antibodies against PE-PilA fusion protein
O.D.
3
2.5
2
1.5
1
0.5
0
1
10
100
1000
10000
Antibody dilution
Normal rabbit sera
Rabbit against PE-PilA

Figure 25

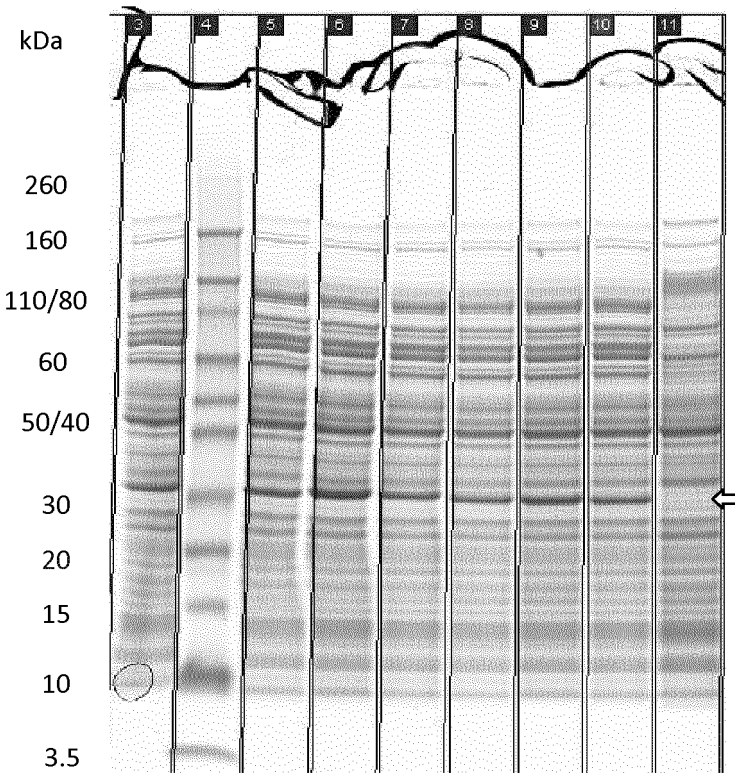
(a) Experiment 1



LEGEND	
1	Novex® standard
2	Soluble fraction of induction #1 for LVL291
3	Soluble fraction of induction #1 for LVL702
4	Soluble fraction of induction #1 for LVL736
5	Soluble fraction of induction #1 for LVL737
6	Soluble fraction of induction #1 for LVL738
7	Soluble fraction of induction #1 for LVL739
8	Soluble fraction of induction #1 for LVL740
9	Soluble fraction of induction #1 for pET26b

Figure 25

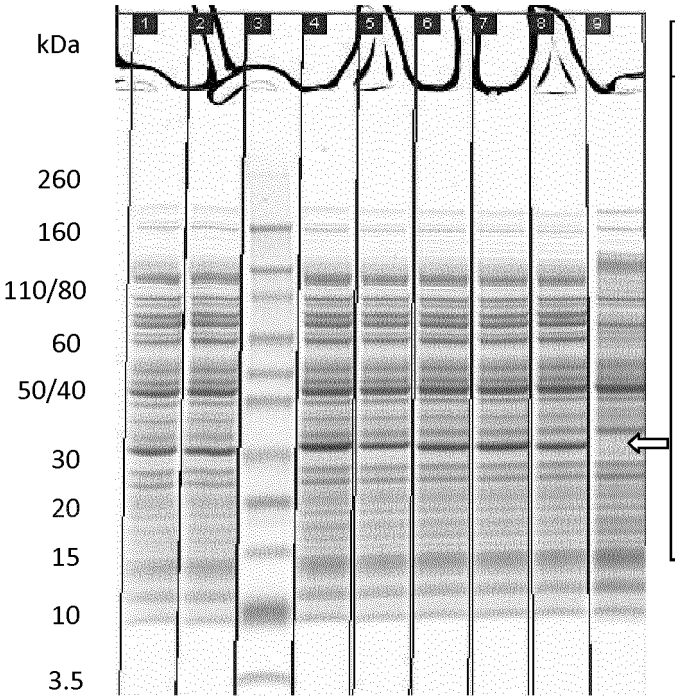
(b) Experiment 2



LEGEND	
3	Soluble fraction of induction #2 for LVL291
4	Novex® standard
5	Soluble fraction of induction #2 for LVL702
6	Soluble fraction of induction #2 for LVL736
7	Soluble fraction of induction #2 for LVL737
8	Soluble fraction of induction #2 for LVL738
9	Soluble fraction of induction #2 for LVL739
10	Soluble fraction of induction #2 for LVL740
11	Soluble fraction of induction #2 for pET26b

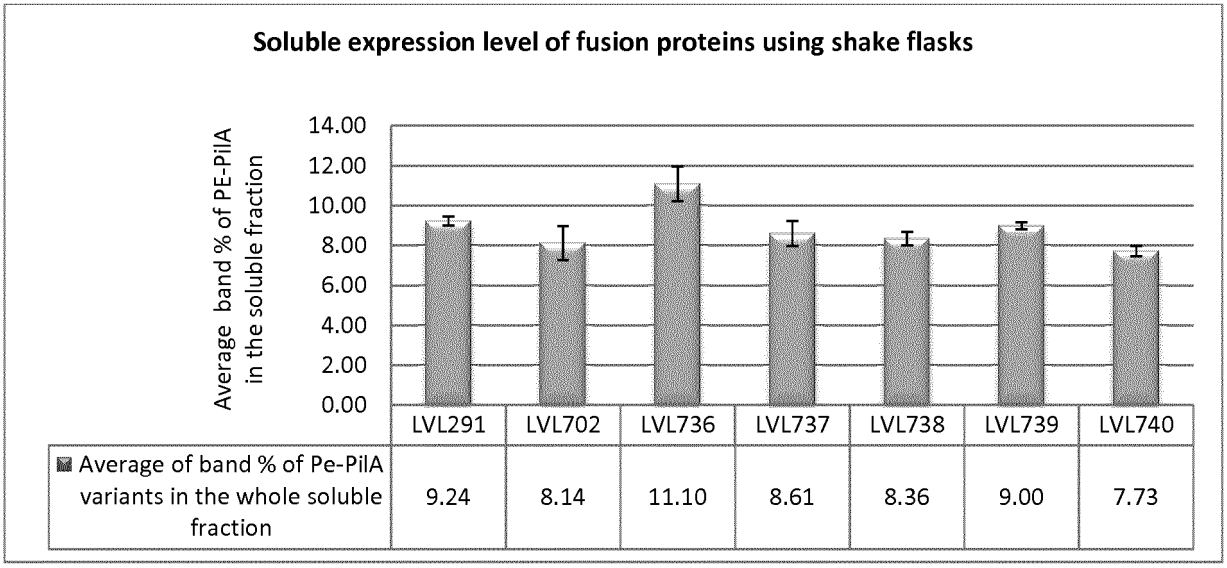
Figure 25

(c) Experiment 3



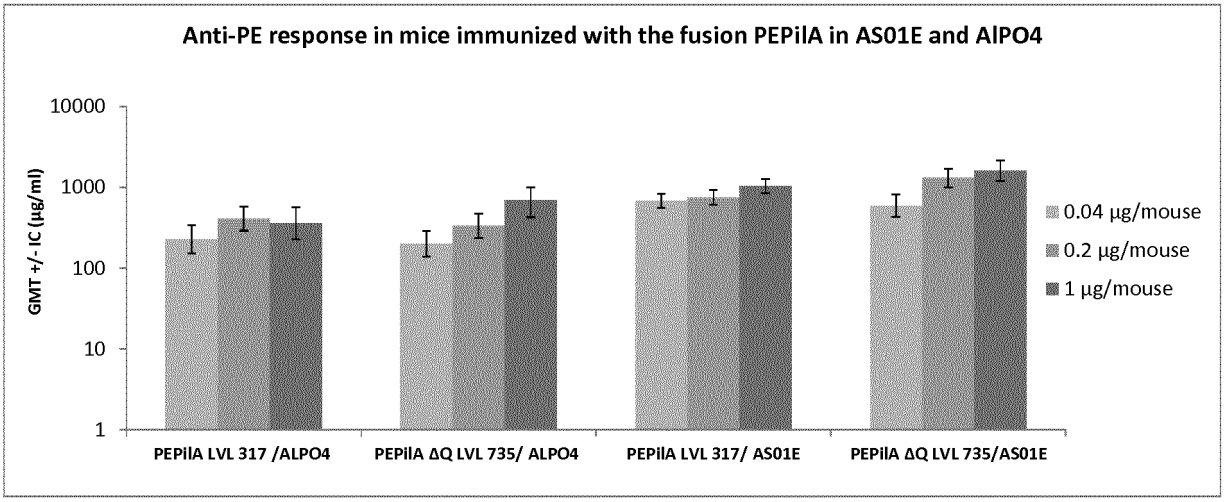
LEGEND	
1	Soluble fraction of induction #3 for LVL291
2	Soluble fraction of induction #3 for LVL702
3	Novex® standard
4	Soluble fraction of induction #3 for LVL736
5	Soluble fraction of induction #3 for LVL737
6	Soluble fraction of induction #3 for LVL738
7	Soluble fraction of induction #3 for LVL739
8	Soluble fraction of induction #3 for LVL740
9	Soluble fraction of induction #3 for pET26b

Figure 26



Construct ID	% fusion protein gel #1	% fusion protein gel #2	% fusion protein gel #3	Average % fusion protein	SD deviation
LVL291	9.3	8.98	9.43	**9.24**	0.231588716
LVL702	7.2	8.85	8.37	**8.14**	0.848704896
LVL736	11.16	10.2	11.94	**11.10**	0.871550343
LVL737	8.18	8.33	9.32	**8.61**	0.619435227
LVL738	8.56	8.55	7.97	**8.36**	0.337786915
LVL739	8.91	8.88	9.21	**9.00**	0.182482876
LVL740	7.69	7.49	8	**7.73**	0.256969518

Figure 27


Anti-PE response in mice immunized with the fusion PEPilA in AS01E and AlPO4
GMT +/- IC (µg/ml)
10000
1000
100
10
1
PEPilA LVL 317 /ALPO4
PEPilA ΔQ LVL 735/ ALPO4
PEPilA LVL 317/ AS01E
PEPilA ΔQ LVL 735/AS01E
0.04 µg/mouse
0.2 µg/mouse
1 µg/mouse

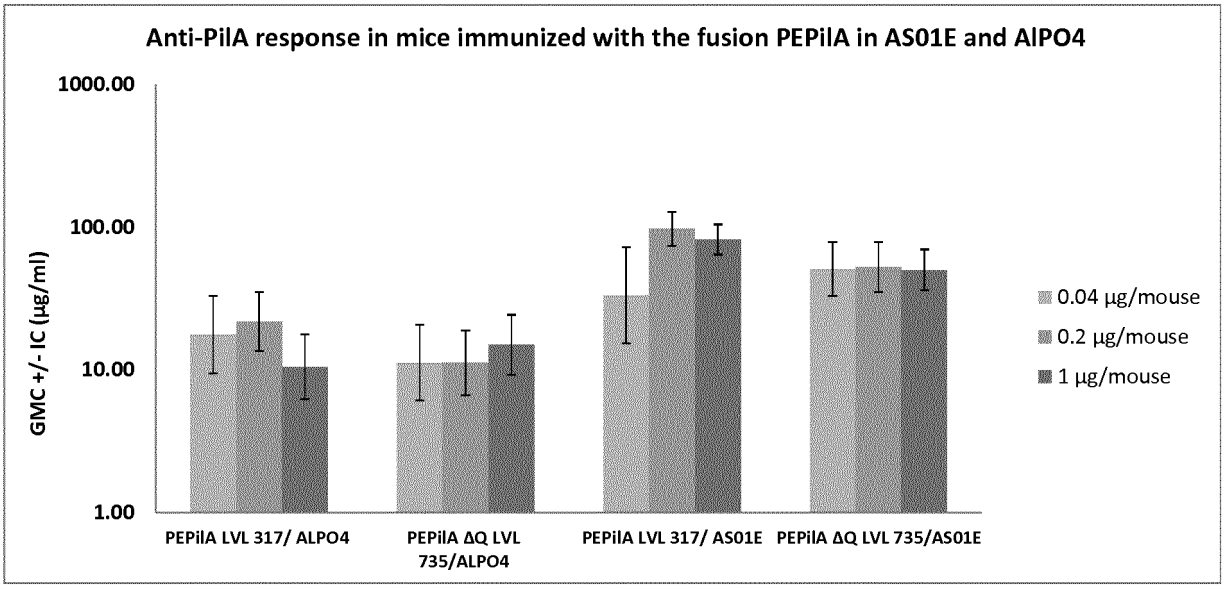

Anti-PilA response in mice immunized with the fusion PEPilA in AS01E and AlPO4
GMC +/- IC (µg/ml)
1000.00
100.00
10.00
1.00
PEPilA LVL 317/ ALPO4
PEPilA ΔQ LVL 735/ALPO4
PEPilA LVL 317/ AS01E
PEPilA ΔQ LVL 735/AS01E
0.04 µg/mouse
0.2 µg/mouse
1 µg/mouse

Figure 28

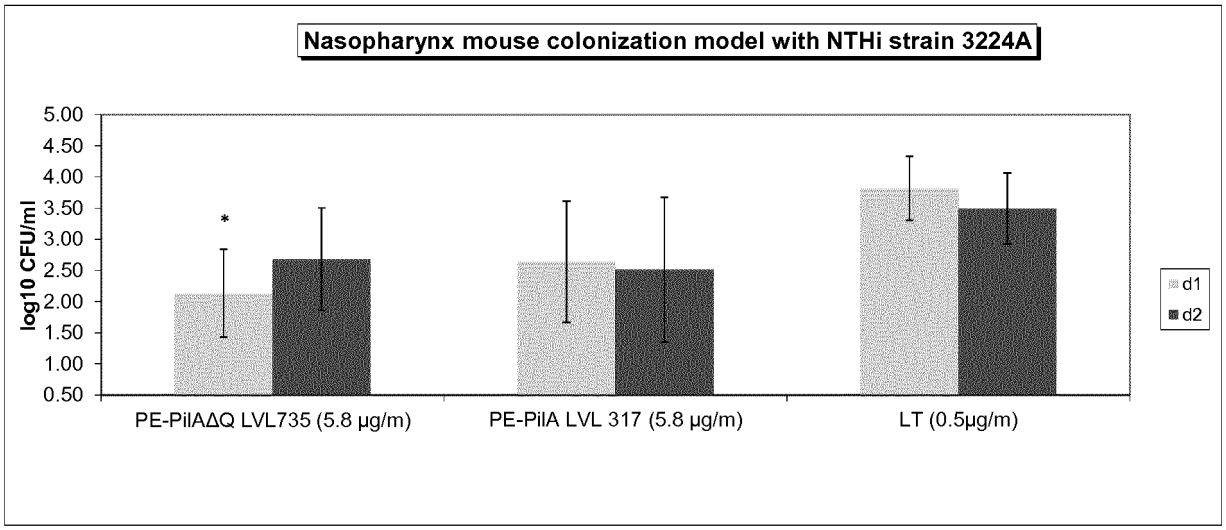
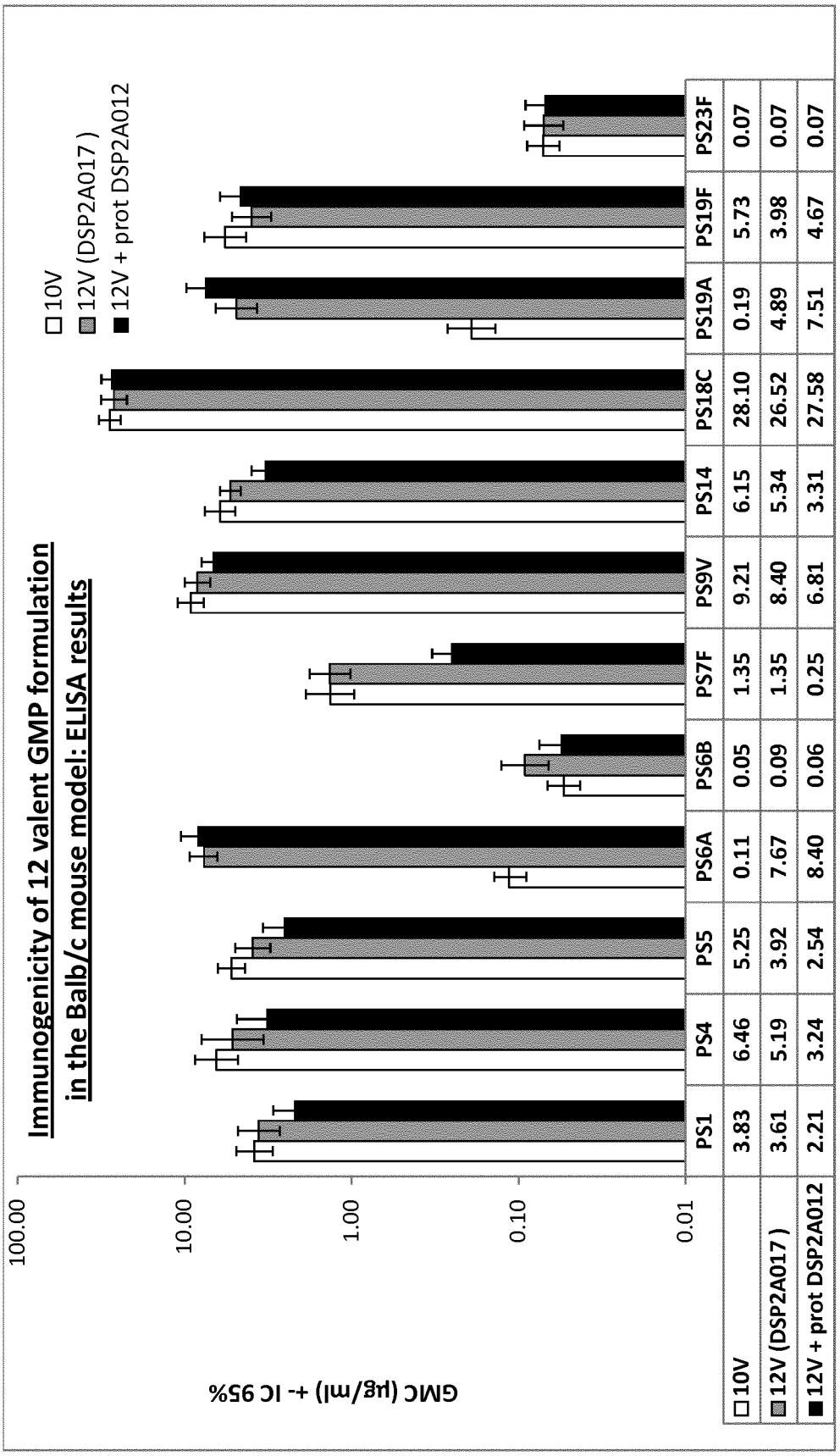

Nasopharynx mouse colonization model with NTHi strain 3224A
log10 CFU/ml
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
*
PE-PilAΔQ LVL735 (5.8 µg/m)
PE-PilA LVL 317 (5.8 µg/m)
LT (0.5µg/m)
d1
d2

Figure 29


Immunogenicity of 12 valent GMP formulation
in the Balb/c mouse model: ELISA results
10V
12V (DSP2A017)
12V + prot DSP2A012
GMC (µg/ml) +- IC 95%
100.00
10.00
1.00
0.10
0.01
PS1 PS4 PS5 PS6A PS6B PS7F PS9V PS14 PS18C PS19A PS19F PS23F
10V 3.83 6.46 5.25 0.11 0.05 1.35 9.21 6.15 28.10 0.19 5.73 0.07
12V (DSP2A017) 3.61 5.19 3.92 7.67 0.09 1.35 8.40 5.34 26.52 4.89 3.98 0.07
12V + prot DSP2A012 2.21 3.24 2.54 8.40 0.06 0.25 6.81 3.31 27.58 7.51 4.67 0.07

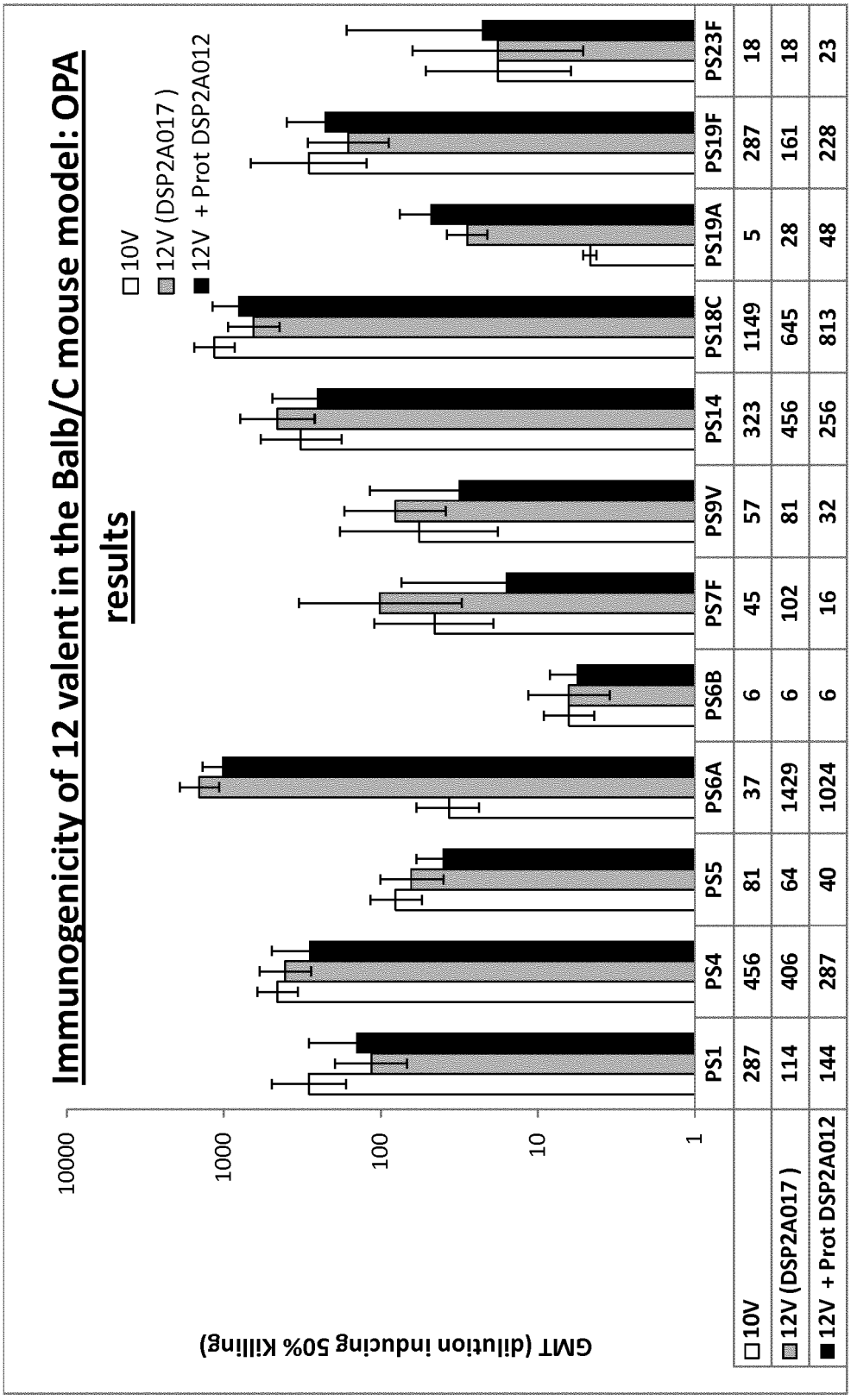

Immunogenicity of 12 valent in the Balb/C mouse model: OPA
results
10V
12V (DSP2A017)
12V + Prot DSP2A012
GMT (dilution inducing 50% Killing)
10000
1000
100
10
1
PS1 PS4 PS5 PS6A PS6B PS7F PS9V PS14 PS18C PS19A PS19F PS23F
10V 287 456 81 37 6 45 57 323 1149 5 287 18
12V (DSP2A017) 114 406 64 1429 6 102 81 456 645 28 161 18
12V + Prot DSP2A012 144 287 40 1024 6 16 32 256 813 48 228 23

Figure 30

Figure 31

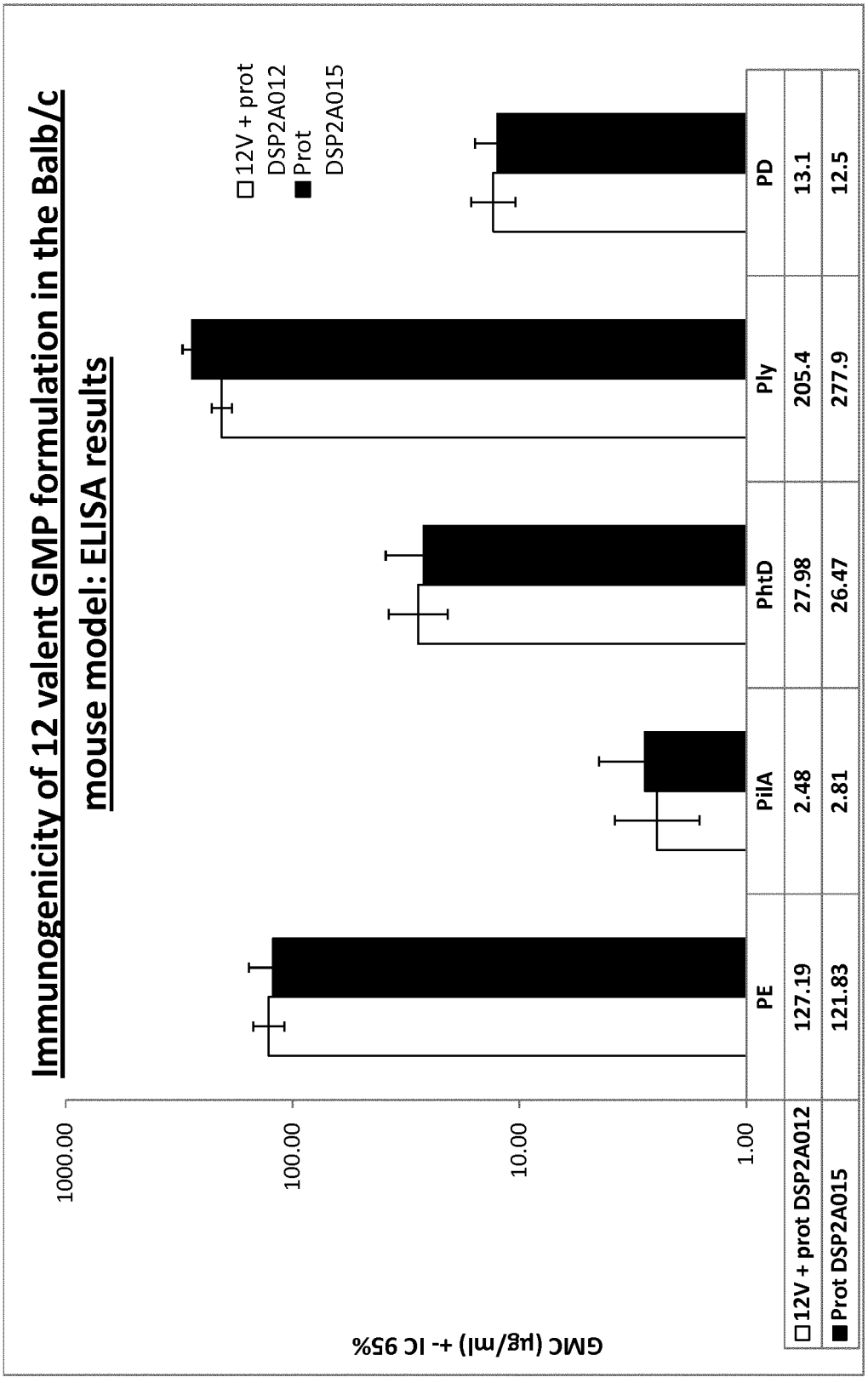
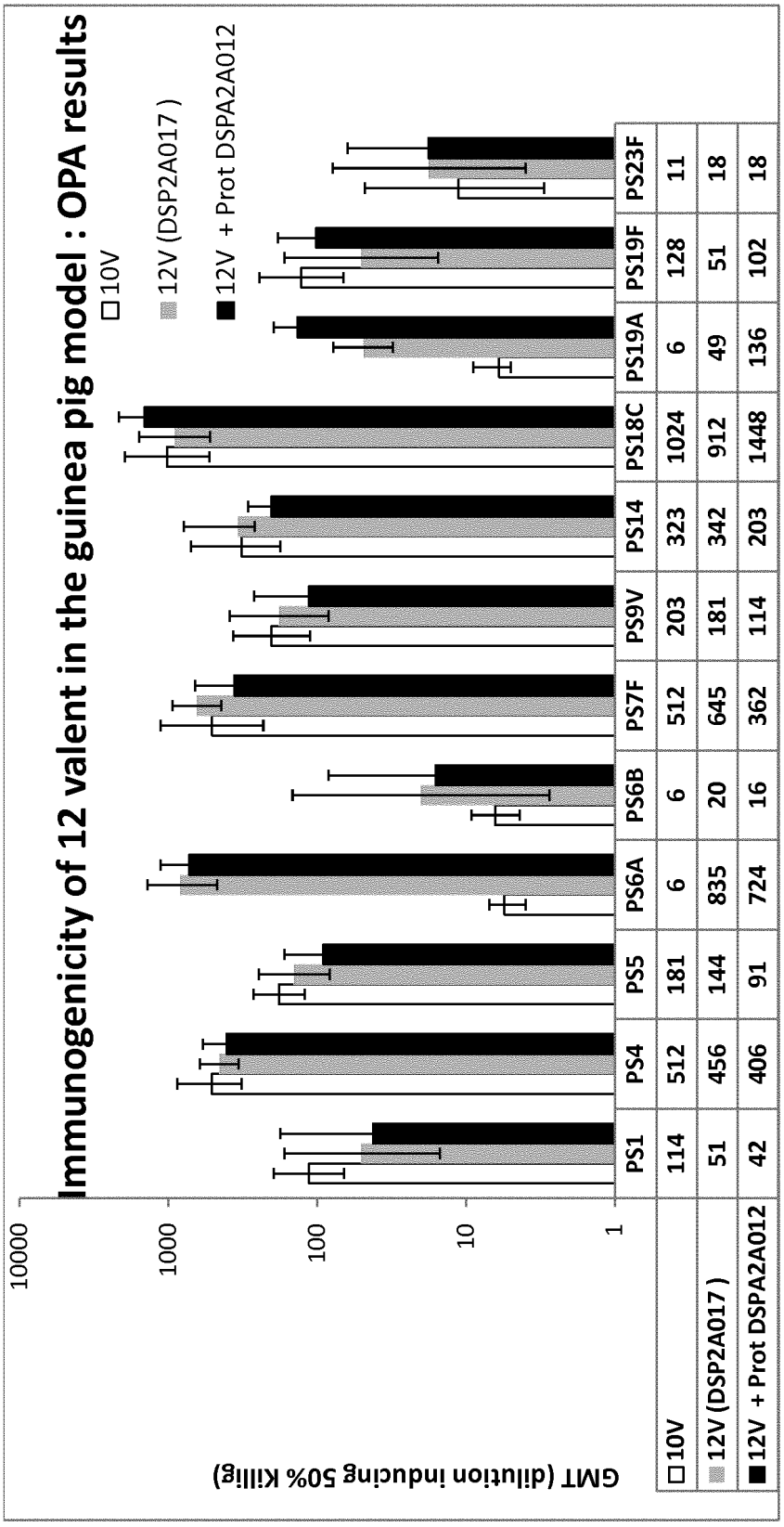

Immunogenicity of 12 valent GMP formulation in the Balb/c mouse model: ELISA results
GMC (µg/ml) +- IC 95%
1000.00
100.00
10.00
1.00
12V + prot DSP2A012
Prot DSP2A015
PE 127.19 121.83
PilA 2.48 2.81
PhtD 27.98 26.47
Ply 205.4 277.9
PD 13.1 12.5

Figure 32


Immunogenicity of 12 valent in the guinea pig model : OPA results
10V
12V (DSP2A017)
12V + Prot DSPA2A012
GMT (dilution inducing 50% killig)
10000
1000
100
10
1
PS1 PS4 PS5 PS6A PS6B PS7F PS9V PS14 PS18C PS19A PS19F PS23F
10V 114 512 181 6 6 512 203 323 1024 6 128 11
12V (DSP2A017) 51 456 144 835 20 645 181 342 912 49 51 18
12V + Prot DSPA2A012 42 406 91 724 16 362 114 203 1448 136 102 18

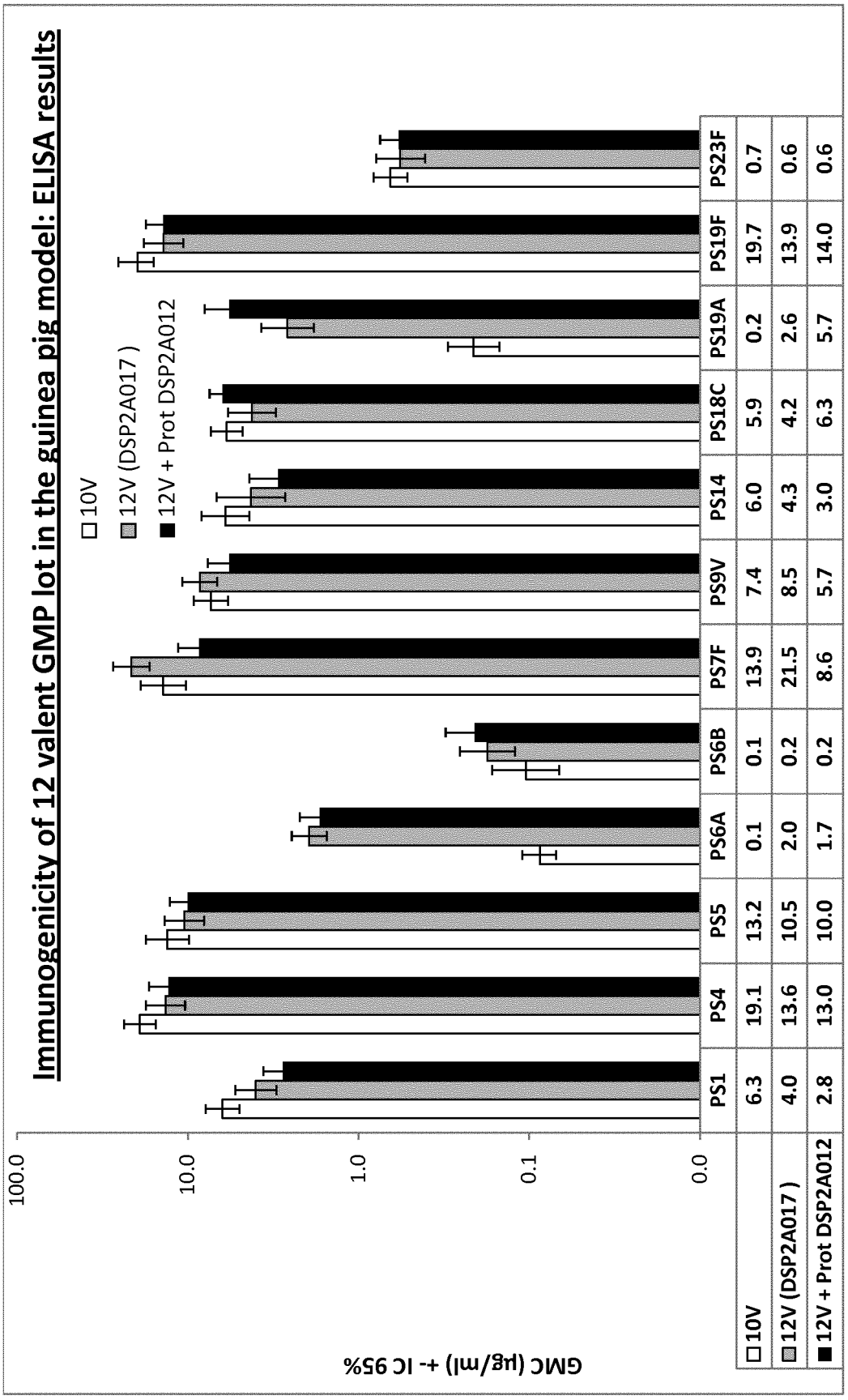

Immunogenicity of 12 valent GMP lot in the guinea pig model: ELISA results
10V
12V (DSP2A017)
12V + Prot DSP2A012
GMC (µg/ml) +- IC 95%
100.0
10.0
1.0
0.1
0.0
PS1 PS4 PS5 PS6A PS6B PS7F PS9V PS14 PS18C PS19A PS19F PS23F
10V 6.3 19.1 13.2 0.1 0.1 13.9 7.4 6.0 5.9 0.2 19.7 0.7
12V (DSP2A017) 4.0 13.6 10.5 2.0 0.2 21.5 8.5 4.3 4.2 2.6 13.9 0.6
12V + Prot DSP2A012 2.8 13.0 10.0 1.7 0.2 8.6 5.7 3.0 6.3 5.7 14.0 0.6

Figure 33

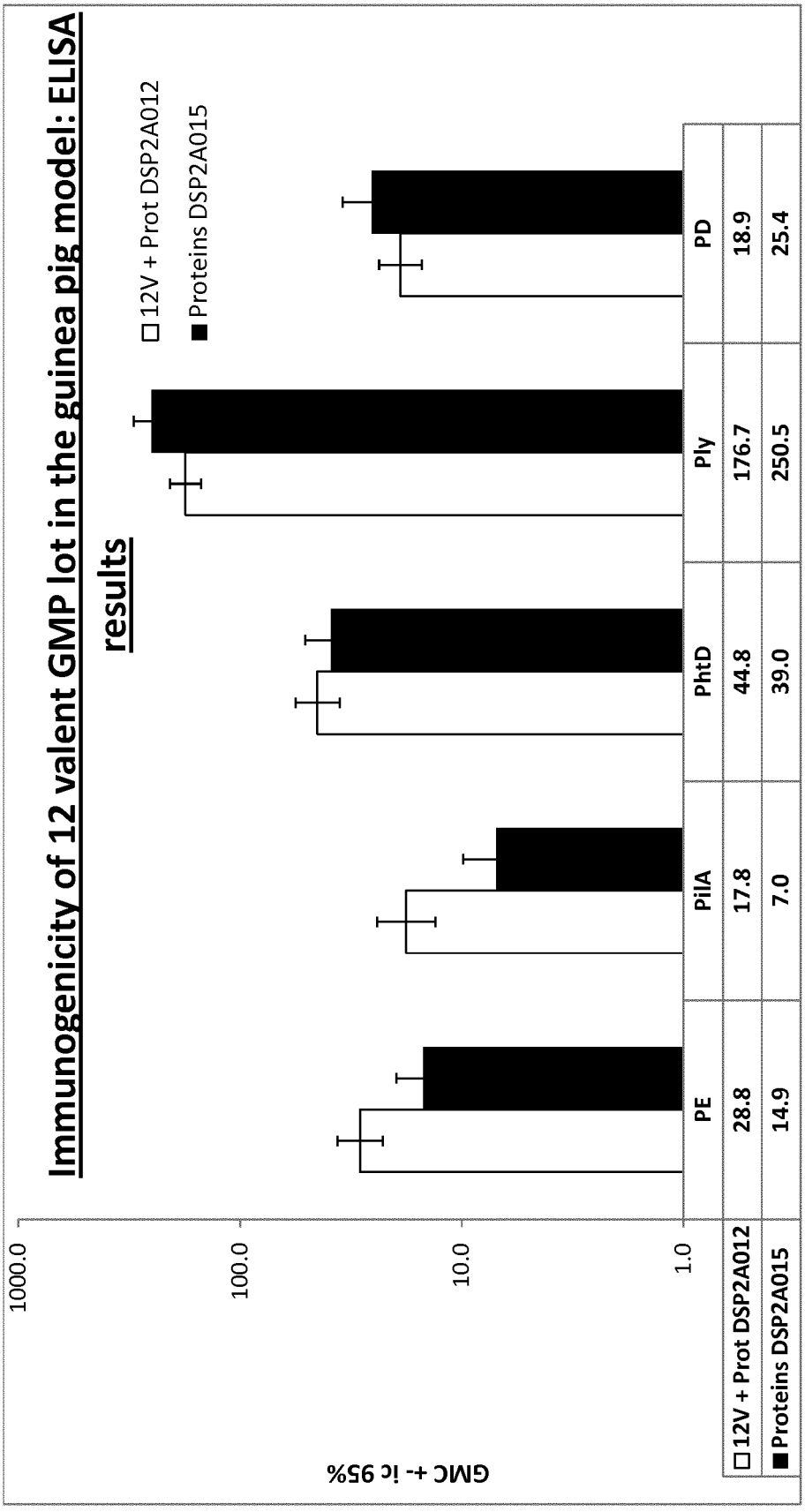

Immunogenicity of 12 valent GMP lot in the guinea pig model: ELISA
results
12V + Prot DSP2A012
Proteins DSP2A015
GMC +- ic 95%
1000.0
100.0
10.0
1.0
PE
PilA
PhtD
Ply
PD
12V + Prot DSP2A012 28.8 17.8 44.8 176.7 18.9
Proteins DSP2A015 14.9 7.0 39.0 250.5 25.4

Figure 34