Figure 1

5

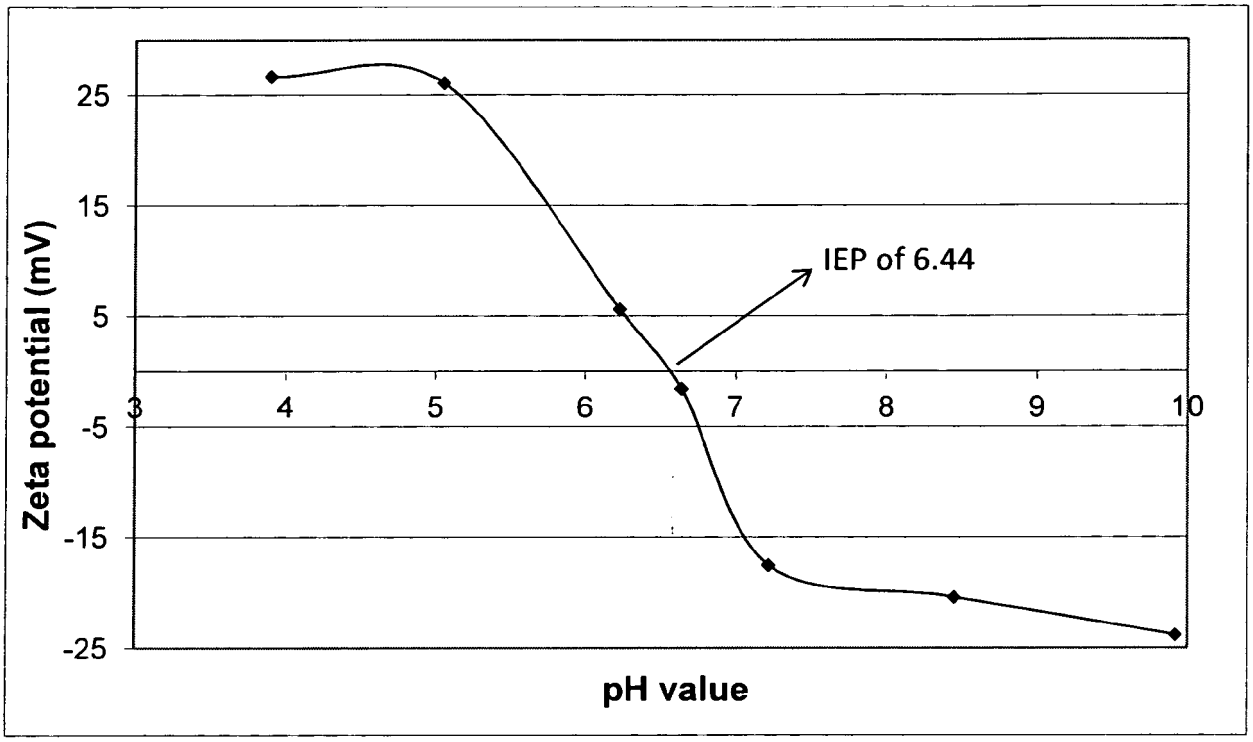
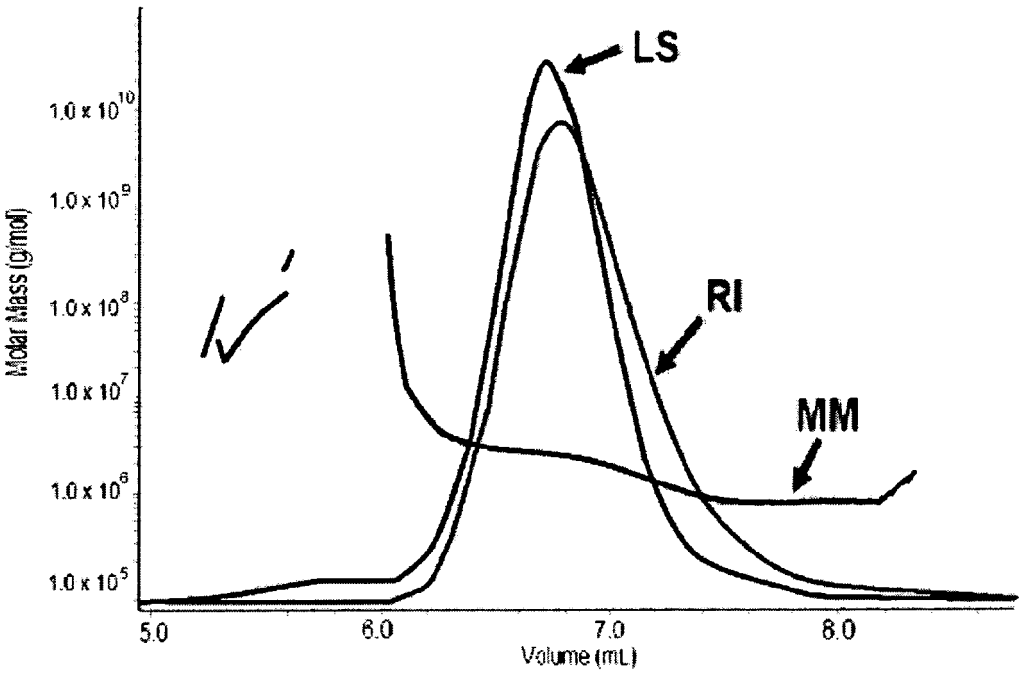


Figure 2


LS
RI
MM
Molar Mass (g/mol)
1.0 x 10^10
1.0 x 10^9
1.0 x 10^8
1.0 x 10^7
1.0 x 10^6
1.0 x 10^5
5.0
6.0
7.0
8.0
Volume (mL)

5

Figure 3

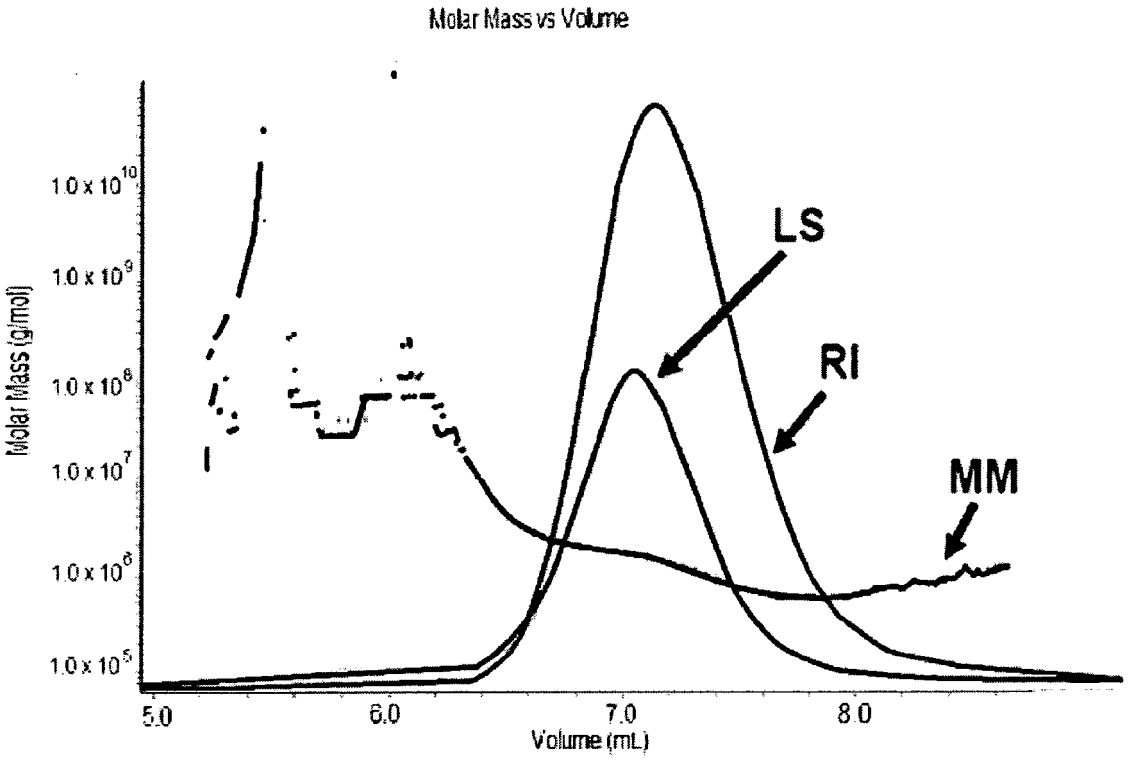

Molar Mass vs Volume
Molar Mass (g/mol)
1.0 x 10^10
1.0 x 10^9
1.0 x 10^8
1.0 x 10^7
1.0 x 10^6
1.0 x 10^5
5.0
6.0
7.0
8.0
Volume (mL)
LS
RI
MM

Figure 4

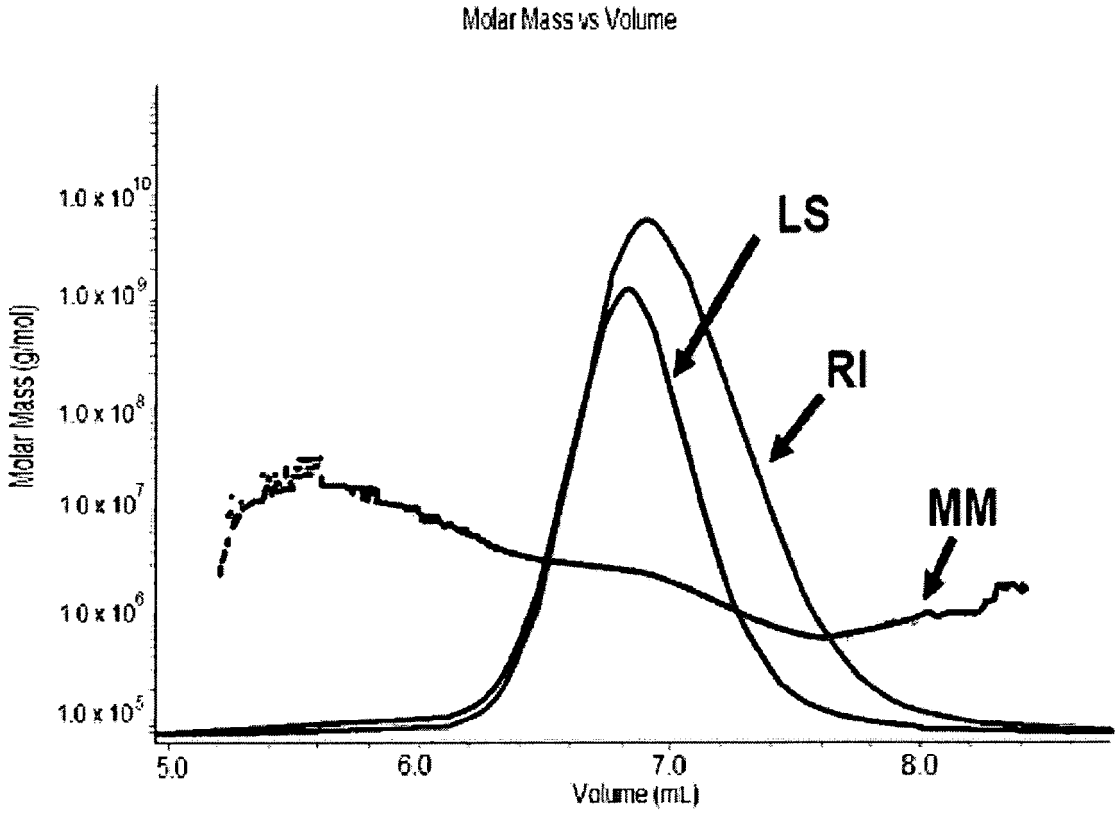

Molar Mass vs Volume
Molar Mass (g/mol)
1.0 x 10^10
1.0 x 10^9
1.0 x 10^8
1.0 x 10^7
1.0 x 10^6
1.0 x 10^5
LS
RI
MM
5.0
6.0
7.0
8.0
Volume (mL)

Figure 5

5

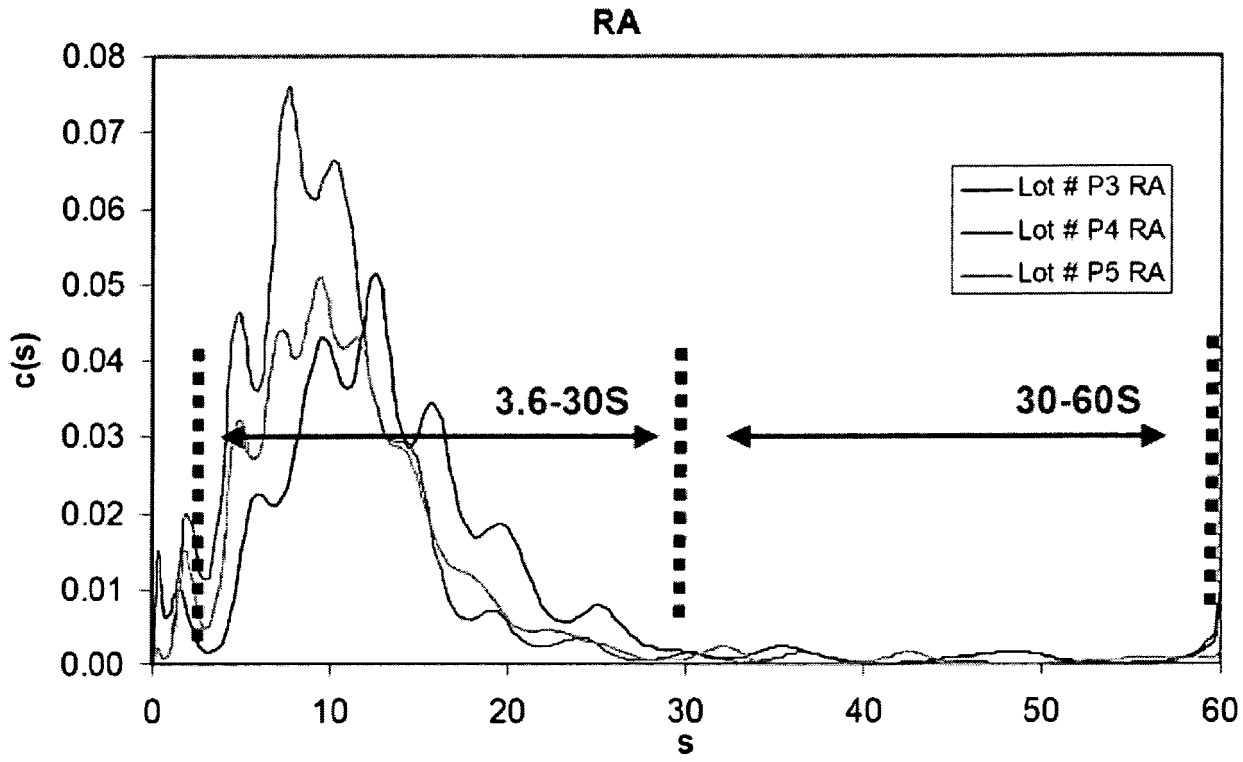
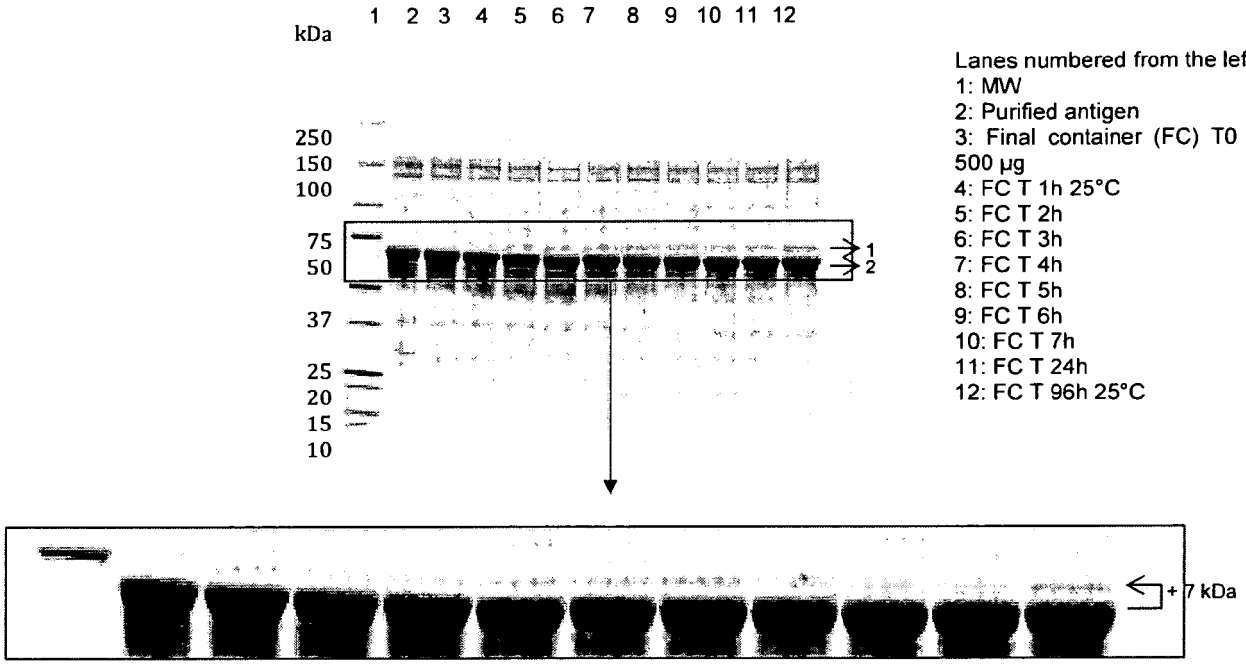

RA
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
c(s)
0
10
20
30
40
50
60
s
3.6-30S
30-60S
Lot # P3 RA
Lot # P4 RA
Lot # P5 RA

Figure 6



	PB	FC T0	T 1h	T 2h	T 3h	T 4h	T 5h	T 6h	T 7h	T 24h	T 96h
Band 1	0%	1.2%	1.3%	1.4%	1.4%	1.7%	2.0%	2.1%	1.6%	2.5%	3.6%
Band 2	100%	98.8%	98.7%	98.6%	98.6%	98.3%	98.0%	97.9%	98.4%	97.5%	96.4%

Figure 7

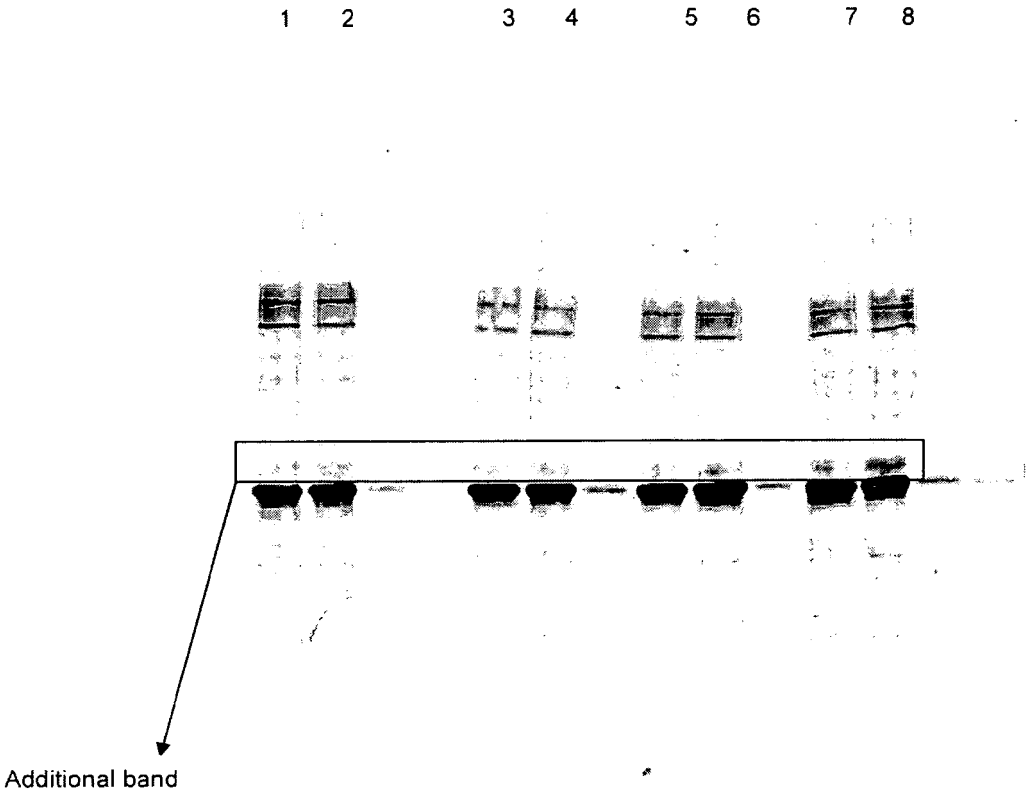


Figure 8

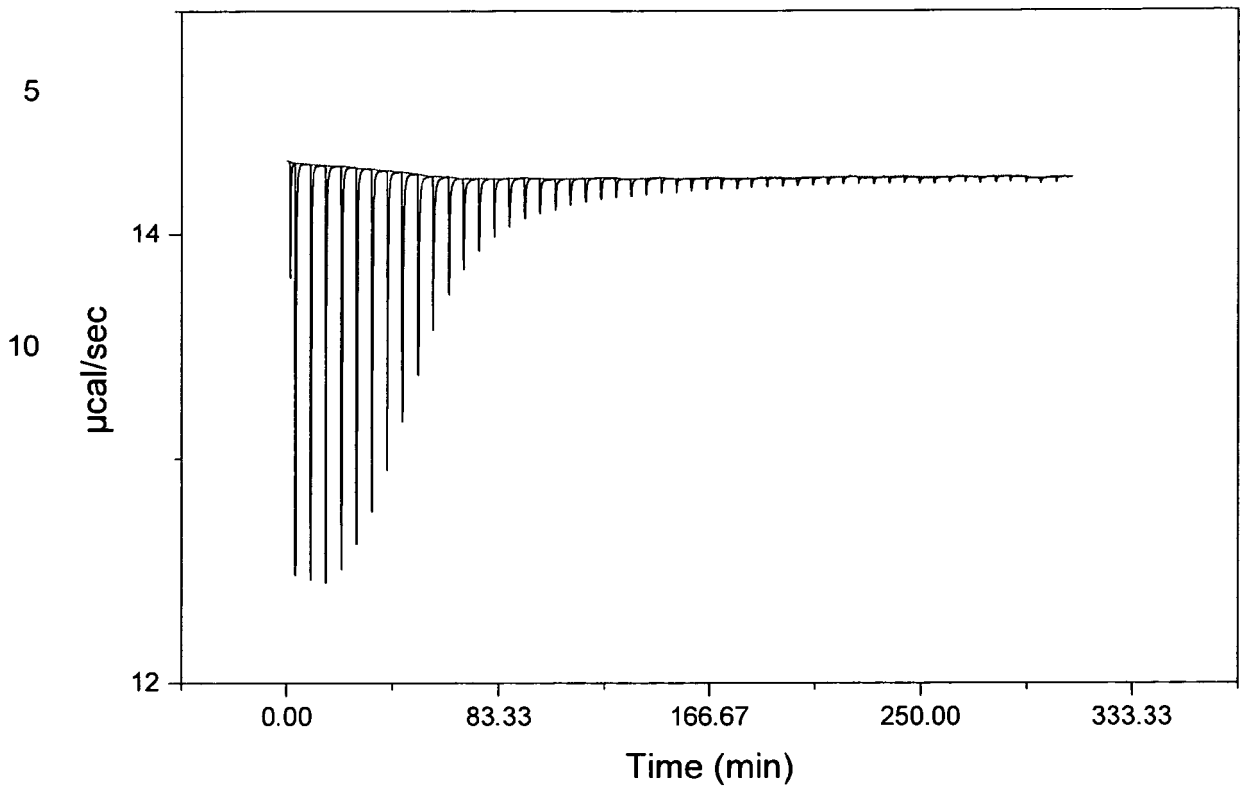
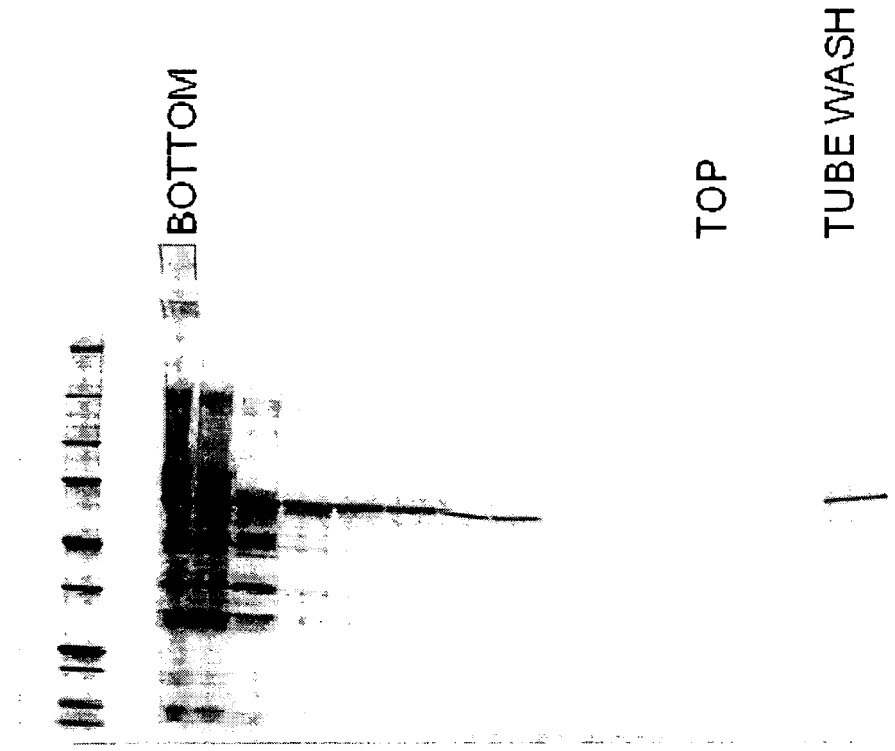


Figure 9


BOTTOM
TOP
TUBE WASH

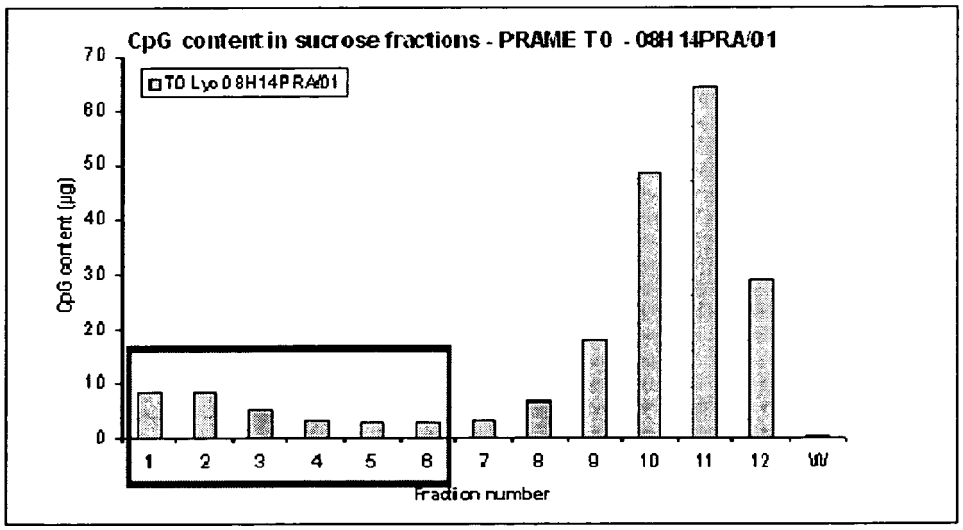

CpG content in sucrose fractions - PRAME T0 - 08H14PRA/01
T0 Lyo 08H14PRA/01
CpG content (µg)
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
W
Fraction number

Figure 10

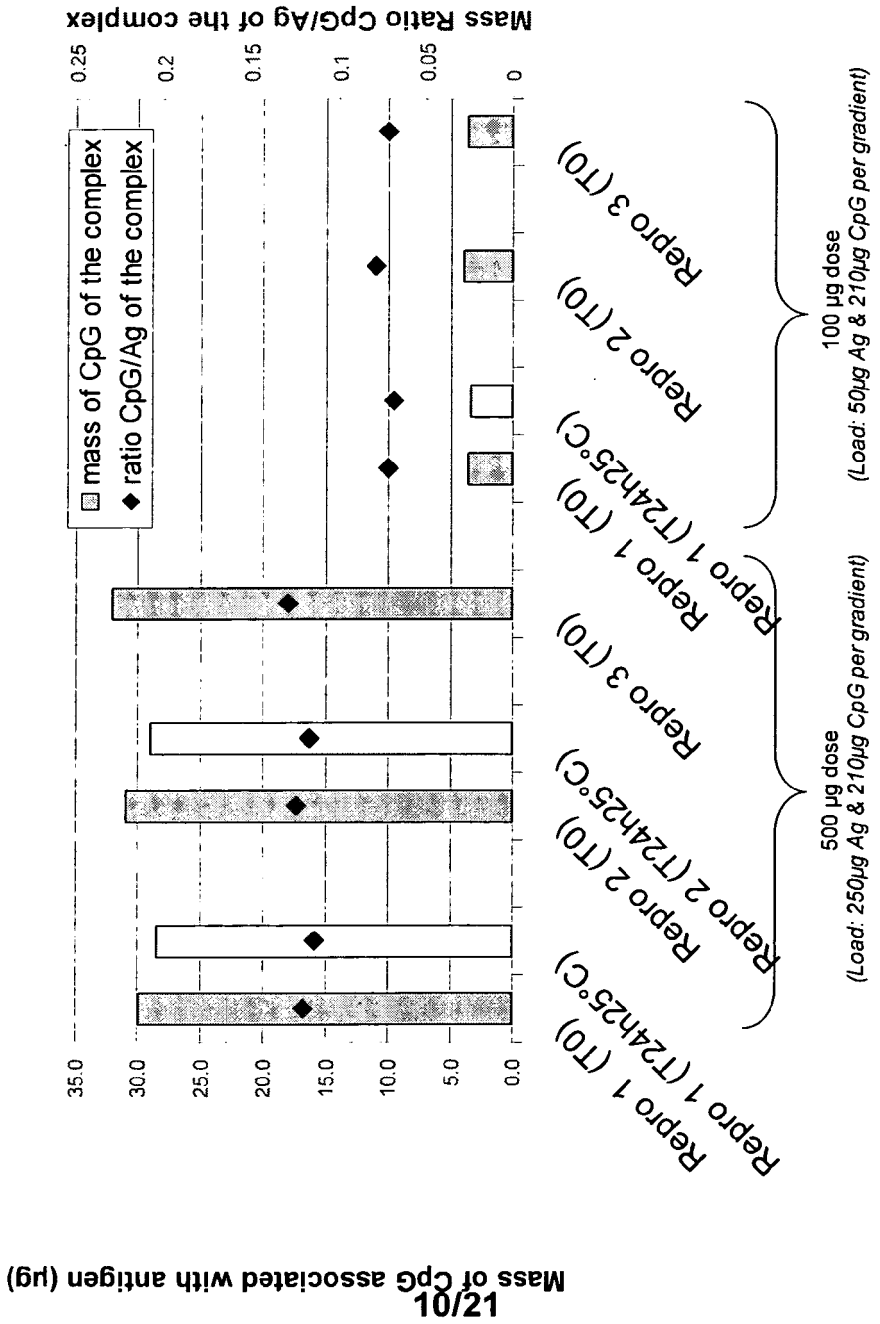

Mass Ratio CpG/Ag of the complex
0.25
0.2
0.15
0.1
0.05
0
mass of CpG of the complex
ratio CpG/Ag of the complex
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Mass of CpG associated with antigen (µg)
Repro 1 (T0)
Repro 1 (T24h25°C)
Repro 2 (T0)
Repro 2 (T24h25°C)
Repro 3 (T0)
Repro 1 (T0)
Repro 1 (T24h25°C)
Repro 2 (T0)
Repro 3 (T0)
500 µg dose
(Load: 250µg Ag & 210µg CpG per gradient)
100 µg dose
(Load: 50µg Ag & 210µg CpG per gradient)

Figure 11

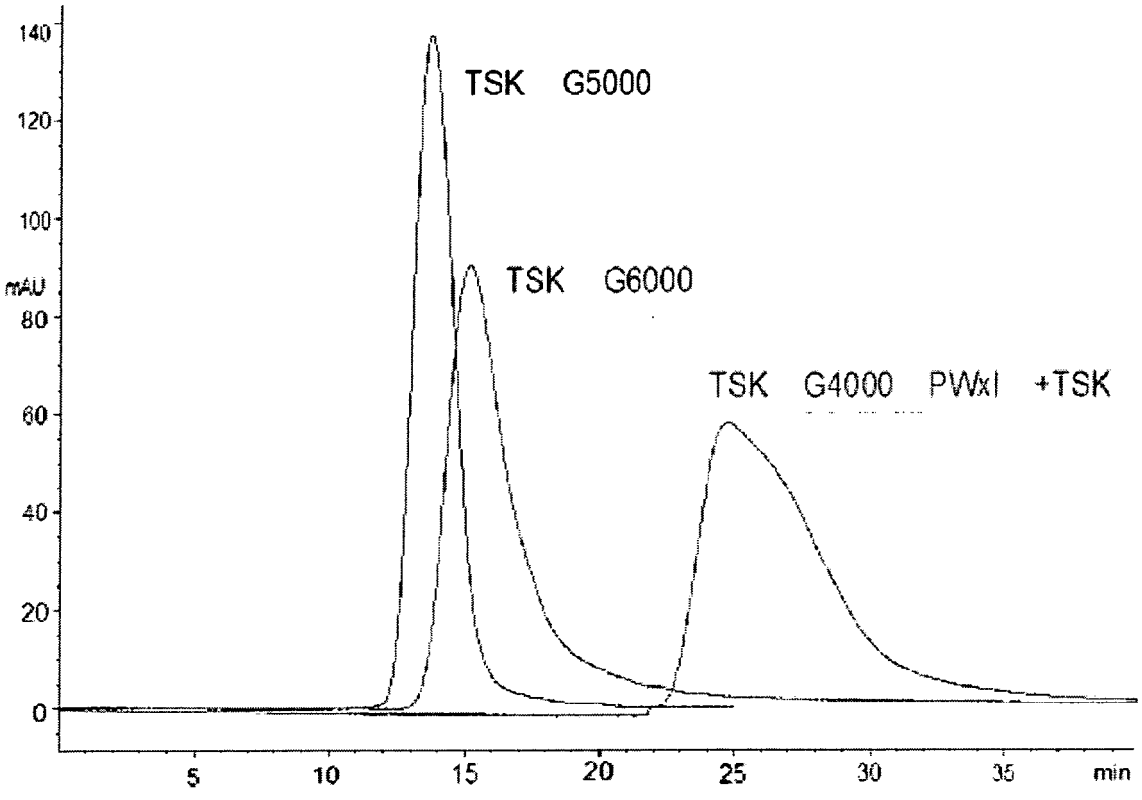

140
120
100
mAU
80
60
40
20
0
TSK G5000
TSK G6000
TSK G4000 PWxl +TSK
5
10
15
20
25
30
35
min

Figure 12

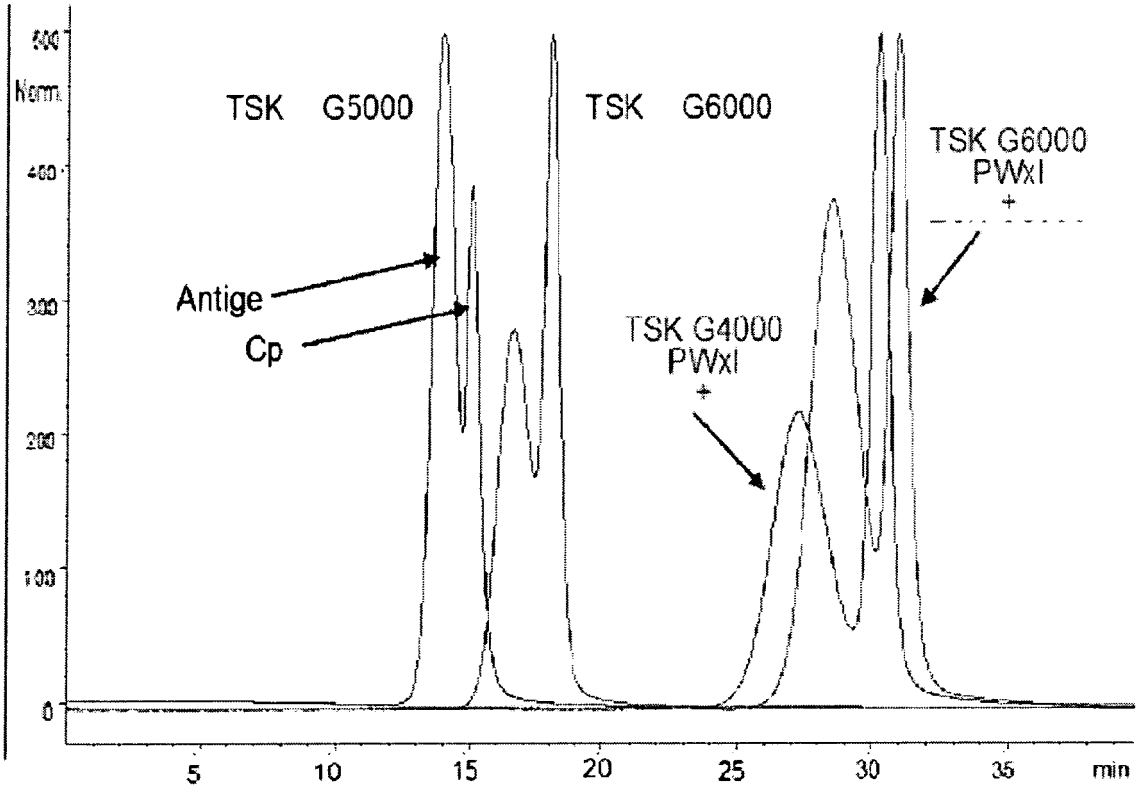

Norm.
TSK G5000
TSK G6000
TSK G6000
PWxl
+
Antige
Cp
TSK G4000
PWxl
+
0
100
200
300
400
5
10
15
20
25
30
35
min

Figure 13

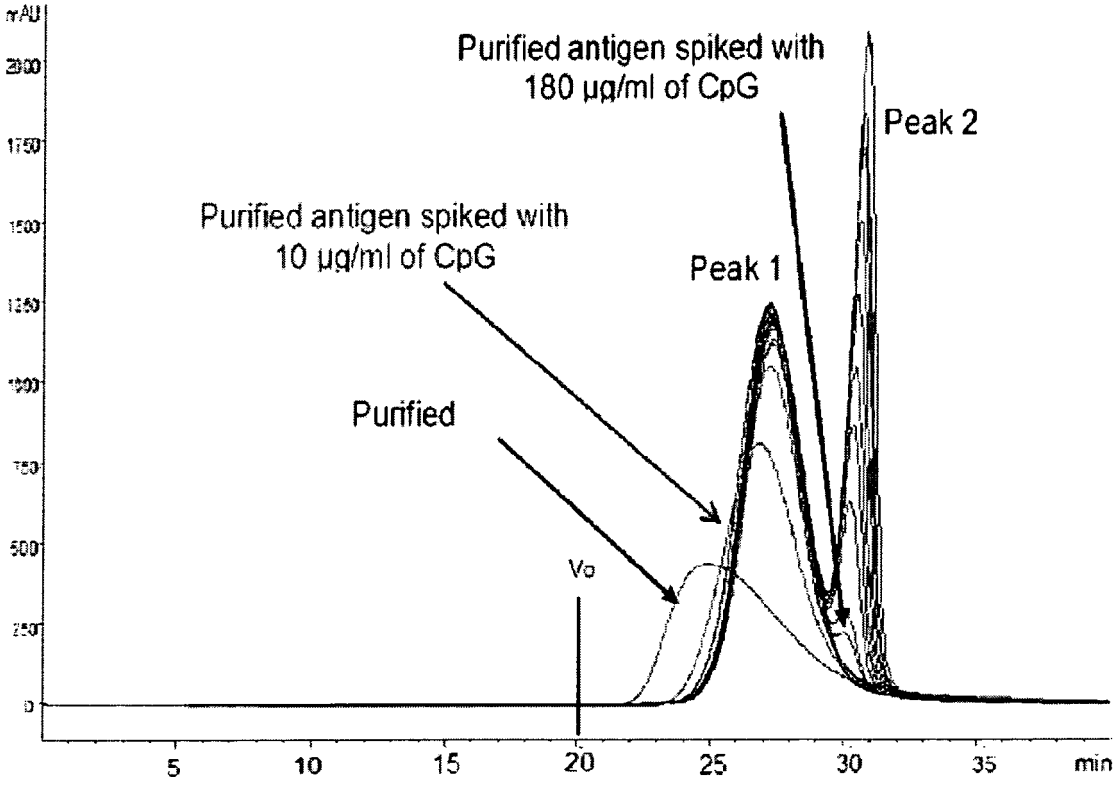

mAU
2000
1750
1500
1250
1000
750
500
250
0
Purified antigen spiked with
180 µg/ml of CpG
Peak 2
Purified antigen spiked with
10 µg/ml of CpG
Peak 1
Purified
Vo
5
10
15
20
25
30
35
min

Figure 14

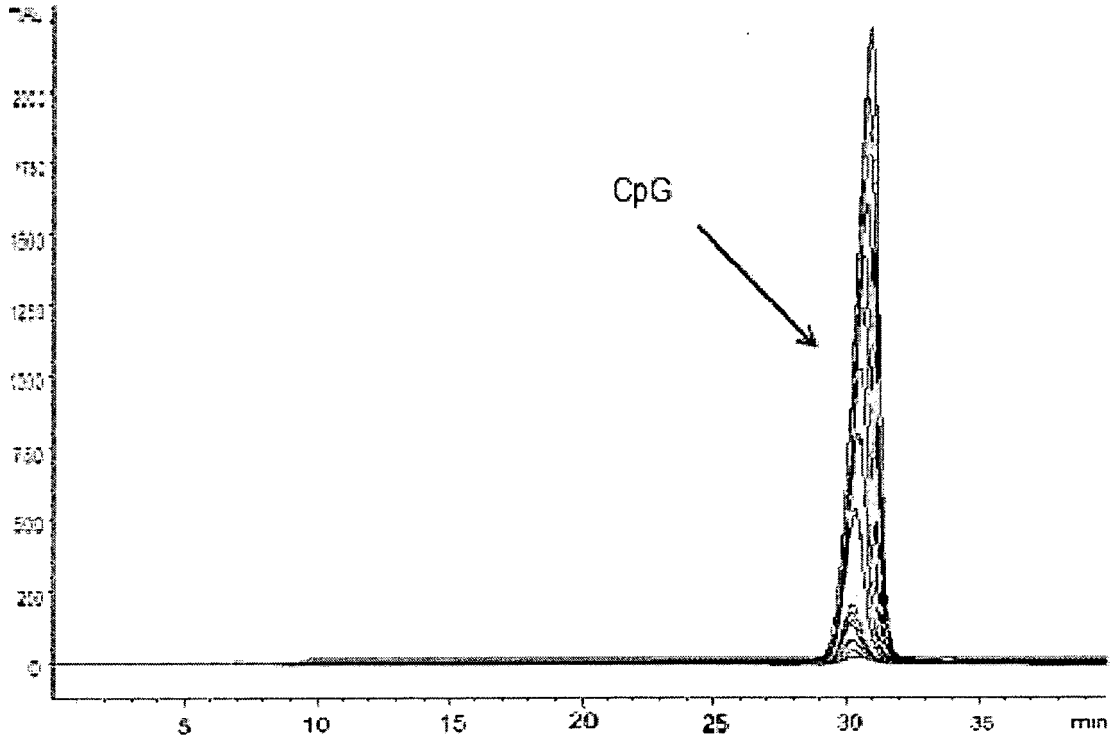
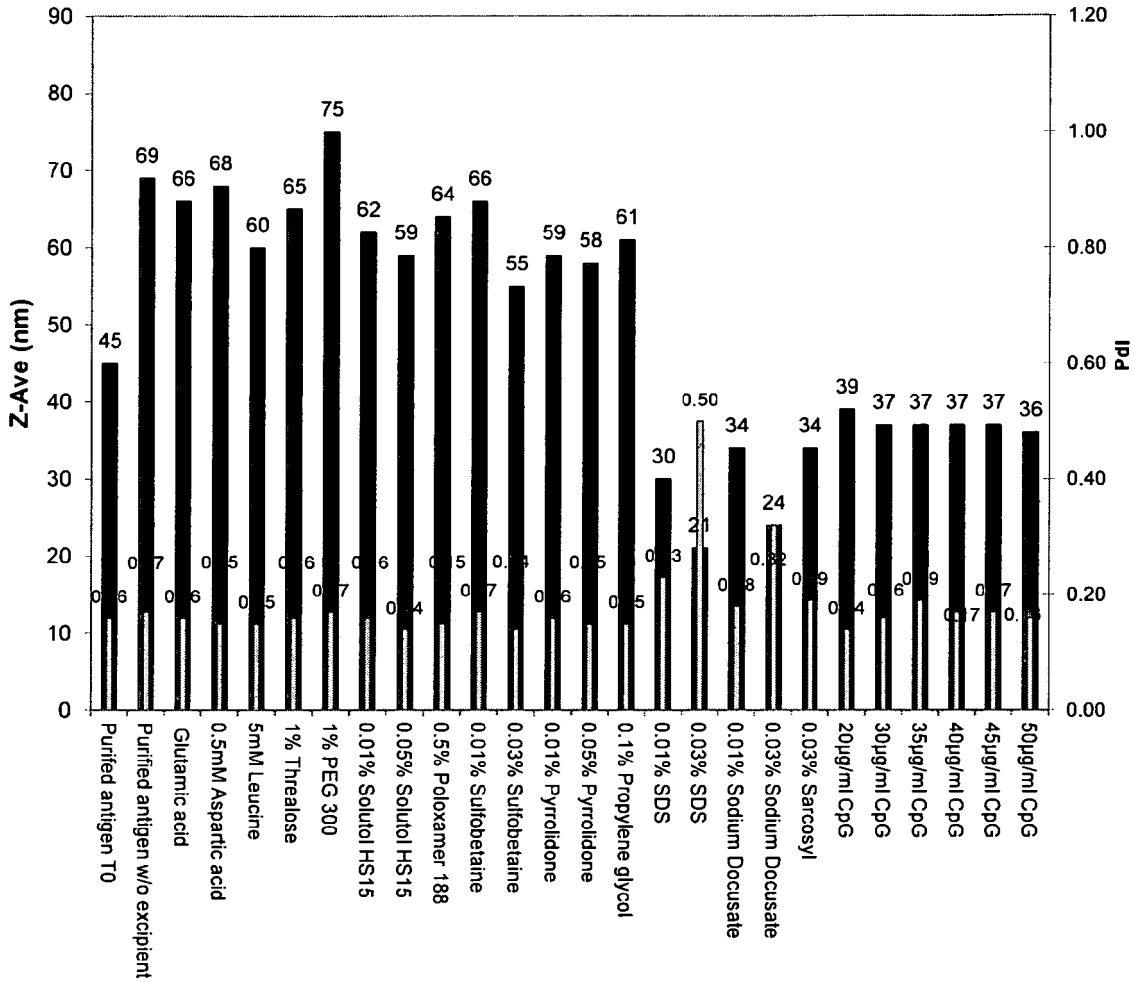


Figure 15


Z-Ave (nm)
PdI
90
80
70
60
50
40
30
20
10
0
1.20
1.00
0.80
0.60
0.40
0.20
0.00
45
69
66
68
60
65
75
62
59
64
66
55
59
58
61
30
0.50
34
24
34
39
37
37
37
37
36
Purified antigen T0
Purified antigen w/o excipient
Glutamic acid
0.5mM Aspartic acid
5mM Leucine
1% Threalose
1% PEG 300
0.01% Solutol HS15
0.05% Solutol HS15
0.5% Poloxamer 188
0.01% Sulfobetaine
0.03% Sulfobetaine
0.01% Pyrrolidone
0.05% Pyrrolidone
0.1% Propylene glycol
0.01% SDS
0.03% SDS
0.01% Sodium Docusate
0.03% Sodium Docusate
0.03% Sarcosyl
20µg/ml CpG
30µg/ml CpG
35µg/ml CpG
40µg/ml CpG
45µg/ml CpG
50µg/ml CpG

5

Figure 16

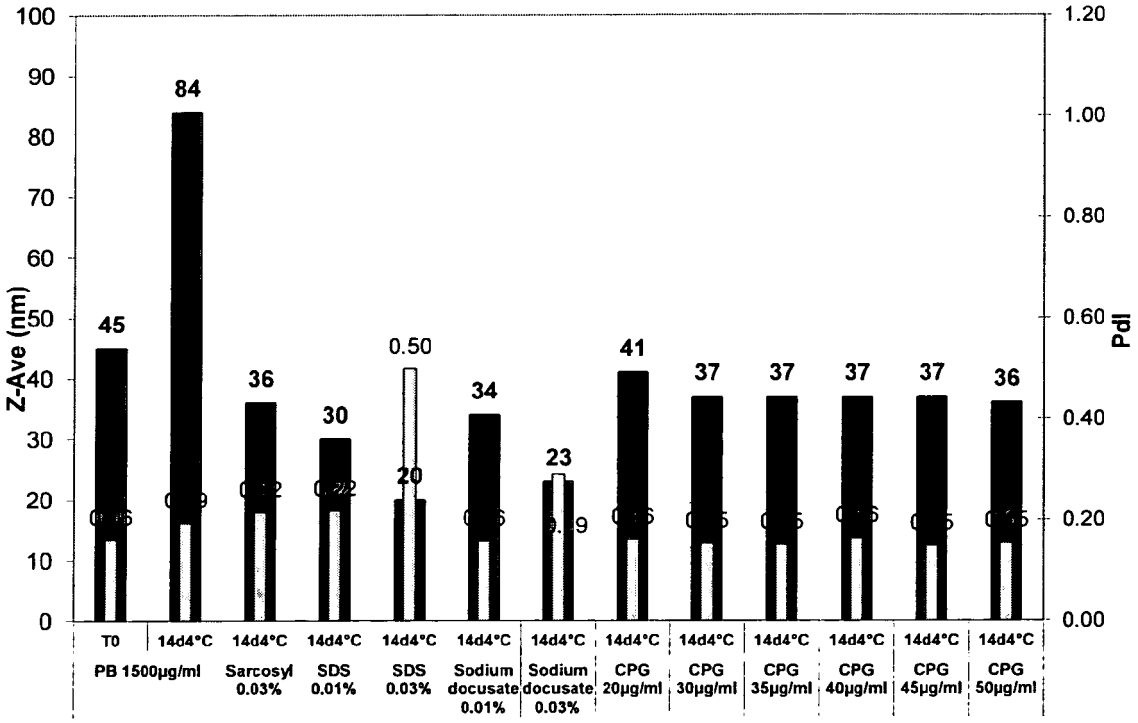
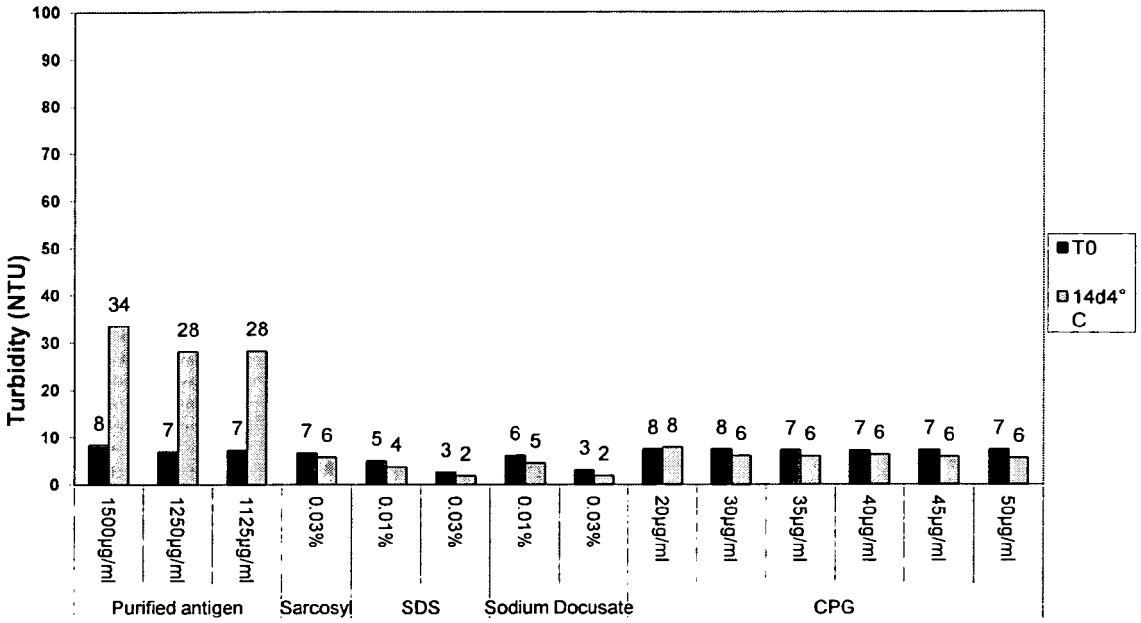
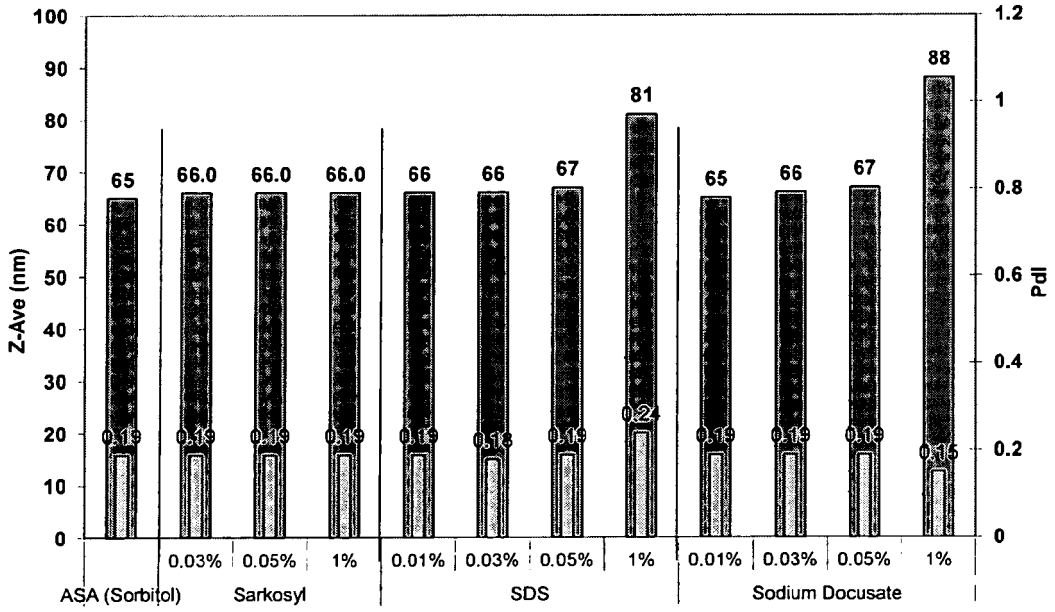

Z-Ave (nm)
PdI
100
90
80
70
60
50
40
30
20
10
0
1.20
1.00
0.80
0.60
0.40
0.20
0.00
45
84
36
30
0.50
20
34
23
41
37
37
37
37
36
T0
14d4°C
PB 1500µg/ml
Sarcosyl 0.03%
SDS 0.01%
SDS 0.03%
Sodium docusate 0.01%
Sodium docusate 0.03%
CPG 20µg/ml
CPG 30µg/ml
CPG 35µg/ml
CPG 40µg/ml
CPG 45µg/ml
CPG 50µg/ml

Figure 17


Turbidity (NTU)
100
90
80
70
60
50
40
30
20
10
0
T0
14d4° C
8 34
7 28
7 28
7 6
5 4
3 2
6 5
3 2
8 8
8 6
7 6
7 6
7 6
7 6
1500µg/ml
1250µg/ml
1125µg/ml
0.03%
0.01%
0.03%
0.01%
0.03%
20µg/ml
30µg/ml
35µg/ml
40µg/ml
45µg/ml
50µg/ml
Purified antigen
Sarcosyl
SDS
Sodium Docusate
CPG

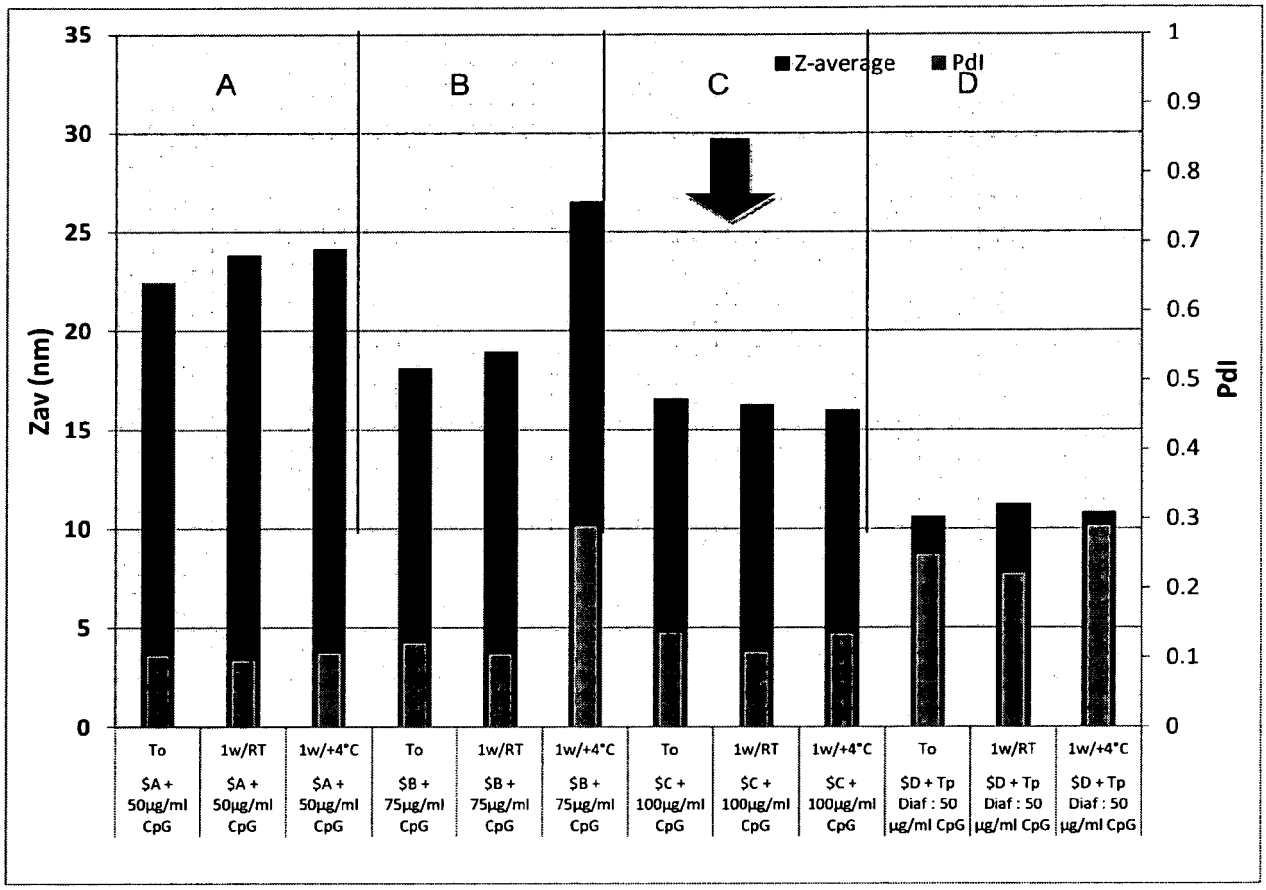
5

Figure 18


Z-Ave (nm)
PdI
100
90
80
70
60
50
40
30
20
10
0
1.2
1
0.8
0.6
0.4
0.2
0
65
66.0
66.0
66.0
66
66
67
81
65
66
67
88
0.19
0.19
0.19
0.19
0.19
0.18
0.19
0.24
0.19
0.19
0.19
0.15
0.03%
0.05%
1%
0.01%
0.03%
0.05%
1%
0.01%
0.03%
0.05%
1%
ASA (Sorbitol)
Sarkosyl
SDS
Sodium Docusate

5

Figure 19


A
B
C
D
Z-average
PdI
Zav (nm)
PdI
35
30
25
20
15
10
5
0
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
To $A + 50µg/ml CpG
1w/RT $A + 50µg/ml CpG
1w/+4°C $A + 50µg/ml CpG
To $B + 75µg/ml CpG
1w/RT $B + 75µg/ml CpG
1w/+4°C $B + 75µg/ml CpG
To $C + 100µg/ml CpG
1w/RT $C + 100µg/ml CpG
1w/+4°C $C + 100µg/ml CpG
To $D + Tp Diaf : 50 µg/ml CpG
1w/RT $D + Tp Diaf : 50 µg/ml CpG
1w/+4°C $D + Tp Diaf : 50 µg/ml CpG

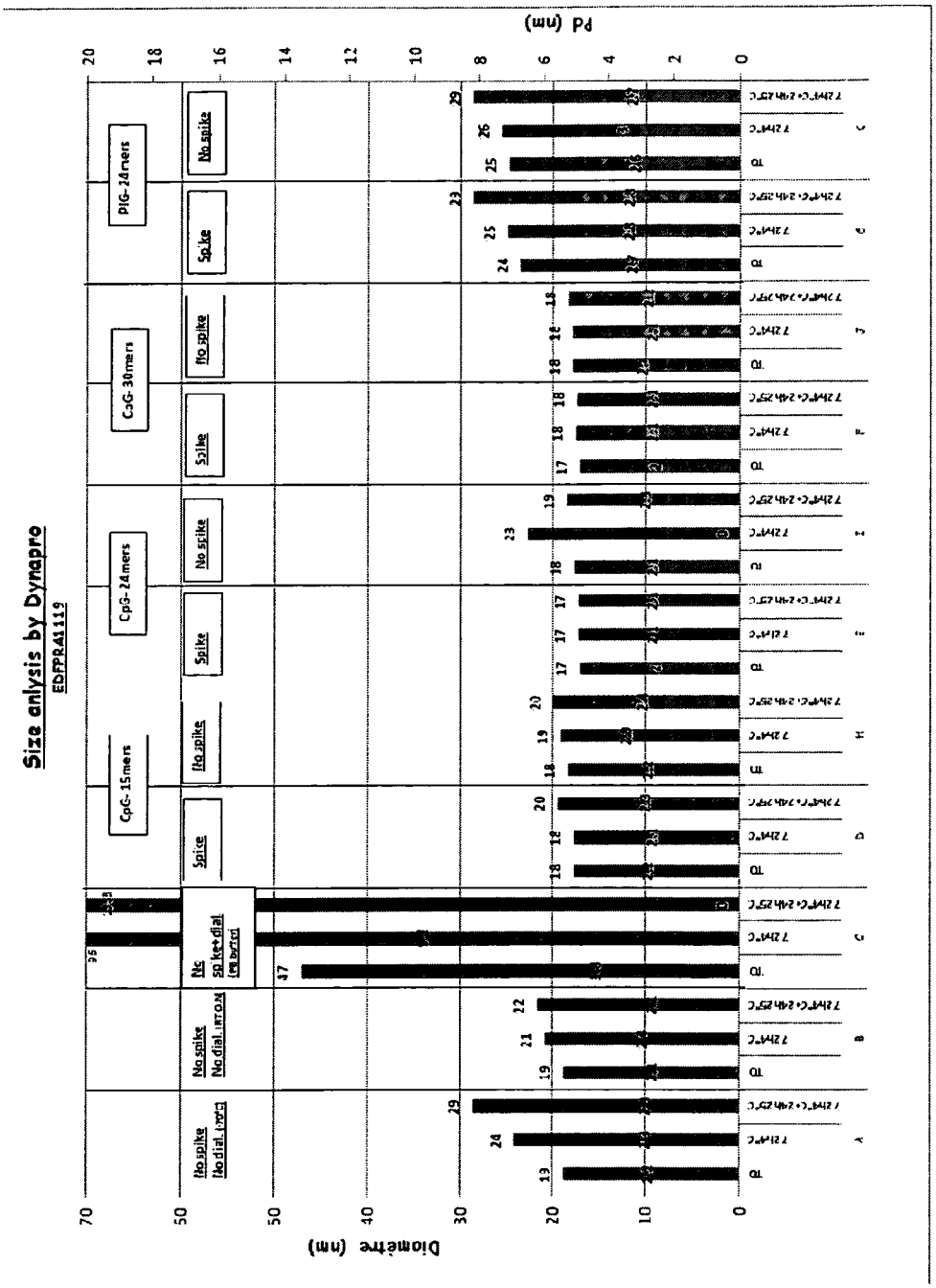
5

Figure 20





Figure 21


Size anlysis by Dynapro
EDFPRA1119
Diometre (nm)
Pd (nm)
No spike
No dial. water
Spike
CpG-15mers
No spike
Spike
CpG-24mers
No spike
Spike
CpG-30mers
No spike
Spike
PIG-24mers
No spike

INTERNATIONAL SEARCH REPORT

WO 2013/014105

International application No
PCT/EPCT/EP2012/064340

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/035360 A1 (LEMOINE DOMINIQUE INGRID [BE]) 5 February 2009 (2009-02-05)	1,2,7, 10-14, 16-21,26
Y	the whole document paragraphs [0053], [0183], [0184]; claims; figure 9; example 3; sequences 1-5	3-6,8,9, 15, 22-25, 27,28

X	WO 2008/087102 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; BLAIS NORMAND [CA]; MARTIN DENIS [CA];) 24 July 2008 (2008-07-24) cited in the application	18-21
Y	the whole document pages 28-30; claims; examples; sequences 36-40	3-6,8,9, 15, 22-25, 27,28

	-/--	

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[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2012	10/09/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Madruga, Jaime

1

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/142686 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; HENDERICKX VERONIQUE [BE]; LEMOINE DOM) 16 December 2010 (2010-12-16) the whole document example 1	18-21
A	US 5 830 753 A (COULIE PIERRE [BE] ET AL) 3 November 1998 (1998-11-03) cited in the application the whole document	1-28
A	IKEDA HIDEYUKI ET AL: "Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor", IMMUNITY, CELL PRESS, US, vol. 6, no. 2, 1 February 1997 (1997-02-01), pages 199-208, XP002216277, ISSN: 1074-7613, DOI: 10.1016/S1074-7613(00)80426-4 cited in the application the whole document	1-28

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

WO 2013/014105

International application No
PCT/EPCT/EP2012/064340

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