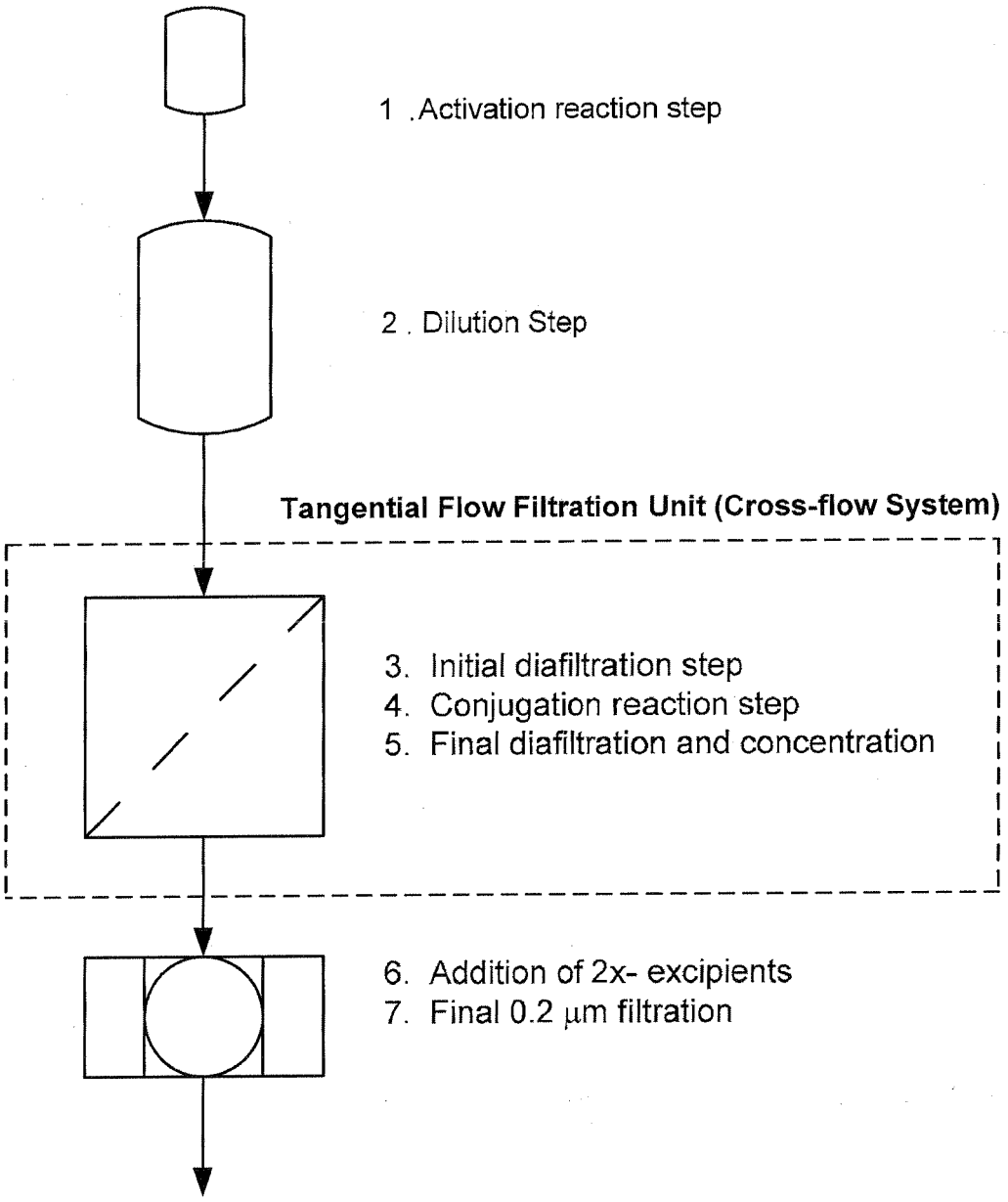

1 .Activation reaction step
2 . Dilution Step
Tangential Flow Filtration Unit (Cross-flow System)
3. Initial diafiltration step
4. Conjugation reaction step
5. Final diafiltration and concentration
6. Addition of 2x- excipients
7. Final 0.2 μm filtration

Figure 1

Run	Amount	Linker:KLH (molar ratio)	Activation Buffer	KLH (mg/mL)	Linker Stock (mg/mL)	Linker Solution	Reaction time (min)
121161-090	100	357	100 mM Na_2PO_4 pH 7.2	10	100	DMSO	45
121161-093	100	100	25 mM Na_2PO_4 pH 7.2		4.4	Water	
121161-096	120	200			8.7		
121161-100	92.5	357	40 mM Na_2PO_4 pH 7.2		180	DMSO	
121161-120	100	200			100		
121161-125	160		10 mM Na_2PO_4 pH 7.2	15			65
121161-130	160	250	40 mM Na_2PO_4 pH 7.2	10			45
121161-147	160	200	10 mM Na_2PO_4 pH 7.2	15			65
121161-165	160		40 mM Na_2PO_4 pH 7.2	10			45
121161-172	1000						

Figure 2A: Summary of key operating parameters – activation reaction

Run	MWCO (kD)	Diafiltration Buffer	Activated KLH (mg/mL)	TMP (bar)	Retentate Flow Rate (mL/min)	Flux rate	Hold Step (min)
121161-090	100	PBS	1.5	1.0	100	109	No hold
121161-093	30					70	
121161-096							
121161-100		100 mM Na_2PO_4 pH 7.2					255
121161-120			2.0	0.5	50	32	
121161-125					100	33	
121161-130	100					60	
121161-147	30					30	
121161-165			1.5			35	No hold
121161-172					500	46	

Figure 2B: Summary of key operating parameters – initial diafiltration step

Run	Peptide Lot	Activated KLH:Peptide (mass ratio)	Peptide Stock Solution (mg/mL)	Reaction Time (min)	Final Diafiltration Buffer
121161-090	CH 06-00453	1:1	100	180	PBS
121161-093			100		
121161-096			60		
121161-100	Api 080616		6	120	10 mM K_2PO_4, pH 7.4
121161-120					
121161-125					
121161-130					
121161-147					
121161-165				180	
121161-172					

Figure 2C: Summary of key operating parameters – conjugation step

Run	Overall Process Yield (%)	No. of peptides/KLH
121161-090	85	68
121161-093	97	28
121161-096	82	44
121161-100	98	67
121161-120	84	36
121161-125	87	36
121161-130	88	44
121161-147	n/a	39
121161-165	84	43
121161-172	85	51
Lot 120957-033 (control - obtained from previous process)		47
Target specifications	n/a	30-64

Figure 3A: Summary of Analytical Results

Run	Peak 1 (%area)	Peak 2 (%area)	Peak 3 (%area)	Peak 4 (%area)	Peak 5 (%area)
121161-090	0.98	11.28	56.41	29.19	2.14
121161-093	0.69	11.69	56.2	29.08	2.35
121161-096	0.38	5.85	47.67	38.89	7.22
121161-100	0.7	6.1	46.0	38.0	9.3
121161-120	0.8	20.2	55.0	22.6	1.5
121161-125	0.4	12.0	63.3	21.4	2.9
121161-130	0.4	23.4	52.6	21.5	2.1
121161-147	0.3	15.5	54.0	27.1	3.2
121161-165	0.2	19.9	54.8	22.2	2.9
121161-172	0.1	8.2	58.3	28.7	4.7
Lot 120957-033 (control - obtained from previous process)	0.9	13.2	64.9	18.8	2.3
Target specifications	<2	8-17	50-60	20-30	1-5

Figure 3B: Summary of Analytical Results: Size Exclusion Chromatography (SEC)

Run	% Peptide	% Peptide Degradents	% Linker	% CDX-110
121161-090	0	1.7	0.7	97.7
121161-093	0	0.6	0	99.4
121161-096	0.3	1.1	0	98.6
121161-100	0.2	0.6	0.3	98.9
121161-120	0	0.2	3.0	96.8
121161-125	0	0.2	0.3	99.4
121161-130	0	0.4	3.4	96.2
121161-147	0	0.7	1.9	97.4
121161-165	0	0.5	2.3	97.2
121161-172	0	0.7	0.2	99.1
Lot 120957-033 (control - obtained from previous process)	0	2.5	0.8	96.8

Figure 3C: Summary of Analytical Results: Anion Exchange (AEX) Results

Run	% Peptide Dimer	% Peptide + Linker	% Peptide + Linker Condensate	% CDX-110	% Other
121161-090	N/A				
121161-093					
121161-096					
121161-100	0.3	0.7	1.2	97.8	0
121161-120	0.1	0.6	0.2	98.9	n/a
121161-125	0.2	0.4	0.1	99.2	0
121161-130	0.0	0.4	0.2	99.4	0
121161-147	0.3	0.5	0.2	99.0	0
121161-165	0.1	0.5	0.2	99.1	0
121161-172	0.0	0.4	0.1	99.4	0.1
Lot 120957-033 (control - obtained from previous process)	0.0	0.0	0.9	95.5	3.6

Figure 3D: Summary of Analytical Results: RRHPLC Results

Exp. No.	Method to Remove Linker	Activation							
		Reaction Buffer	Temp	Volume	Linker Stock Solution	% DMSO	Linker:KLH Molar ratio	KLH Concentration	Activation Time (min)
Optimized Process	TFF	100 mM Sodium Phosphate pH 7.2	15°C	-	100 mg/mL in DMSO	1.9%	200	10 mg/mL	45
122123-065	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	1.5 mL	8 mg/mL in water	0%	100, 125, 150, 200, 250, 357	10 mg/mL	20, 44, 85, 186
122123-097	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	1.0 mL	8 mg/mL in water	0%	100, 440	10 mg/mL	5, 15, 45
122123-109	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	0.3 mL	30 mg/mL in DMSO	3.5% 9.6% 15.6%	100, 270, 440	10 mg/mL	5, 15, 45
122123-112	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	0.36 mL	30 mg/mL in DMSO	15.6%	100, 270, 440	10 mg/mL	5, 15, 45
122123-115	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	0.13-0.28 mL	121.8 mg/mL in DMSO	See Figure 4C	See Figure 4C	See Figure 4C	45
122123-190	One-Pot Reaction	100 mM Sodium Phosphate pH 7.2	22°C	0.4 – 1 mL	100 mg/mL in DMSO	< 3.4%	25, 50, 100, 200, 300, 350	10 mg/mL	21, 35, 45, 60, 126, 245

Figure 4A

Exp. No.	Conjugation		
	Volume	Peptide:KLH Molar Ratio	KLH Concentration
Optimized Process	-	~275 (1g peptide/ 1g initial KLH)	1.5 mg/mL
122123-065	5 mL	242	1.39 mg/mL
122123-097	0.65 mL	2.42 x Linker	1.39 mg/mL
122123-109	0.75 mL	4 x Linker	1.39 mg/mL
122123-112	0.75 mL	4 x Linker	1.39 mg/mL
122123-115	1 – 2 mL	4 x Linker	1.39 mg/mL
122123-190	1.5 mL	4 x Linker	0.9 mg/mL

Figure 4B

Exp Number	% DMSO	Linker:KLH Molar Ratio	Protein Concentration (mg/ml)
1	15.6	357	5
2	4.7	357	5
3	15.6	357	15
4	15.6	200	15
5	10.1	279	10
6	4.7	200	15
7	4.7	200	5
8	10.1	279	10
9	4.7	357	15
10	15.6	200	5

Figure 4C

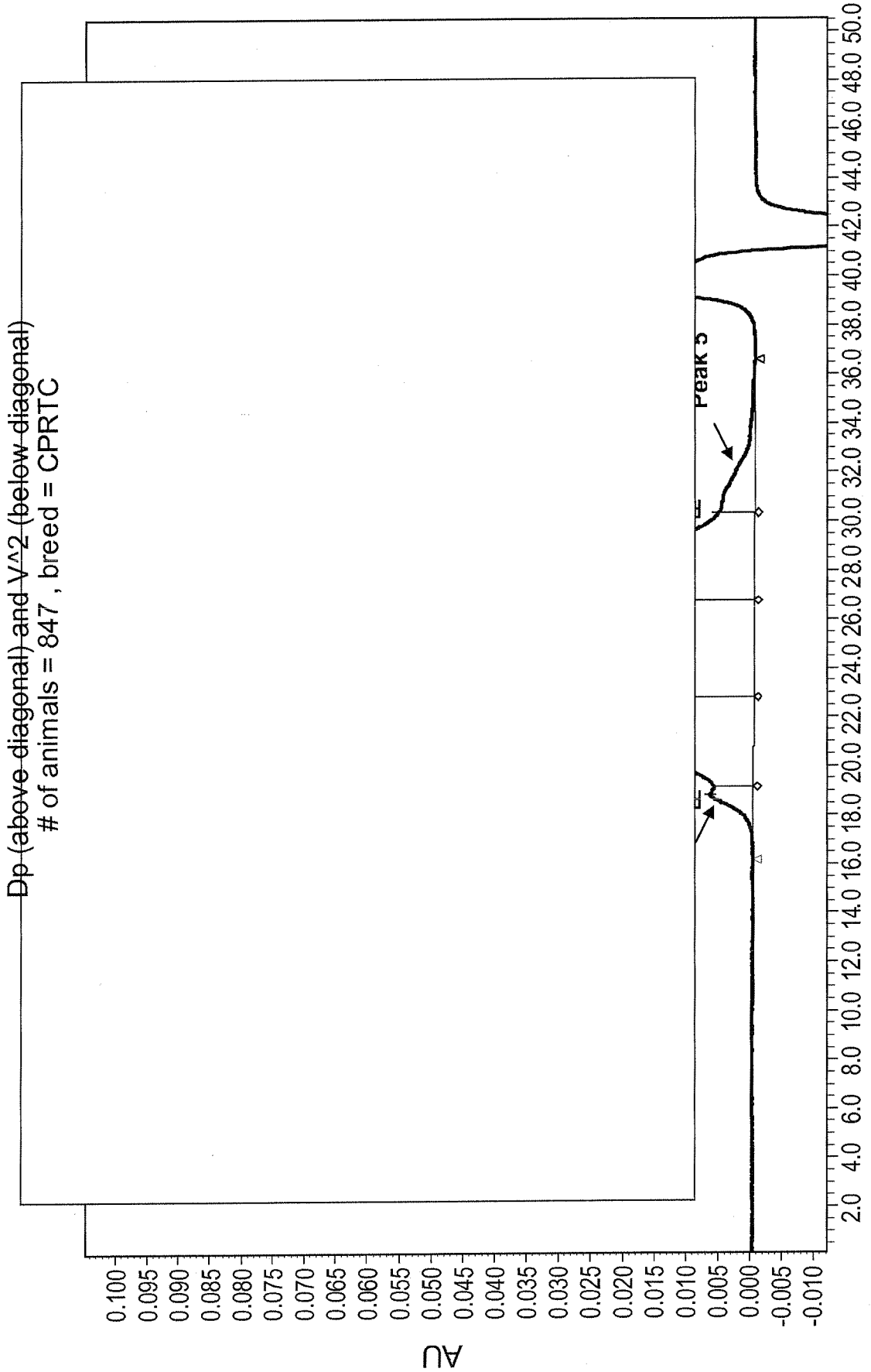

Dp (above diagonal) and V^2 (below diagonal)
# of animals = 847 , breed = CPRTC
Peak 3
AU
Minutes
0.100
0.095
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0
32.0
34.0
36.0
38.0
40.0
42.0
44.0
46.0
48.0
50.0

Figure 5

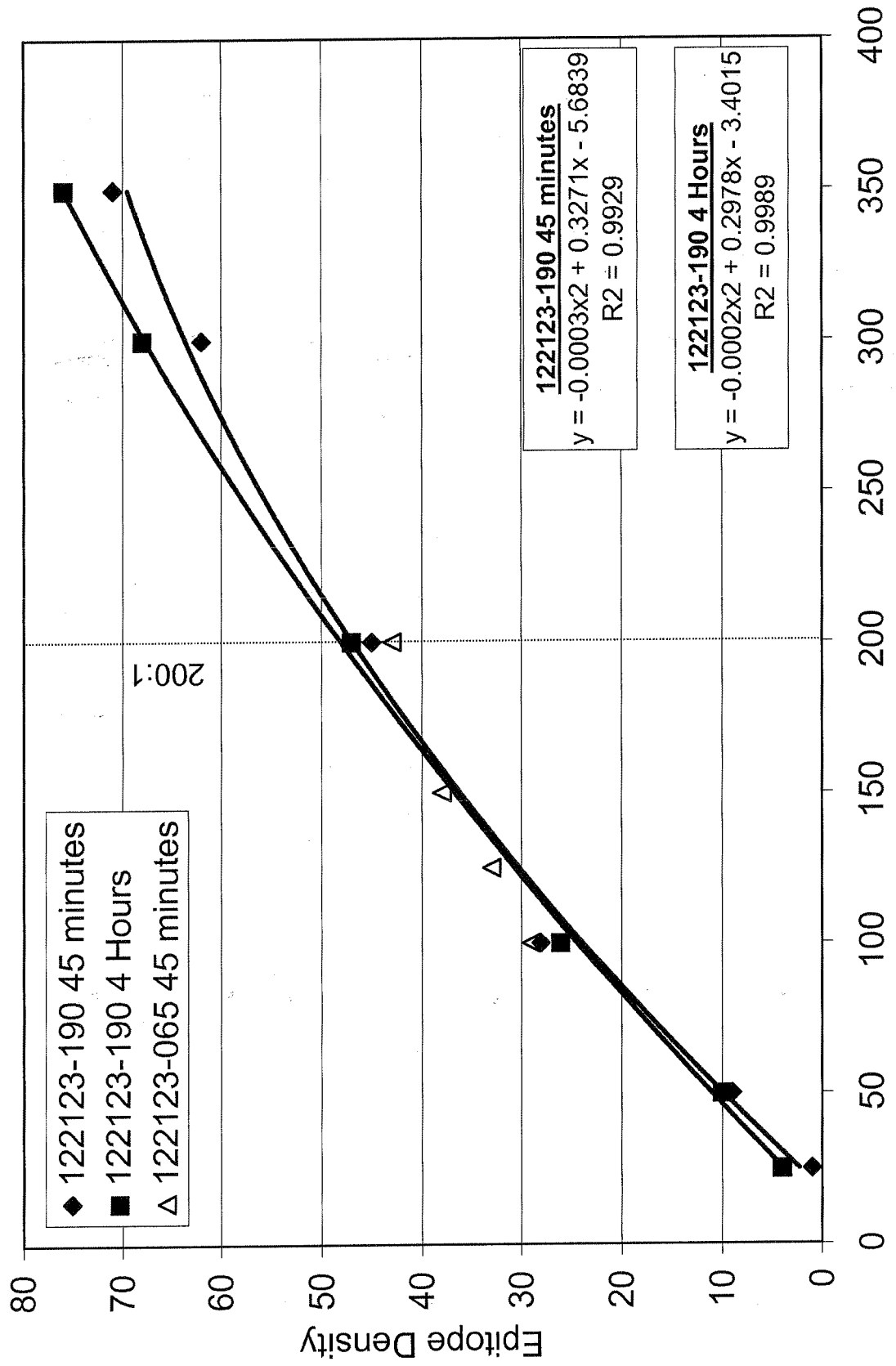

Epitope Density
Sulfo-SMCC Ratio
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300
350
400
200:1
◆ 122123-190 45 minutes
■ 122123-190 4 Hours
Δ 122123-065 45 minutes
122123-190 45 minutes
y = -0.0003x2 + 0.3271x - 5.6839
R2 = 0.9929
122123-190 4 Hours
y = -0.0002x2 + 0.2978x - 3.4015
R2 = 0.9989

Figure 6

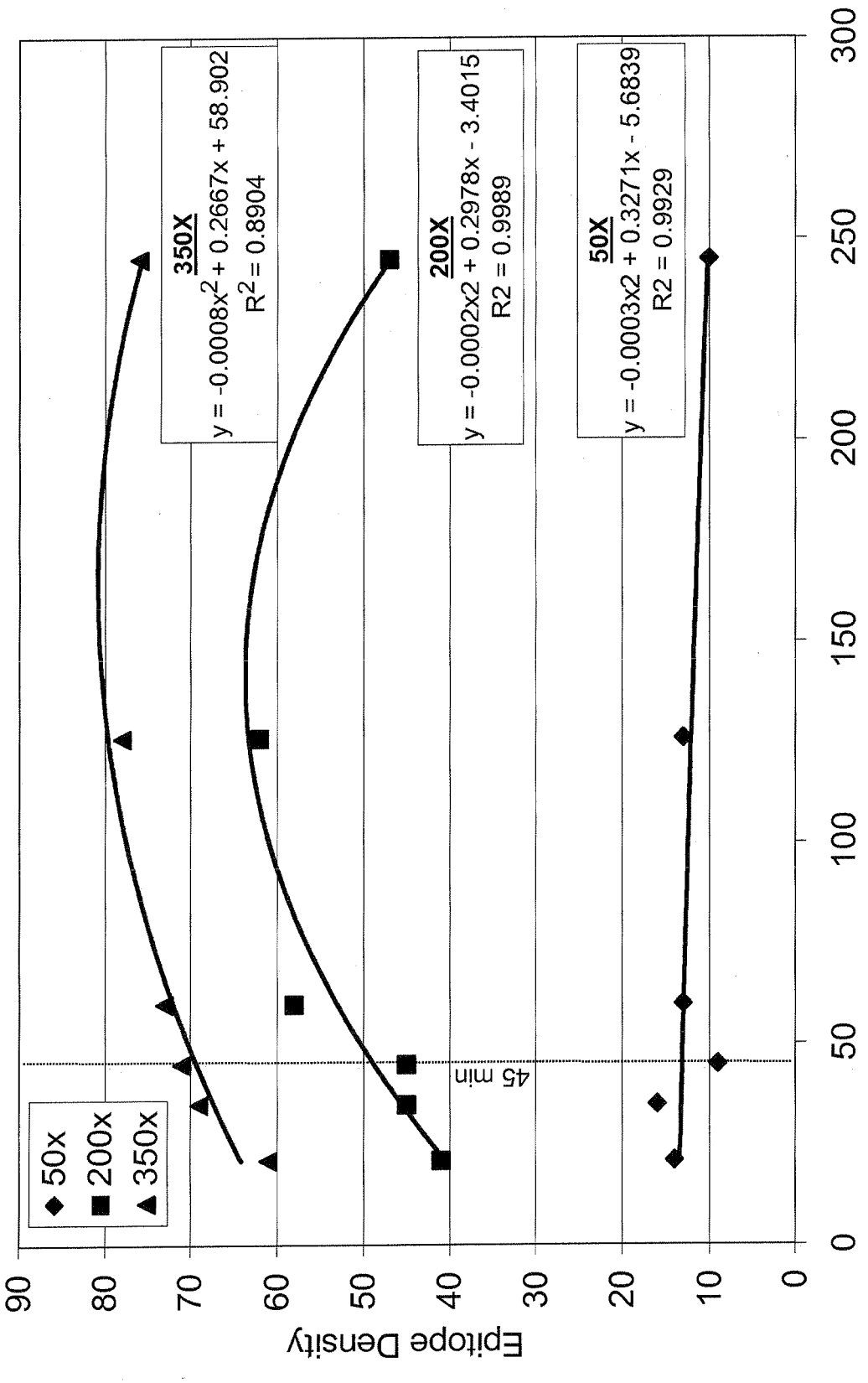

Epitope Density
Activation Time (min)
50x
200x
350x
45 min
350X
y = -0.0008x2 + 0.2667x + 58.902
R2 = 0.8904
200X
y = -0.0002x2 + 0.2978x - 3.4015
R2 = 0.9989
50X
y = -0.0003x2 + 0.3271x - 5.6839
R2 = 0.9929
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300

Figure 7

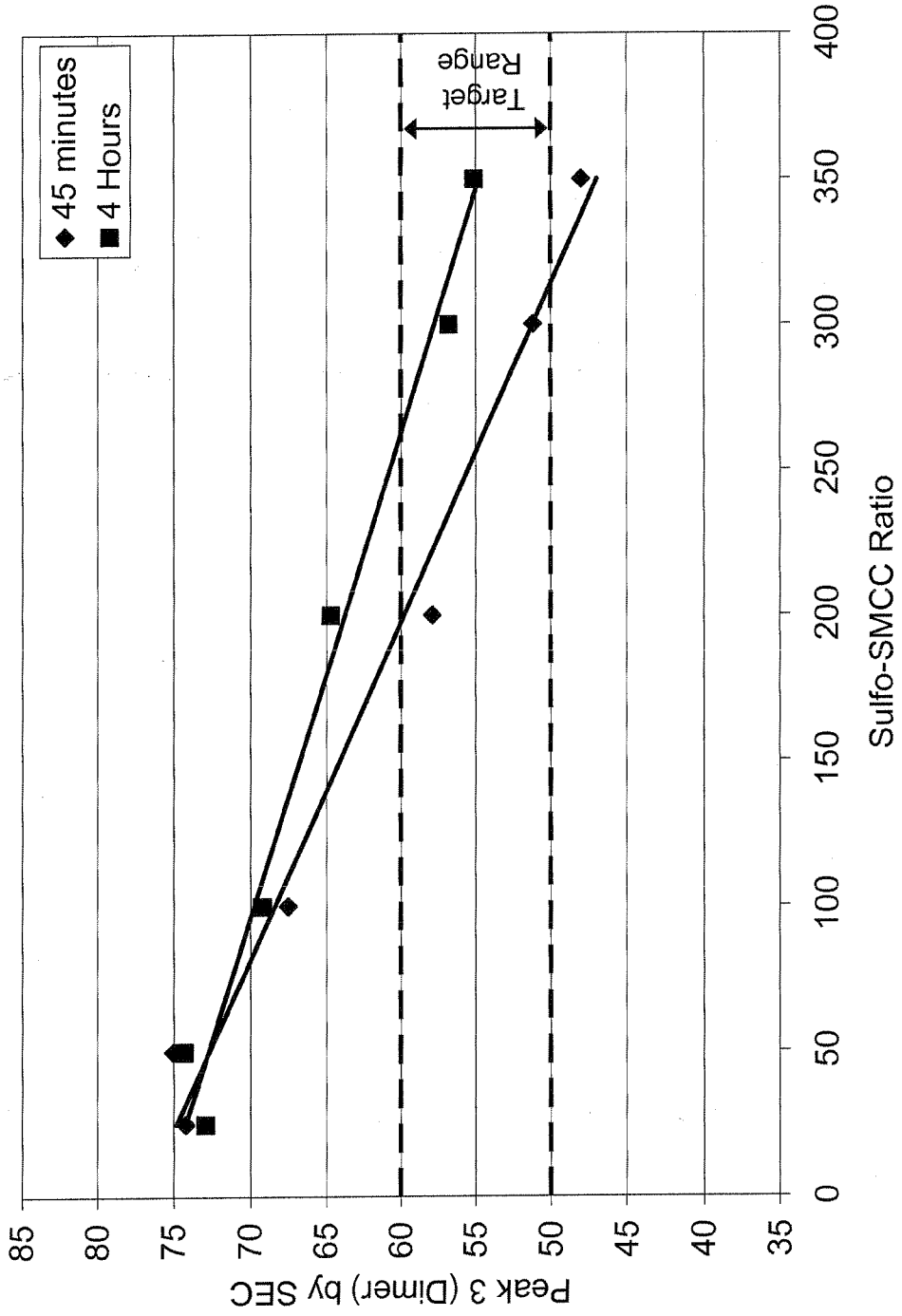

45 minutes
4 Hours
Target Range
Peak 3 (Dimer) by SEC
Sulfo-SMCC Ratio
85
80
75
70
65
60
55
50
45
40
35
0
50
100
150
200
250
300
350
400

Figure 8A

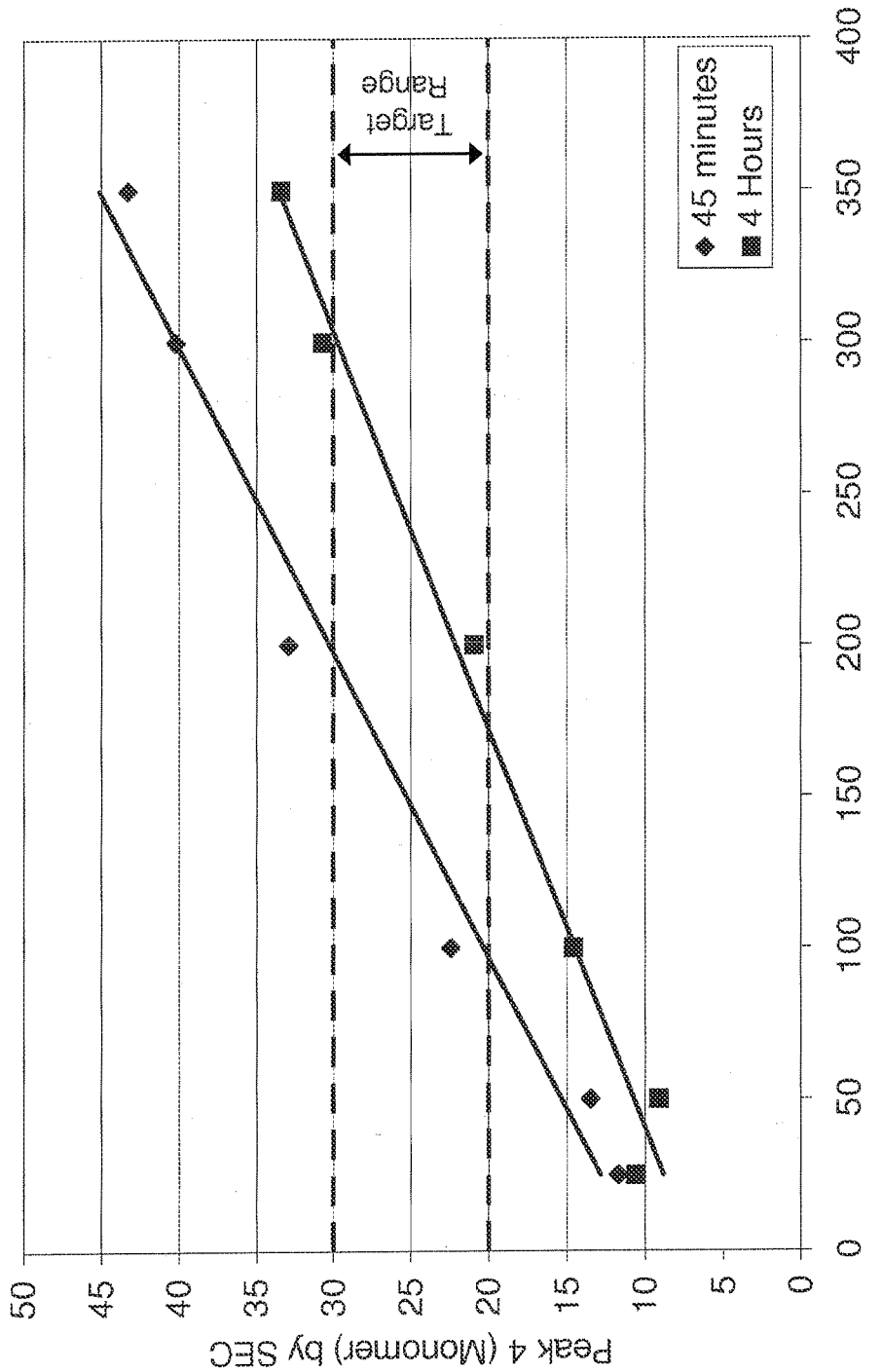

Peak 4 (Monomer) by SEC
Sulfo-SMCC Ratio
45 minutes
4 Hours
Target Range
0
5
10
15
20
25
30
35
40
45
50
0
50
100
150
200
250
300
350
400

Figure 8B

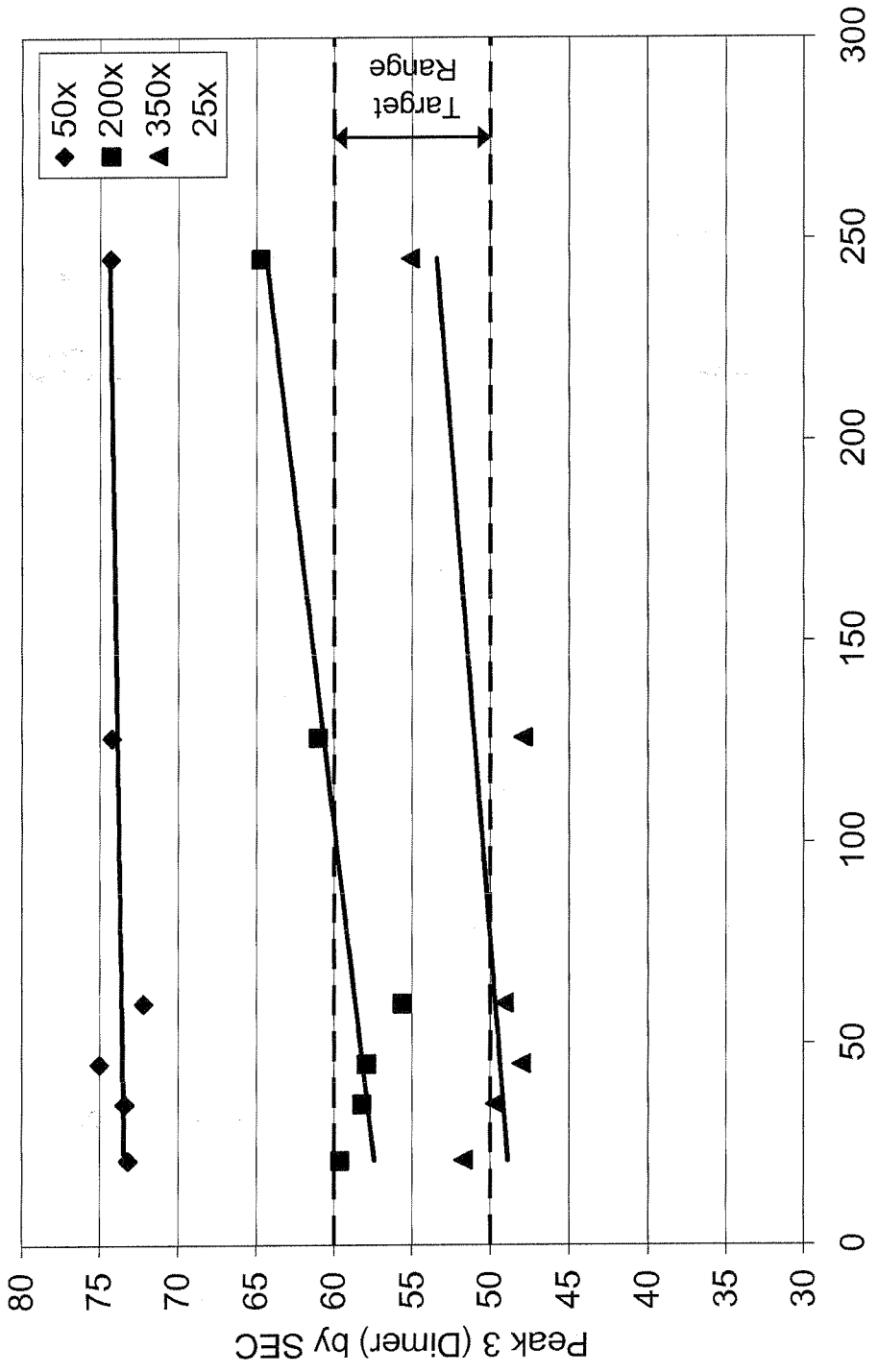

Peak 3 (Dimer) by SEC
Activation Time
Target Range
50x
200x
350x
25x
80
75
70
65
60
55
50
45
40
35
30
0
50
100
150
200
250
300

Figure 9A

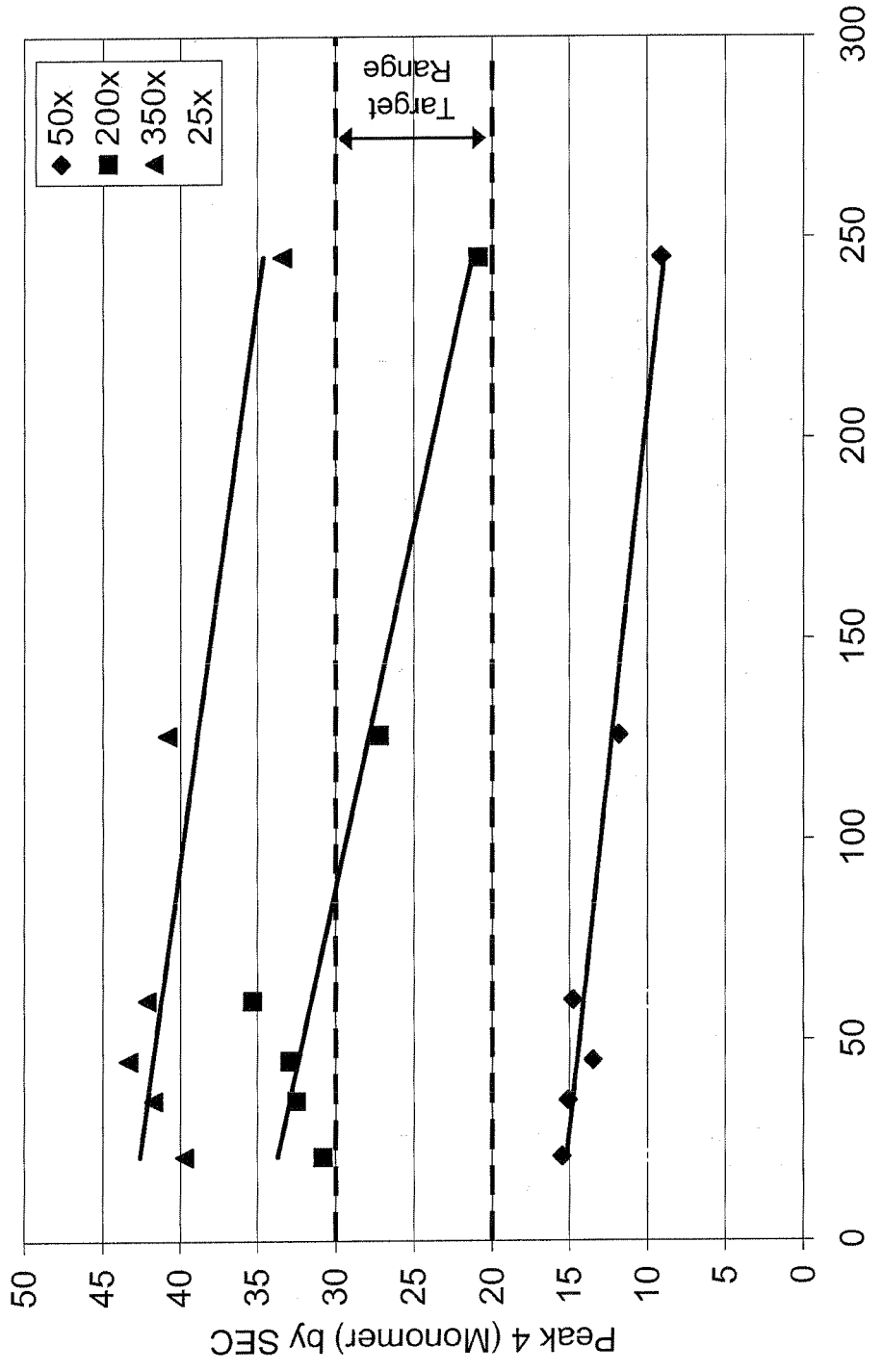

Peak 4 (Monomer) by SEC
Activation Time
Target Range
50x
200x
350x
25x
0
5
10
15
20
25
30
35
40
45
50
0
50
100
150
200
250
300

Figure 9B

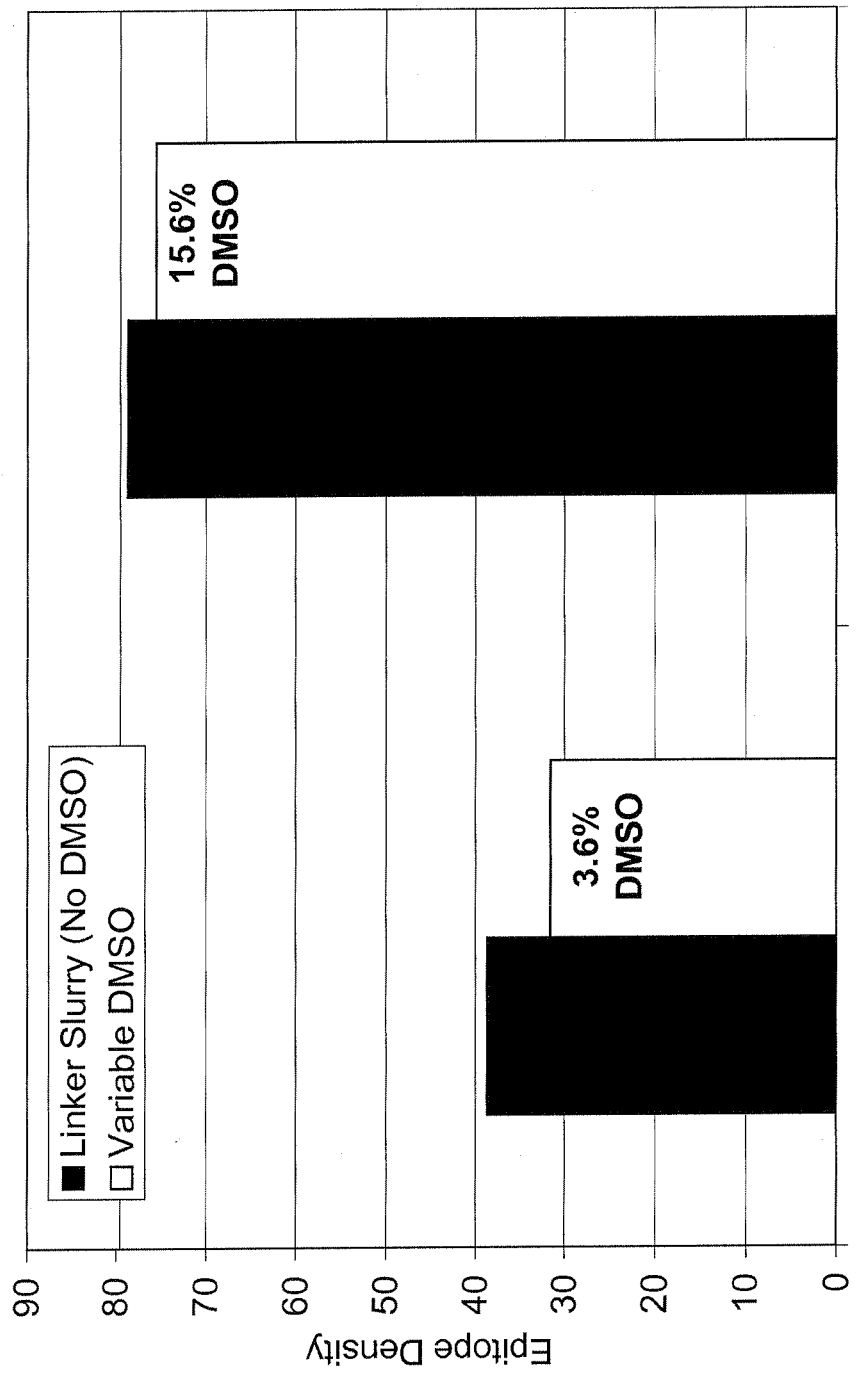

Linker Slurry (No DMSO)
Variable DMSO
Epitope Density
0
10
20
30
40
50
60
70
80
90
3.6% DMSO
15.6% DMSO
100
440
Sulfo-SMCC Ratio

Figure 10

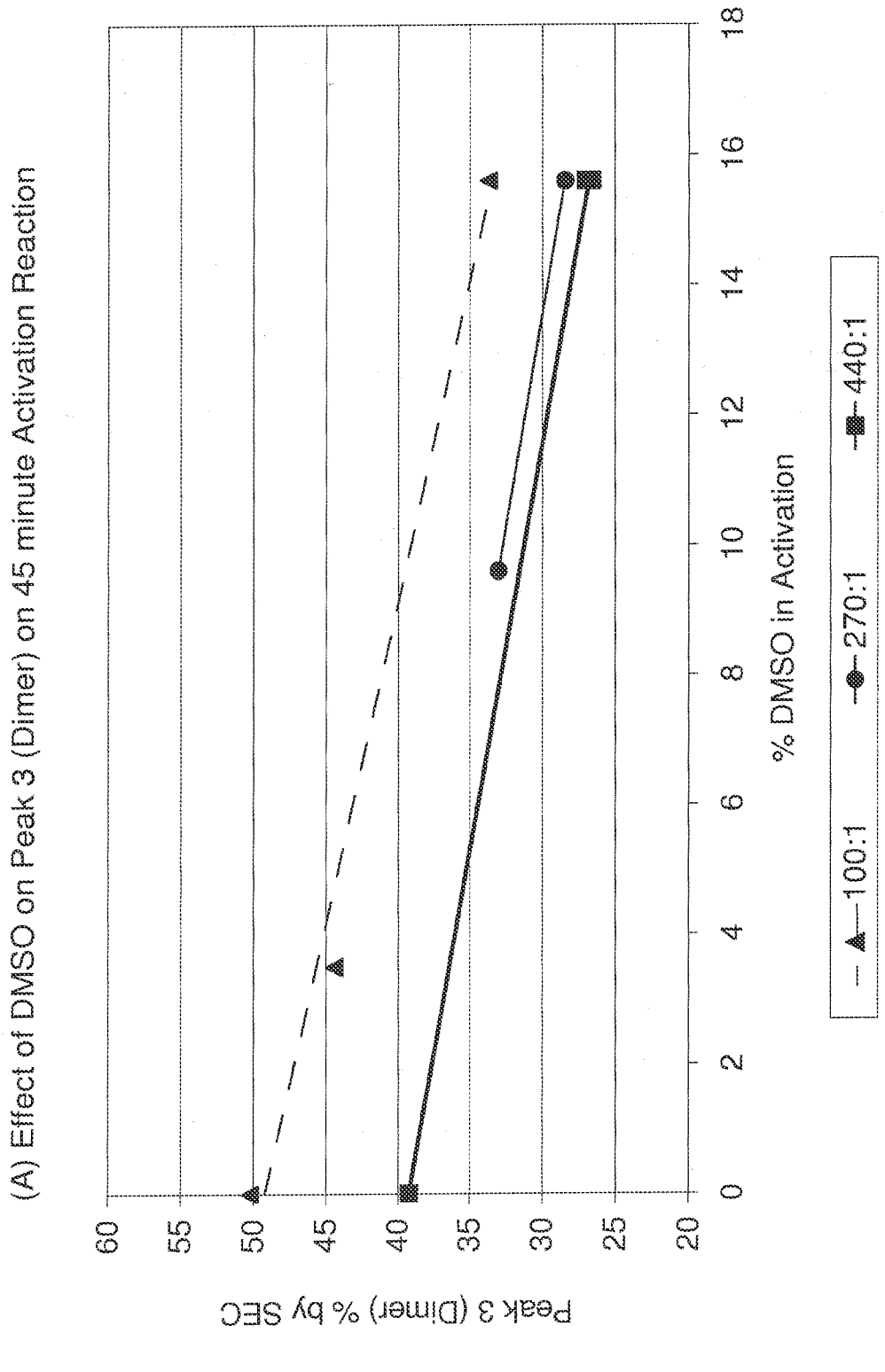

(A) Effect of DMSO on Peak 3 (Dimer) on 45 minute Activation Reaction
Peak 3 (Dimer) % by SEC
60
55
50
45
40
35
30
25
20
0
2
4
6
8
10
12
14
16
18
% DMSO in Activation
100:1
270:1
440:1

Figure 11A

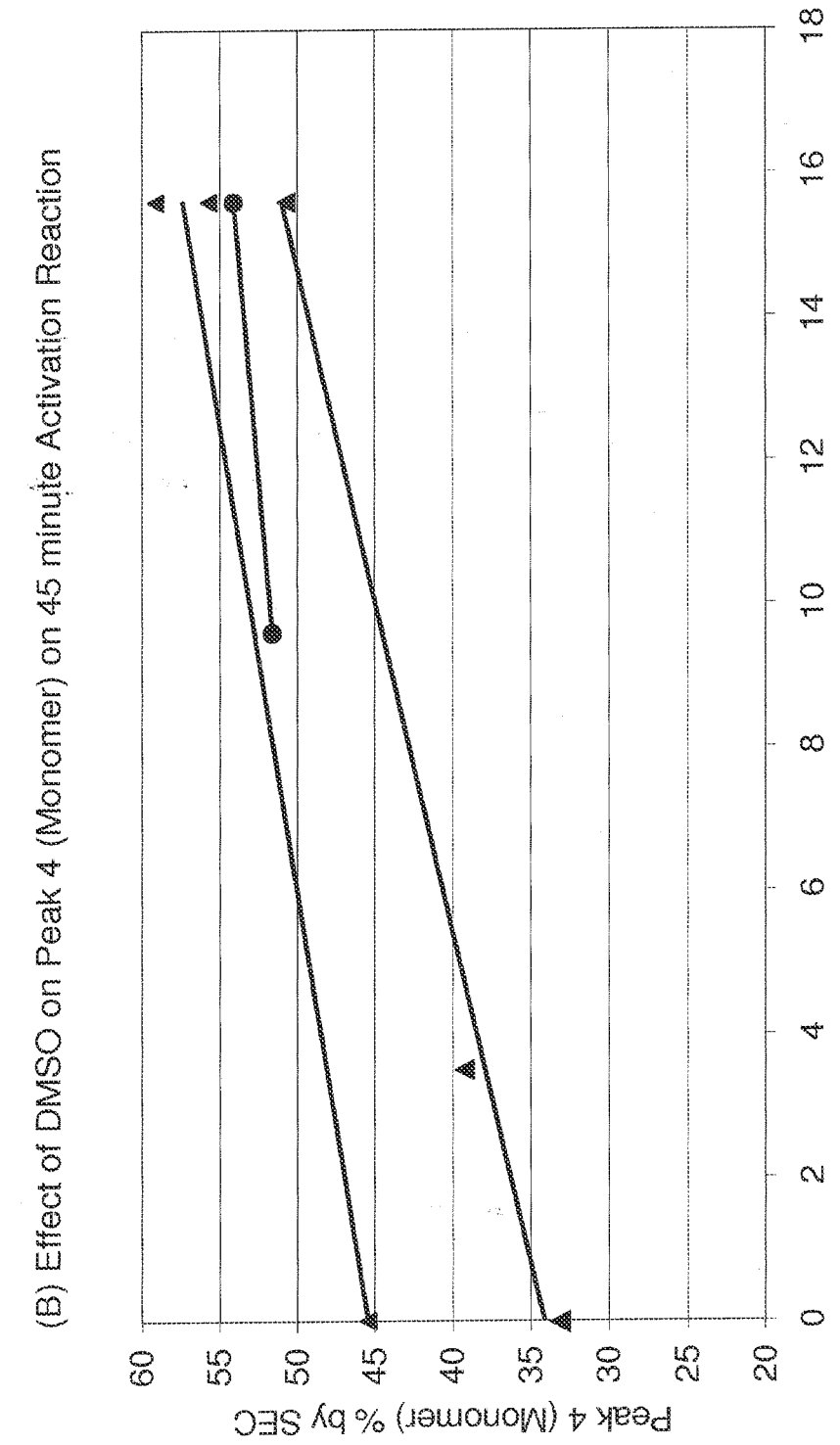

(B) Effect of DMSO on Peak 4 (Monomer) on 45 minute Activation Reaction
Peak 4 (Monomer) % by SEC
60
55
50
45
40
35
30
25
20
0
2
4
6
8
10
12
14
16
18
% DMSO
100:1
270:1
440:1

Figure 11B

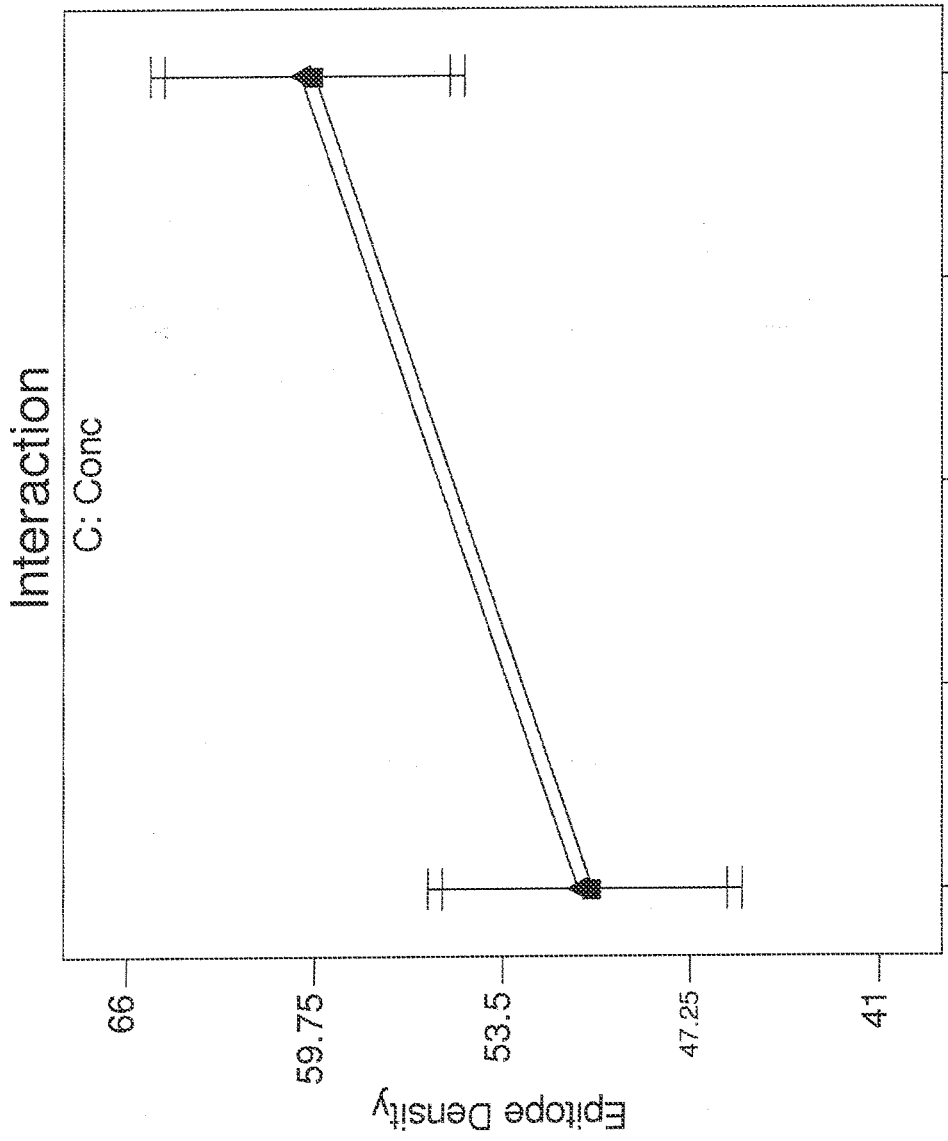

Interaction
C: Conc
66
59.75
53.5
47.25
41
Epitope Density
200.00
239.25
278.50
317.75
357.00
B: Linker:KLH

Figure 12A

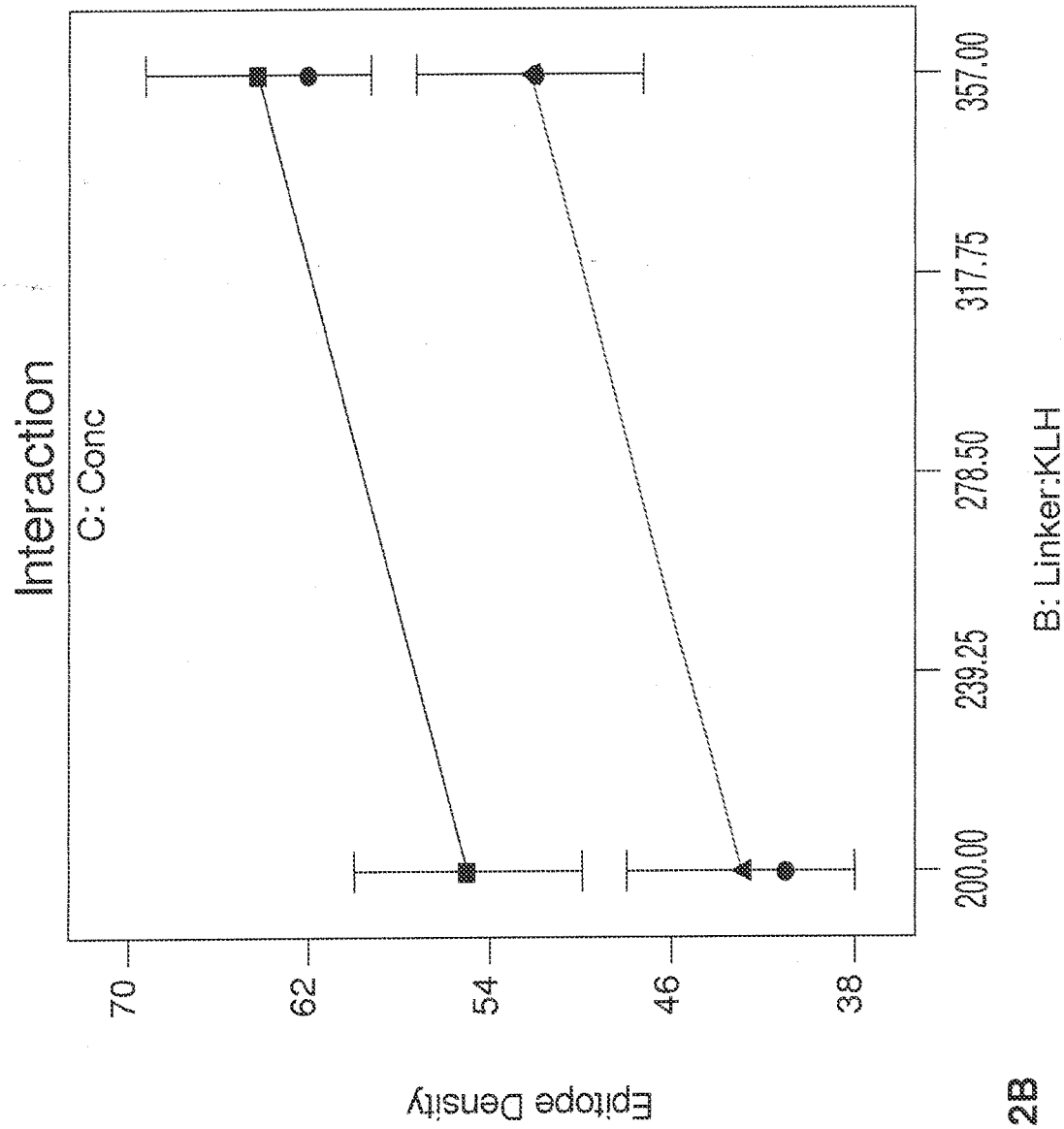

Interaction
C: Conc
Epitope Density
70
62
54
46
38
200.00
239.25
278.50
317.75
357.00
B: Linker:KLH

Figure 12B

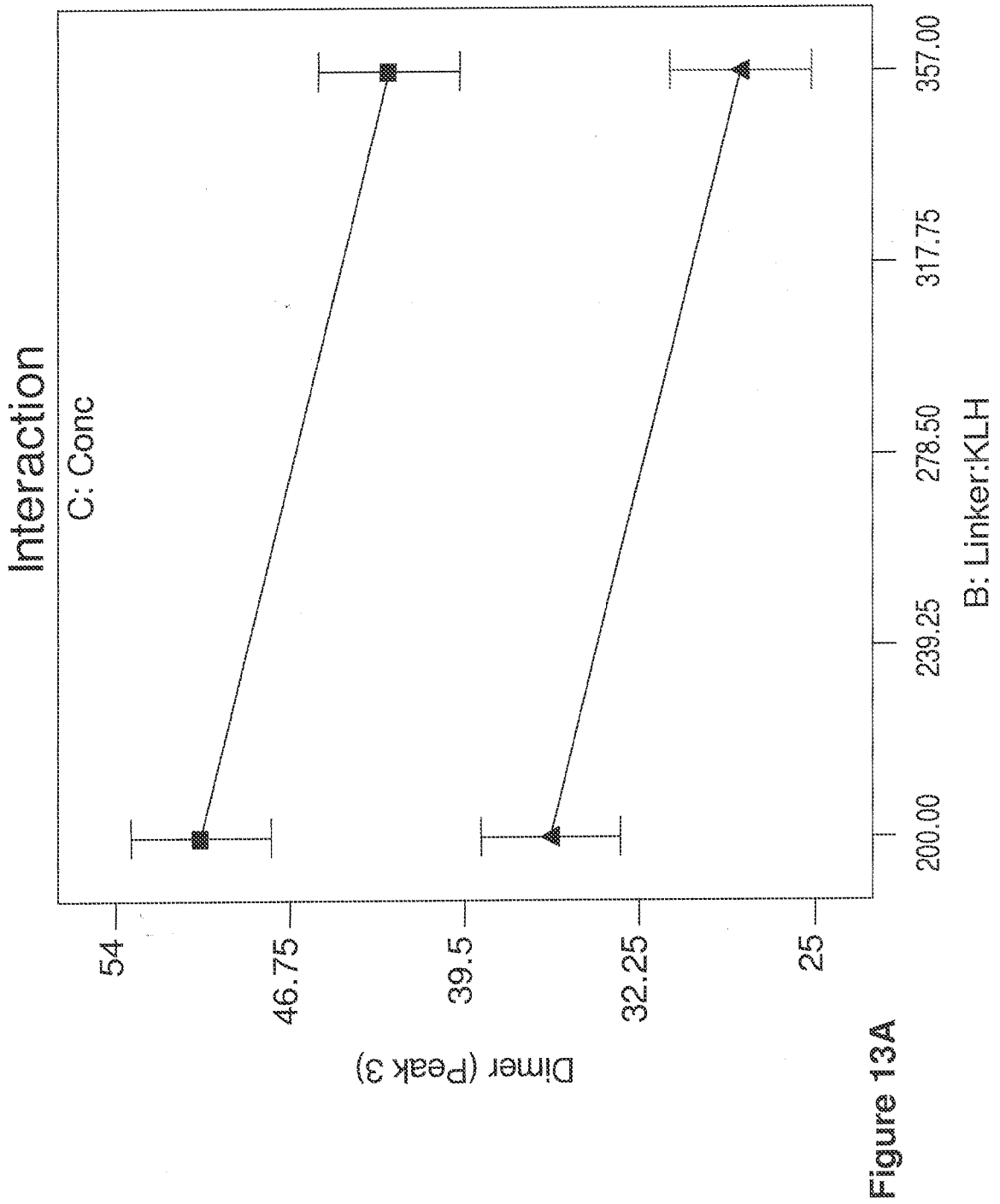

Interaction
C: Conc
Dimer (Peak 3)
54
46.75
39.5
32.25
25
200.00
239.25
278.50
317.75
357.00
B: Linker:KLH

Figure 13A

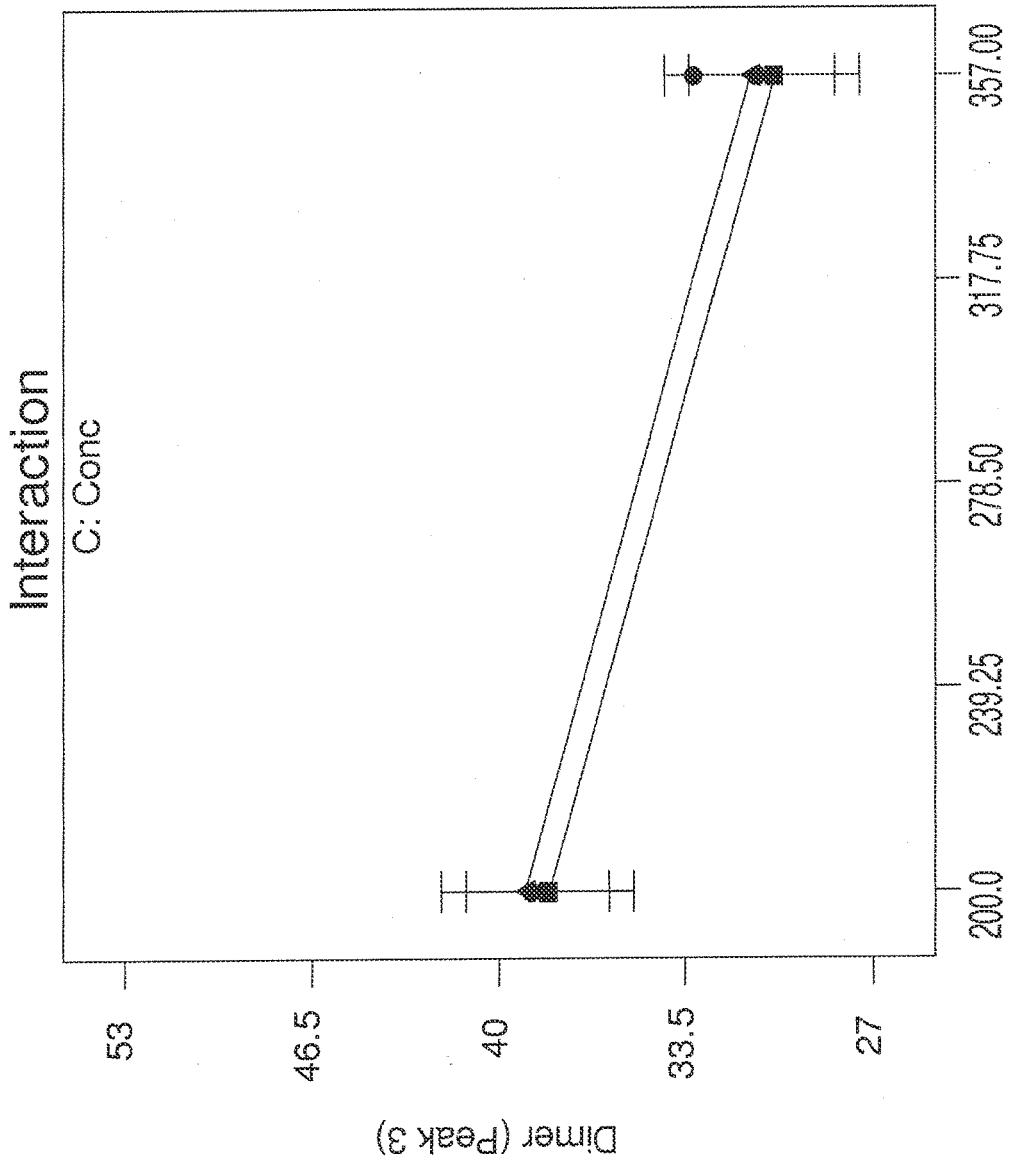

Interaction
C: Conc
Dimer (Peak 3)
53
46.5
40
33.5
27
200.0
239.25
278.50
317.75
357.00
B: Linker:KLH

Figure 13B

Sulfo-SMCC Dissolution		
Parameter	Original process	Optimized process
Sulfo-SMCC Concentration	10 mg/mL	**100 mg/mL**
Dissolution Buffer	100 mM Sodium Phosphate, pH 7.2	**Anhydrous DMSO**

Activation		
Parameter	Original process	Optimized process
Sulfo-SMCC:KLH Molar Ratio	357 X	**200 X**
KLH Concentration	10 mg/mL	10 mg/mL
Temperature	Room Temp	**15℃**
Time	45 minutes	45 minutes
Buffer	~40mM Sodium Phosphate, pH 7.2	40mM Sodium Phosphate, pH 7.2

Peptide Solution Preparation		
Parameter	Original process	Optimized process
Concentration	2 mg/mL	**6 mg/mL**
Dissolution Buffer	100 mM Sodium Phosphate, pH 7.2	100 mM Sodium Phosphate, pH 7.2

Peptide Coupling (Conjugation)		
Parameter	Original process	Optimized process
Peptide:KLH Excess (g/g initial KLH)	1:1	1:1
KLH Concentration	~1.5 mg/mL	1.5 mg/mL
Temperature	Room Temp	**15℃**
Time	3 hours	3 hours
Buffer	100mM Sodium Phosphate, pH 7.2	100mM Sodium Phosphate, pH 7.2

Figure 14

SEQUENCE LISTING

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<120> Vaccine Compositions

<130> SG3238A

<150> 61/289083
<151> 2009-12-22

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1 5 10

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1 5 10