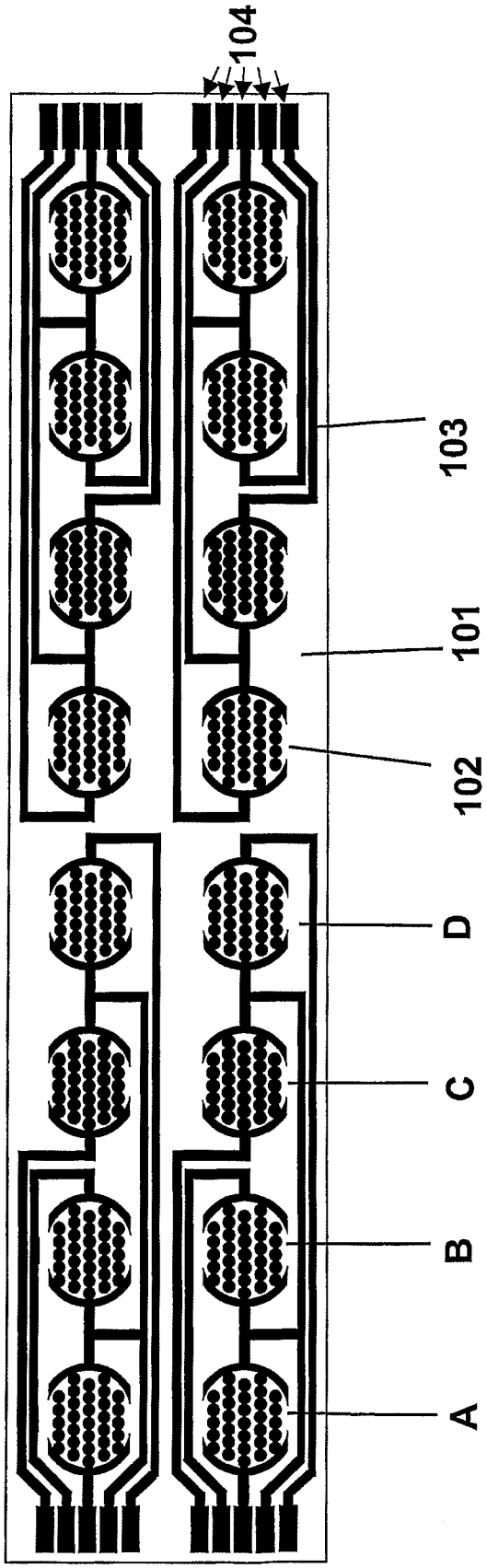


Figure 1A

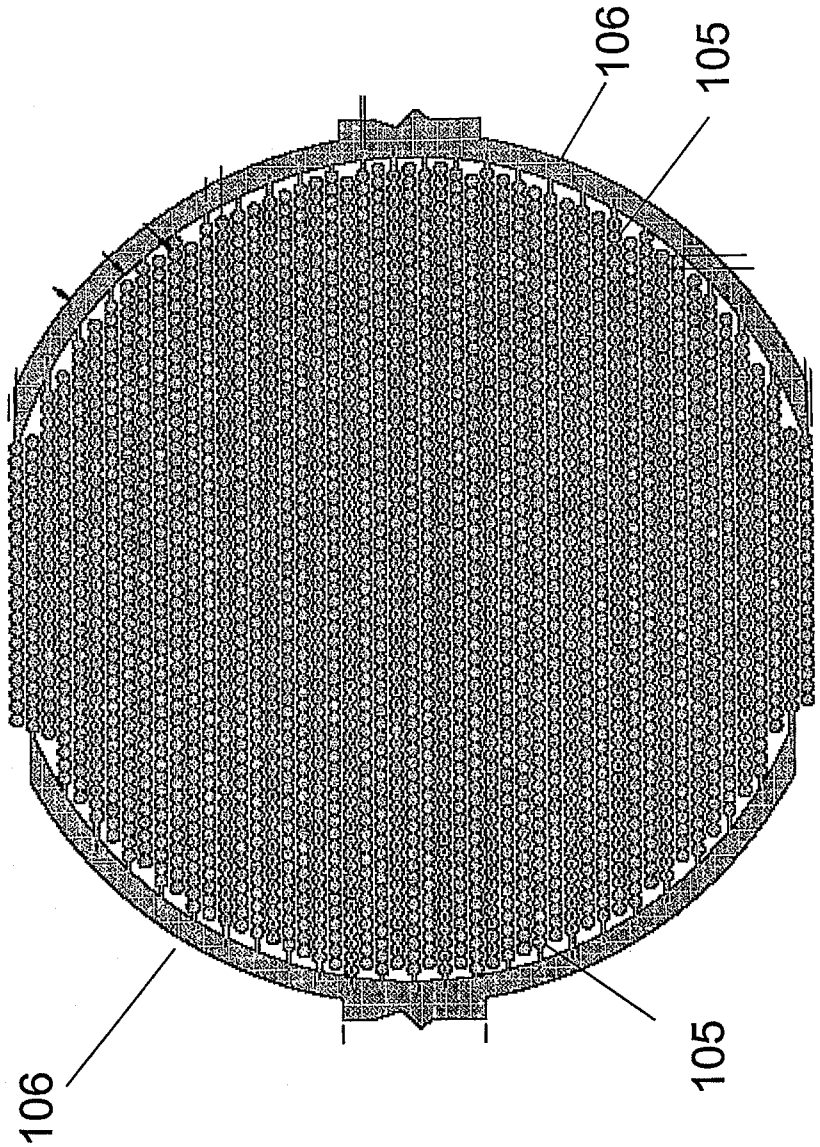


Figure 1B

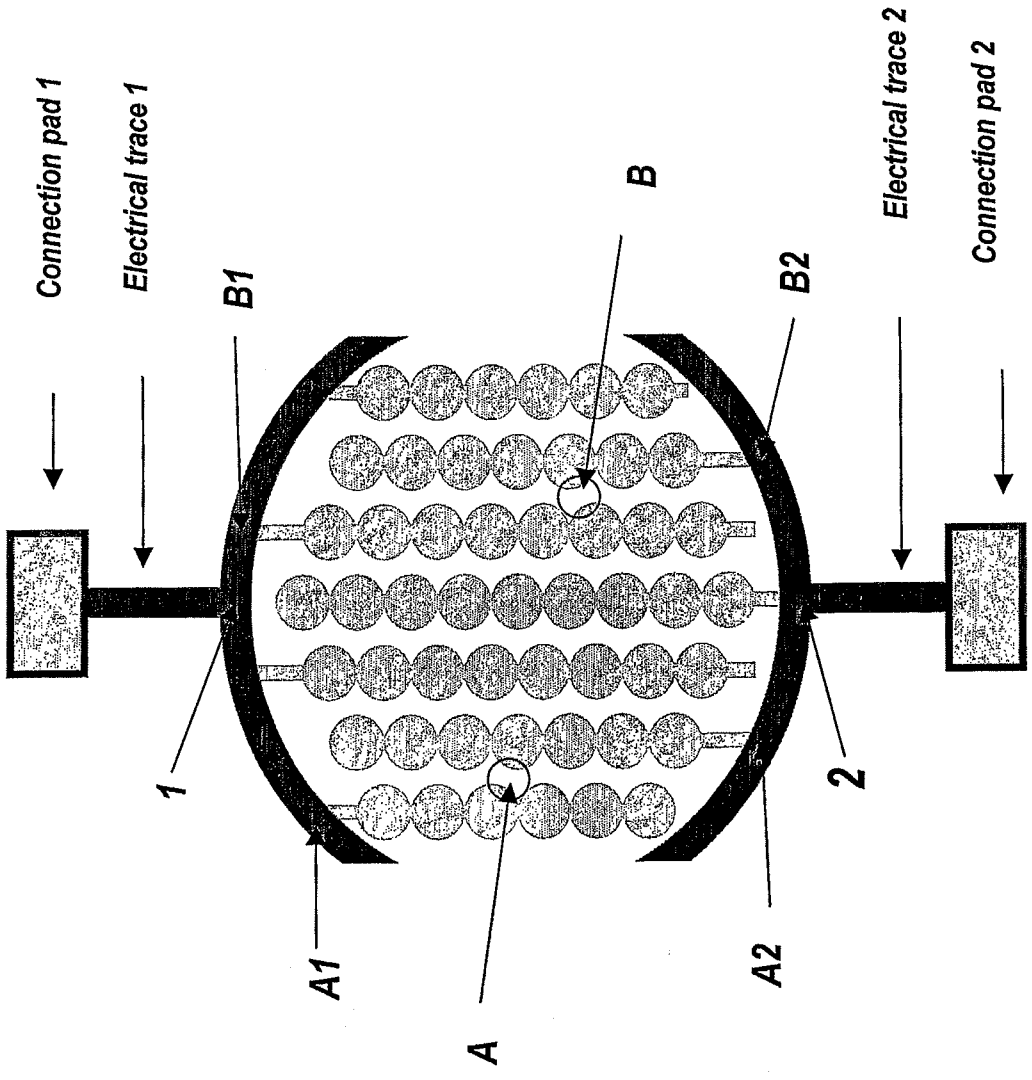

Connection pad 1
Electrical trace 1
B1
B
B2
Electrical trace 2
Connection pad 2
1
2
A1
A
A2

Figure 1C

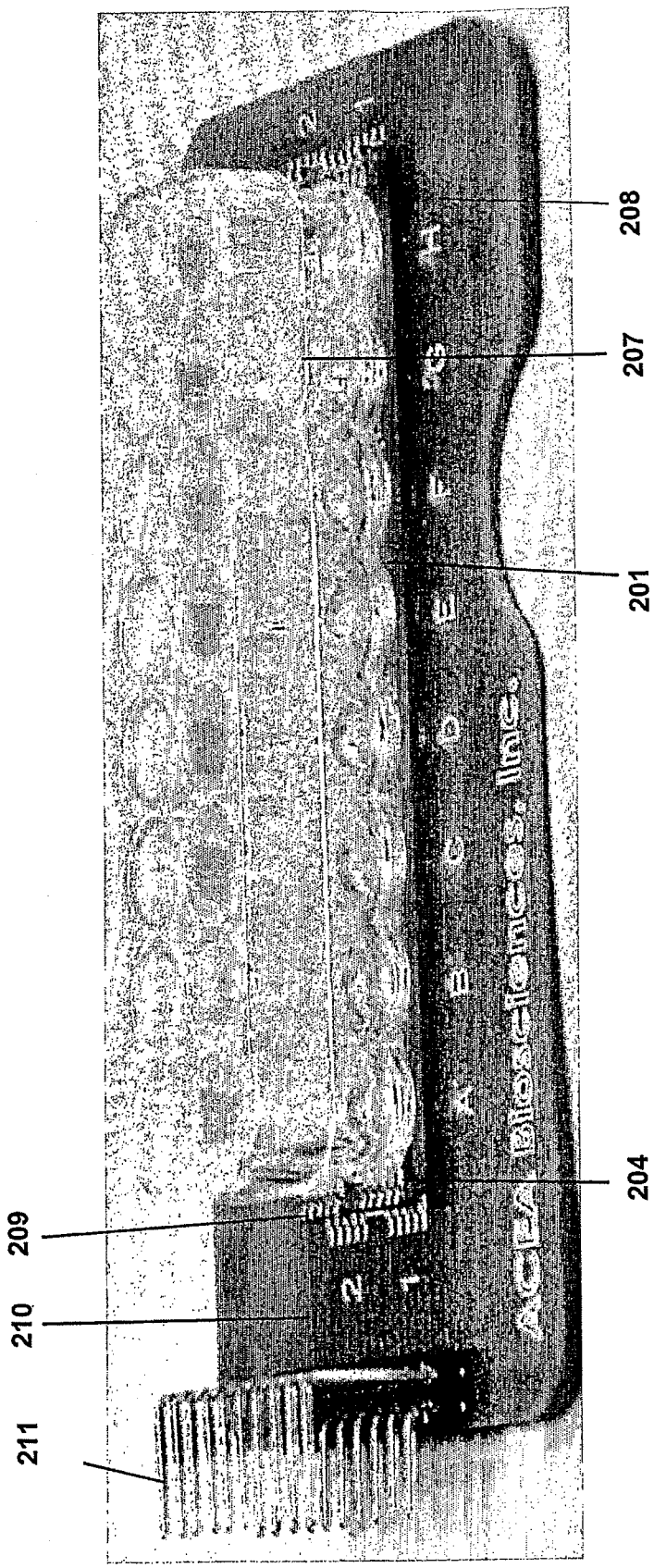


Figure 2.

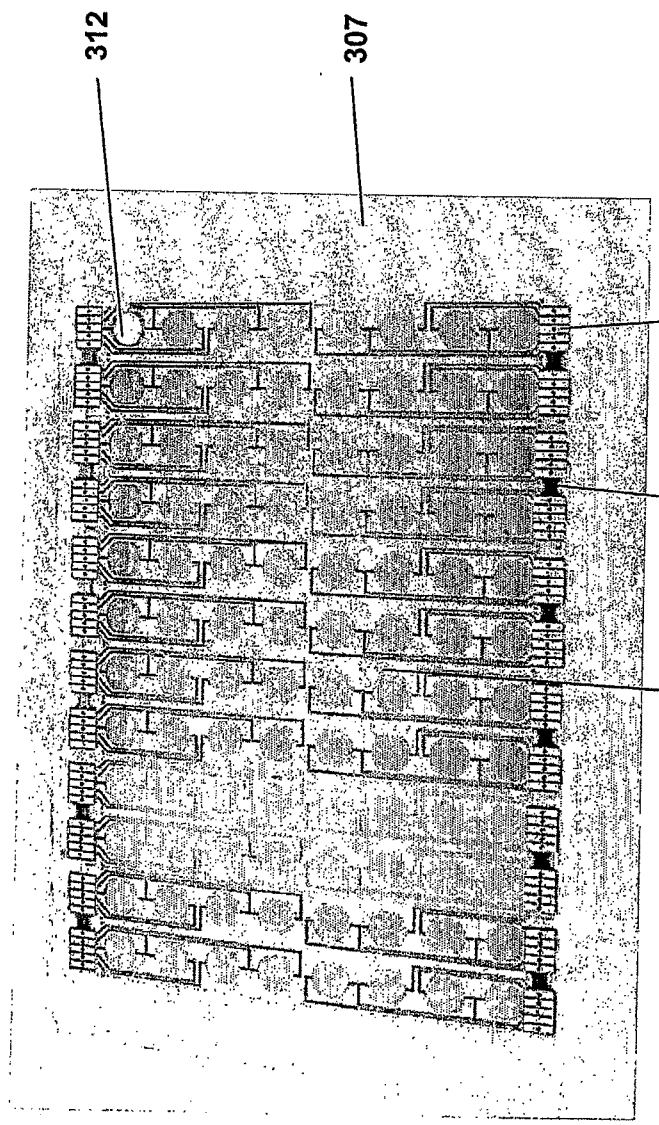


Figure 3A.

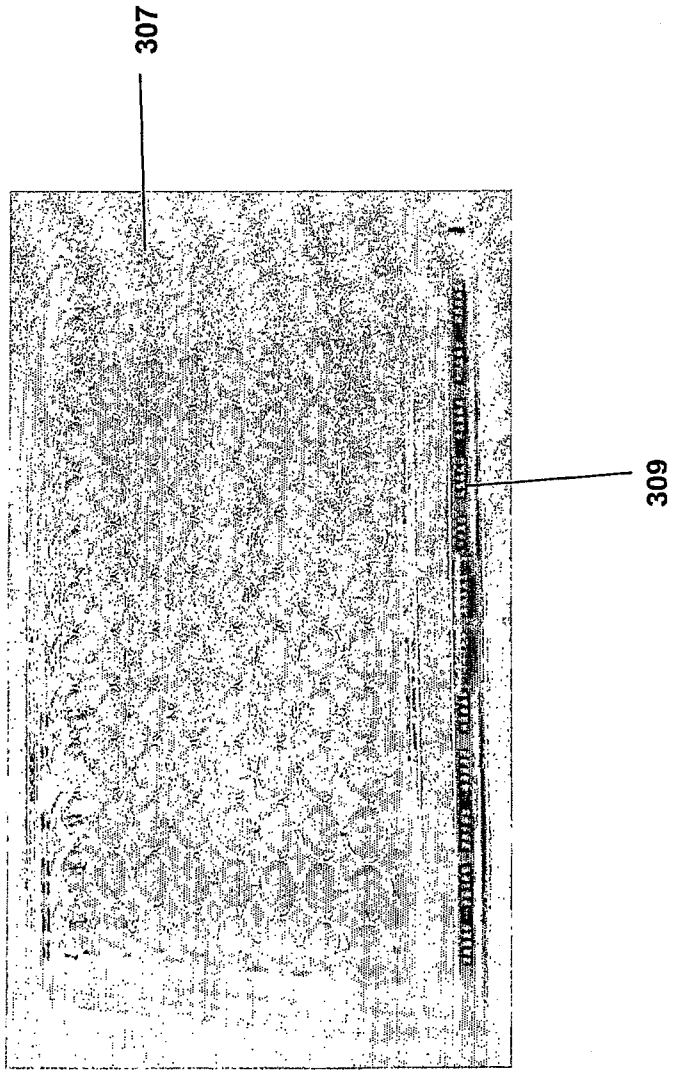


Figure 3B.

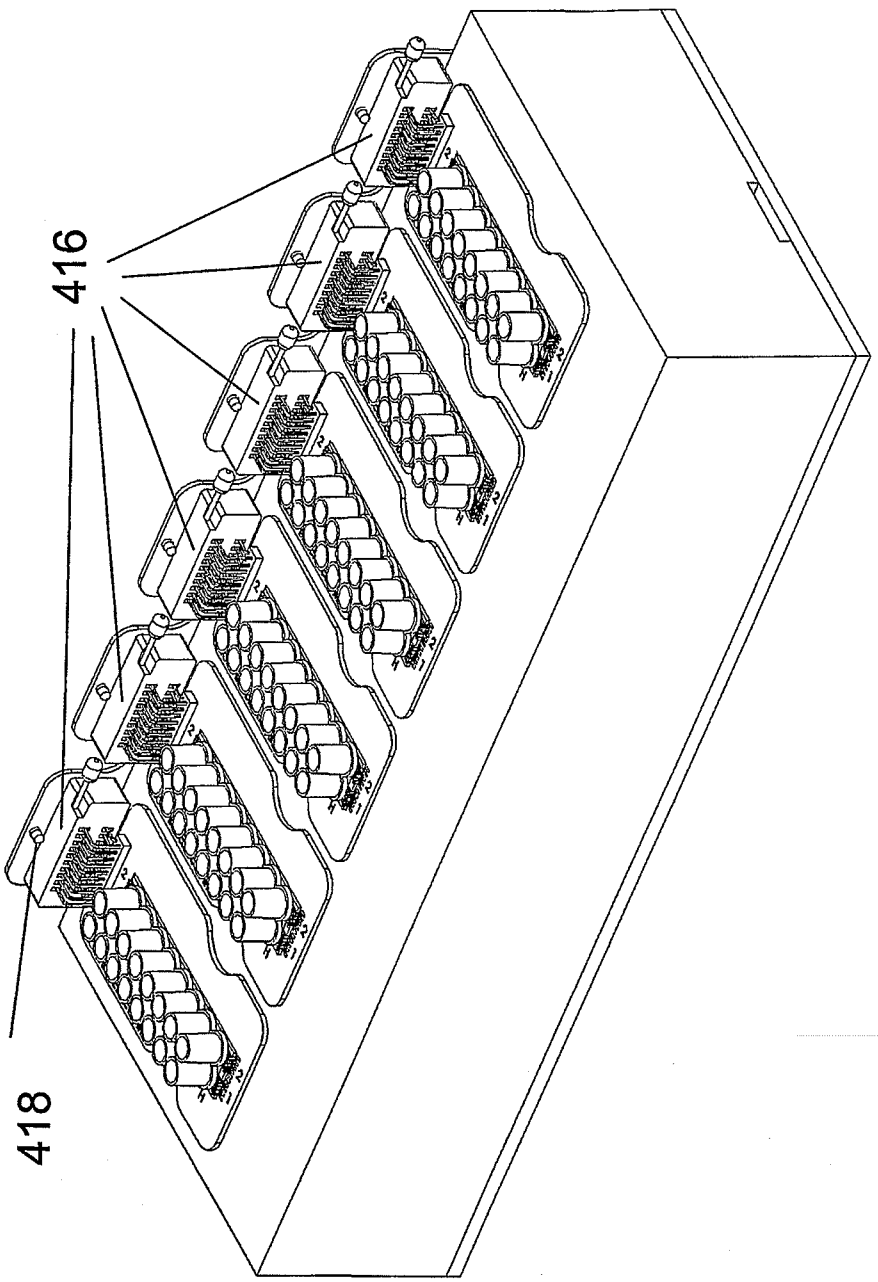


Figure 4.

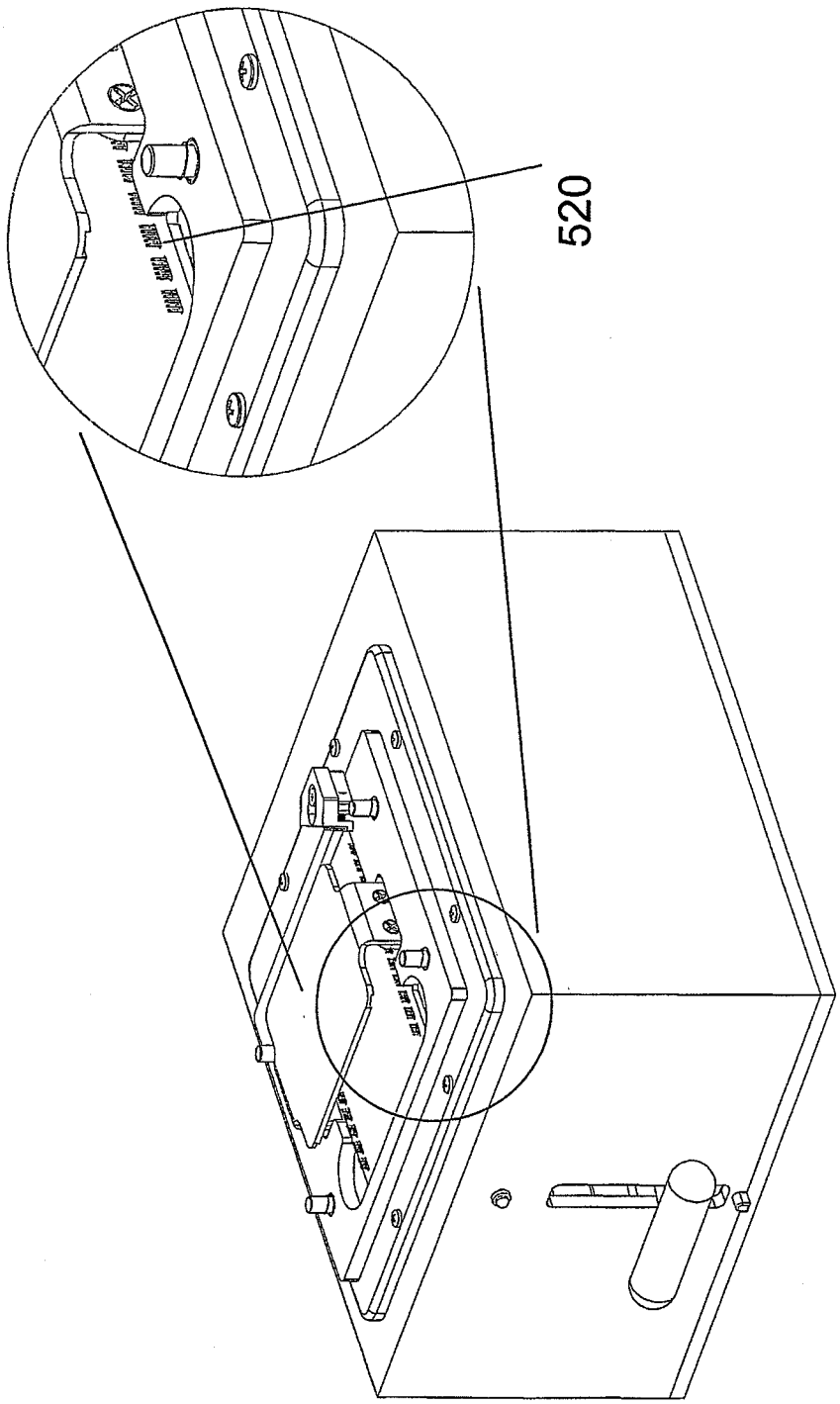


Figure 5.

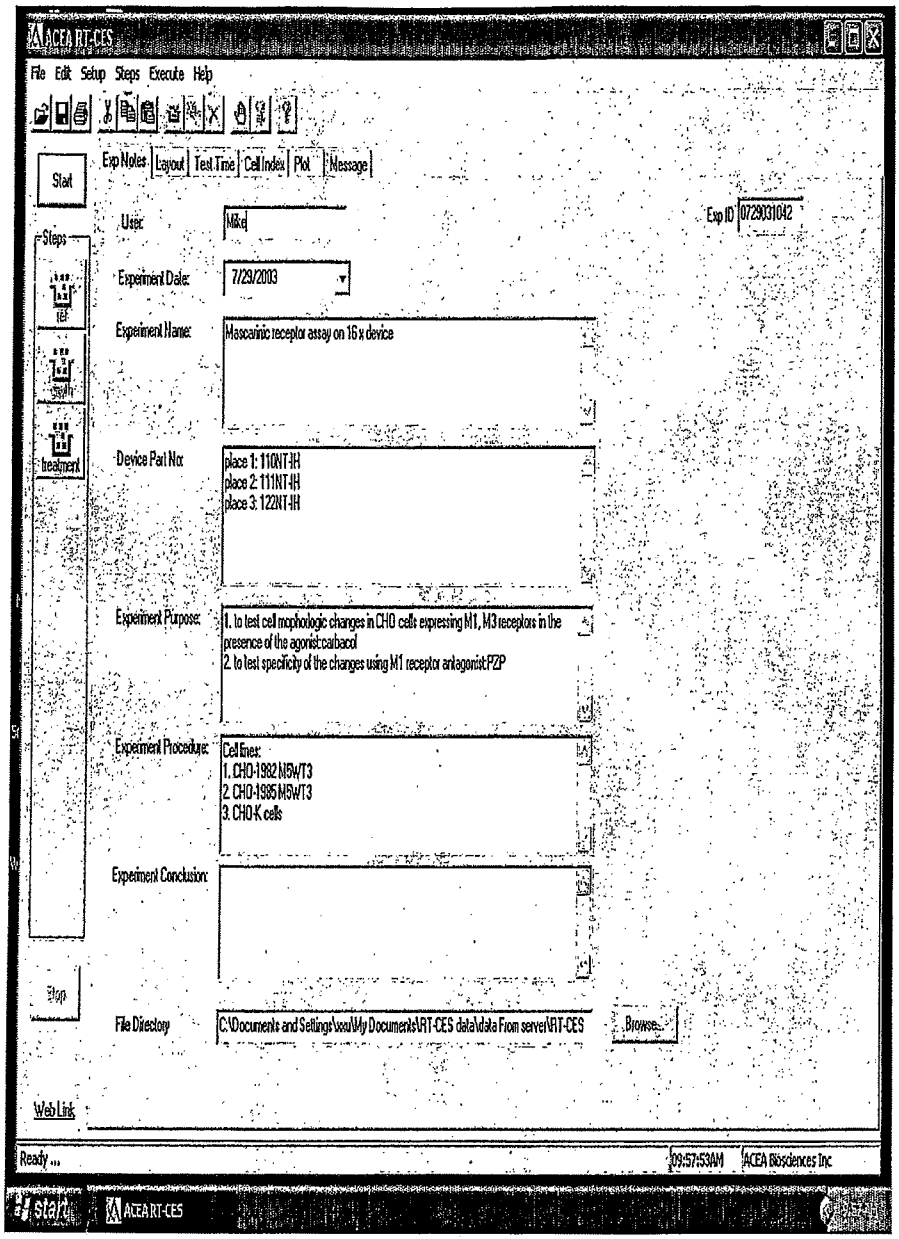

ACEA RT-CES
File Edit Setup Steps Execute Help
Start
Exp Notes
Layout
Test Time
Cell Index
Plot
Message
Steps
User:
Mike
Exp ID
0729031042
Experiment Date:
7/29/2003
Experiment Name:
Mascarinic receptor assay on 16 x device
Device Part No:
place 1: 110NT-IH
place 2: 111NT-IH
place 3: 122NT-IH
Experiment Purpose:
1. to test cell mophologic changes in CHO cells expressing M1, M3 receptors in the presence of the agonist:carbacol
2. to test specificity of the changes using M1 receptor antagonist:PZP
Experiment Procedure:
Cell lines:
1. CHO-1982 M5WT3
2. CHO-1985 M5WT3
3. CHO-K cells
Experiment Conclusion:
File Directory
C:\Documents and Settings\xxu\My Documents\RT-CES data\data From server\RT-CES
Browse...
Web Link
Ready ...
09:57:53AM
ACEA Biosciences Inc
start
ACEA RT-CES

Figure 6A

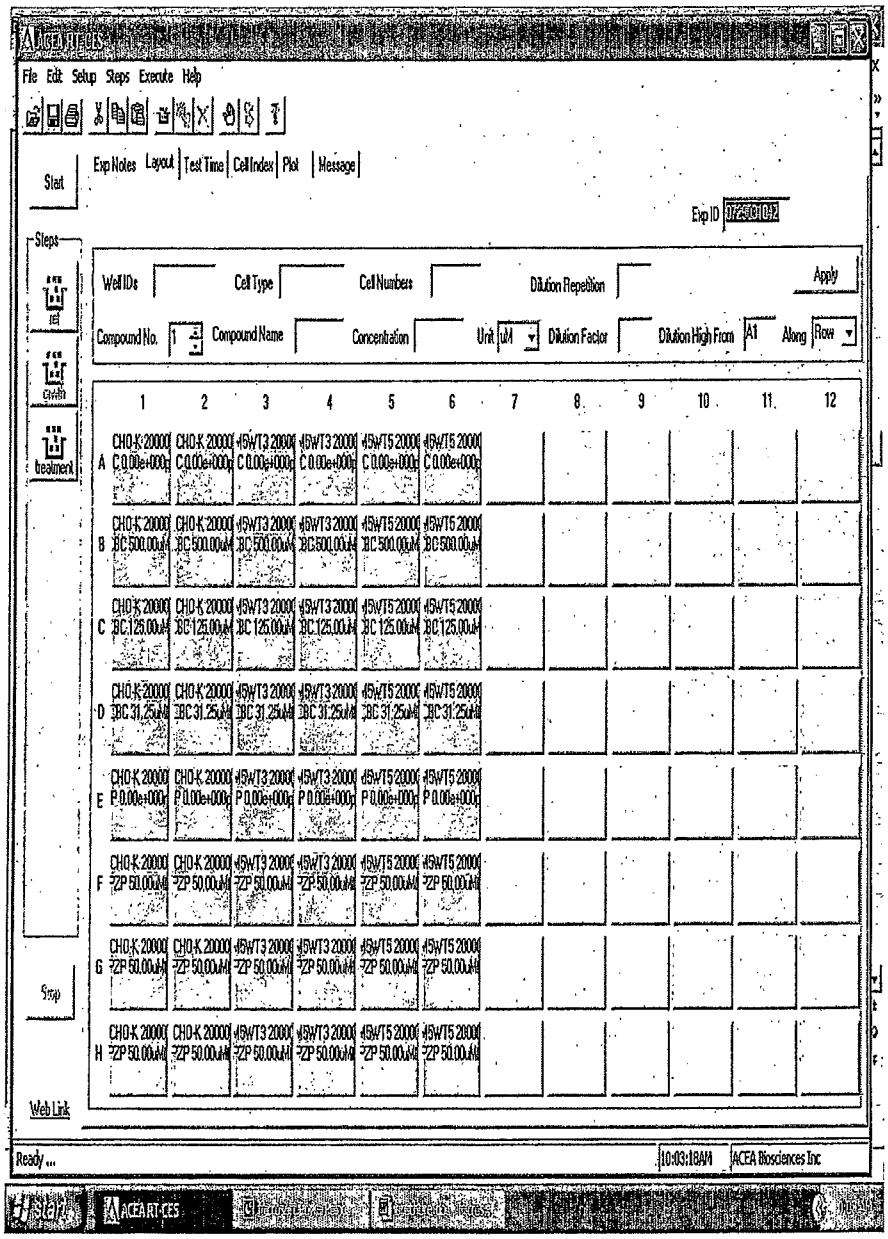

File Edit Setup Steps Execute Help
Start
Exp Notes Layout Test Time Cell Index Plot Message
Exp ID
Steps
Well IDs
Cell Type
Cell Number
Dilution Repetition
Apply
Compound No.
Compound Name
Concentration
Unit
Dilution Factor
Dilution High From
Along
Row
1 2 3 4 5 6 7 8 9 10 11 12
A CHO-K 20000 C 0.00e+000
B CHO-K 20000 BC 500.00uM
C CHO-K 20000 BC 125.00uM
D CHO-K 20000 BC 31.25uM
E CHO-K 20000 P 0.00e+000
F CHO-K 20000 ZP 50.00uM
G CHO-K 20000 ZP 50.00uM
H CHO-K 20000 ZP 50.00uM
Stop
Web Link
Ready ...
10:03:18AM
ACEA Biosciences Inc

Figure 6B

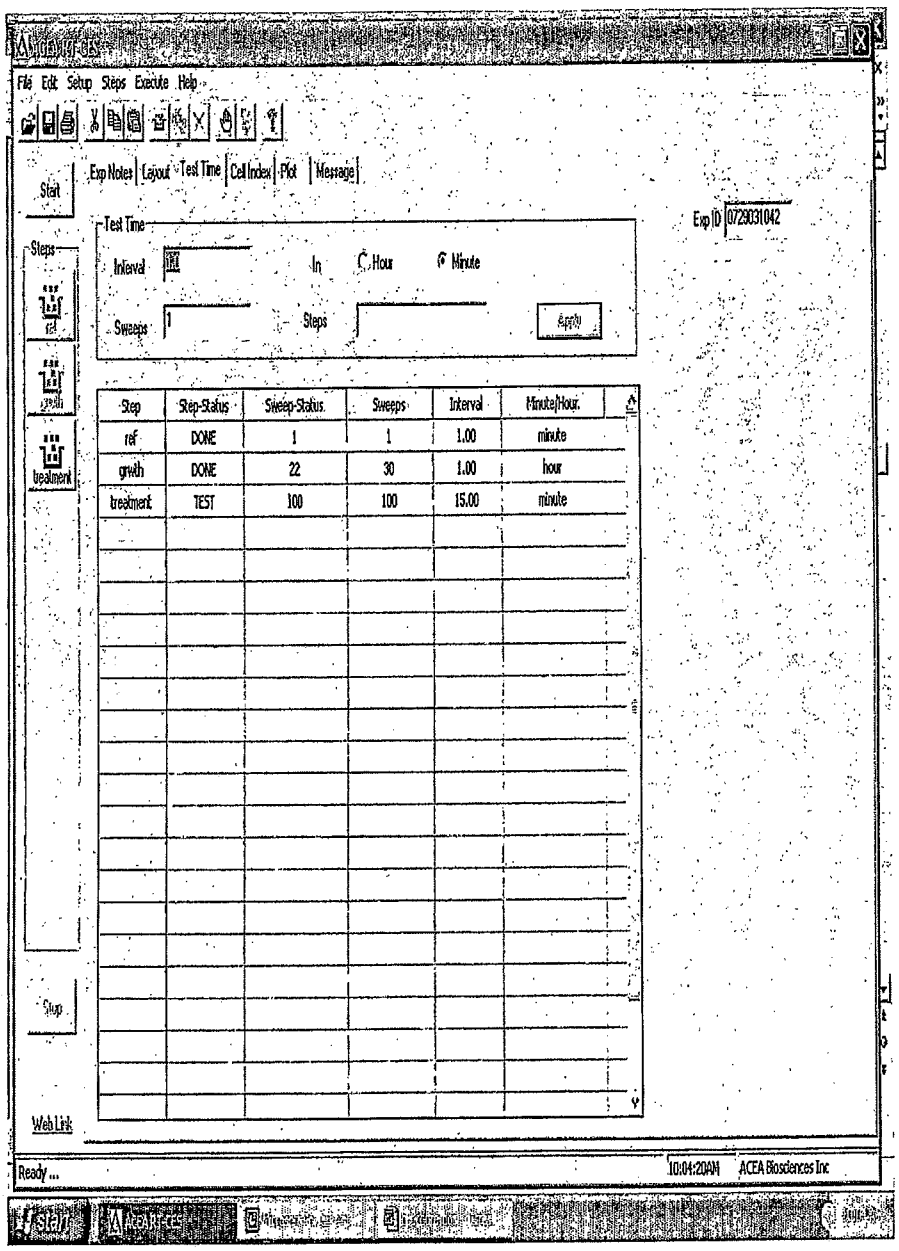

File Edit Setup Steps Execute Help
Start
Exp Notes | Layout | Test Time | Cell Index | Plot | Message
Exp ID 0729031042
Test Time
Interval
In
Hour
Minute
Sweeps
Steps
Apply
Steps
ref
growth
treatment
Stop
Web Link
Step | Step-Status | Sweep-Status | Sweeps | Interval | Minute/Hour
ref | DONE | 1 | 1 | 1.00 | minute
growth | DONE | 22 | 30 | 1.00 | hour
treatment | TEST | 100 | 100 | 15.00 | minute
Ready ...
10:04:20 AM
ACEA Biosciences Inc
start

Figure 6C

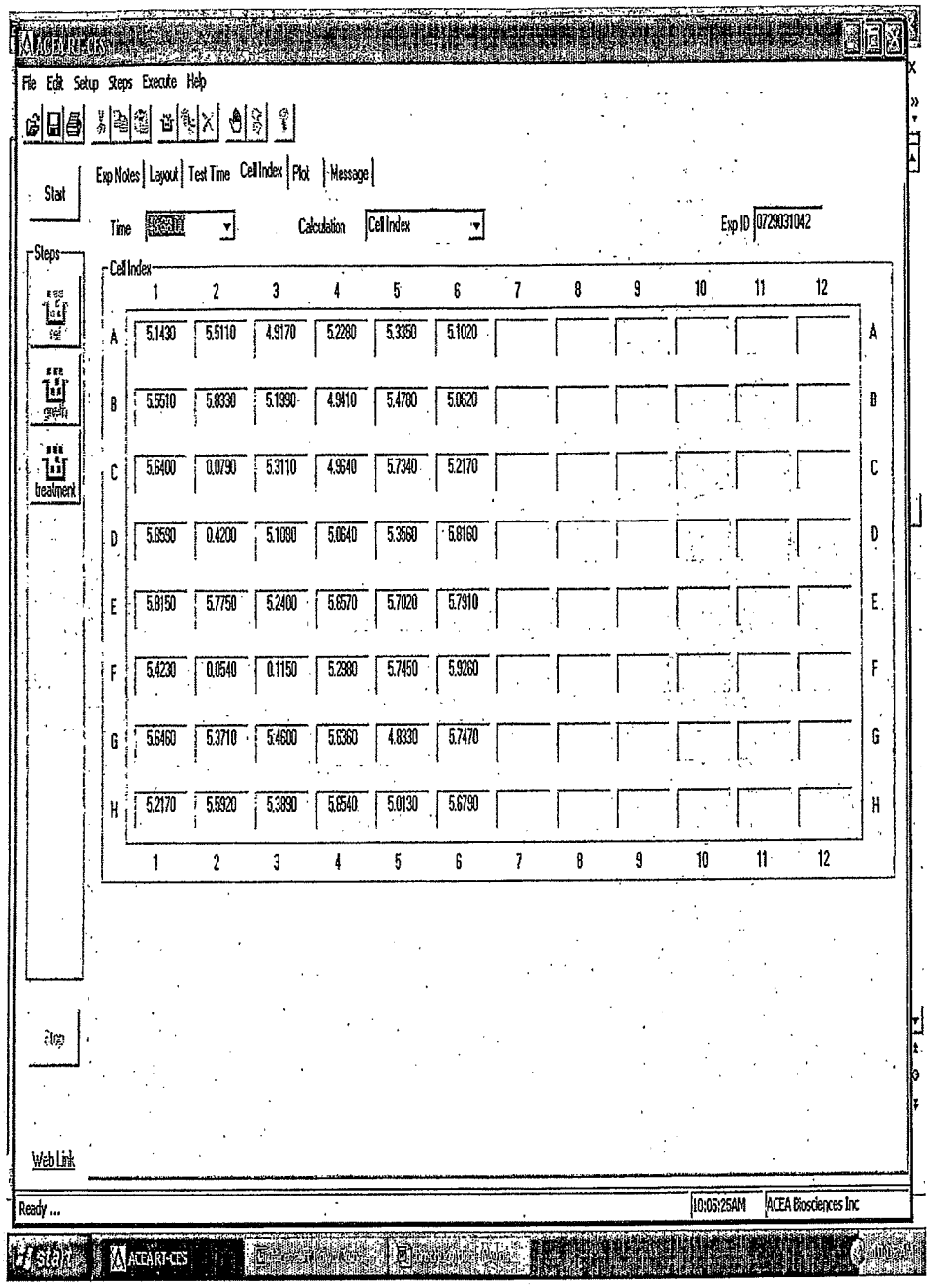

File Edit Setup Steps Execute Help
Start
Exp Notes | Layout | Test Time | Cell Index | Plot | Message
Time
Calculation
Cell Index
Exp ID 0729931042
Steps
Cell Index
1 2 3 4 5 6 7 8 9 10 11 12
A 5.1430 5.5110 4.9170 5.2280 5.3350 5.1020
B 5.5510 5.8330 5.1990 4.9410 5.4780 5.0620
C 5.6400 0.0790 5.3110 4.9640 5.7340 5.2170
D 5.8590 0.4200 5.1090 5.0640 5.3560 5.8160
E 5.8150 5.7750 5.2400 5.6570 5.7020 5.7910
F 5.4230 0.0540 0.1150 5.2980 5.7450 5.9260
G 5.6460 5.3710 5.4600 5.6360 4.8330 5.7470
H 5.2170 5.5920 5.3890 5.6540 5.0130 5.6790
Stop
Web Link
Ready ...
10:05:25AM
ACEA Biosciences Inc
start
ACEA RT-CES

Figure 6D

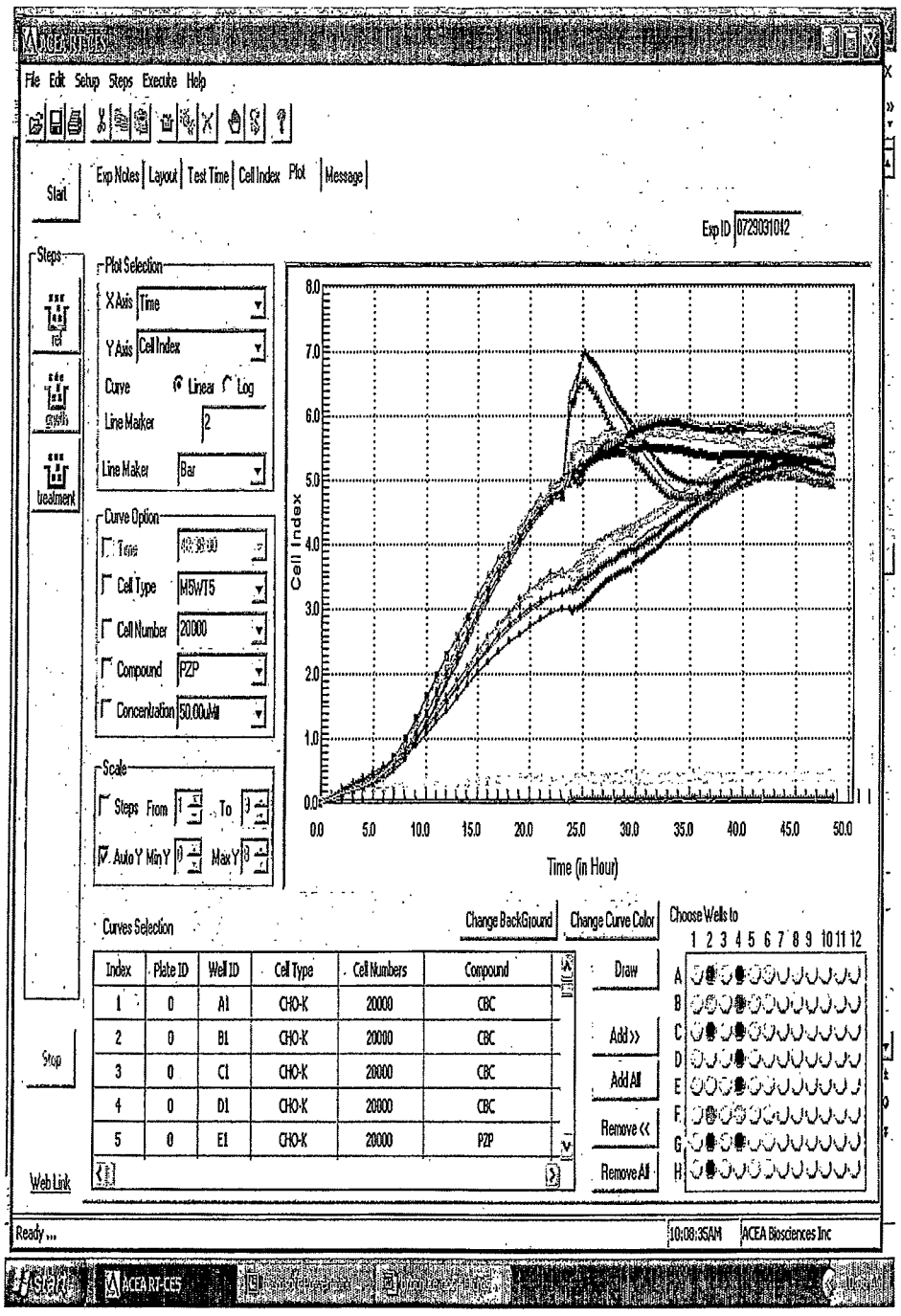

File Edit Setup Steps Execute Help
Start
Exp Notes
Layout
Test Time
Cell Index
Plot
Message
Exp ID 0729031042
Steps
Plot Selection
X Axis Time
Y Axis Cell Index
Curve Linear Log
Line Marker 2
Line Maker Bar
Curve Option
Time
Cell Type M5WT5
Cell Number 20000
Compound PZP
Concentration 50.00uM
Scale
Steps From 1 To 3
Auto Y Min Y 0 Max Y 3
Cell Index
Time (in Hour)
Curves Selection
Change BackGround
Change Curve Color
Choose Wells to
Index Plate ID Well ID Cell Type Cell Numbers Compound
1 0 A1 CHO-K 20000 CBC
2 0 B1 CHO-K 20000 CBC
3 0 C1 CHO-K 20000 CBC
4 0 D1 CHO-K 20000 CBC
5 0 E1 CHO-K 20000 PZP
Draw
Add
Add All
Remove
Remove All
Stop
Web Link
Ready ...
10:08:35AM
ACEA Biosciences Inc
ACEA RT-CES

Figure 6E

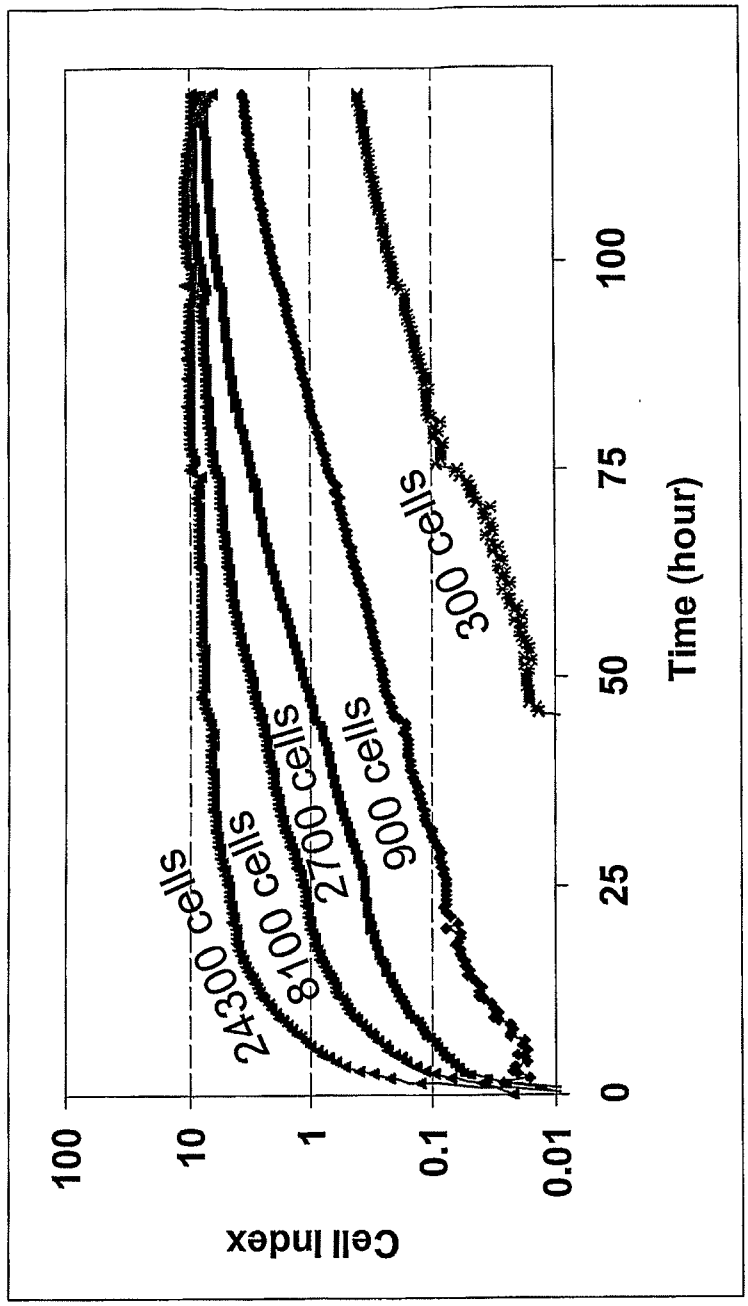

Cell Index
100
10
1
0.1
0.01
0
25
50
75
100
Time (hour)
24300 cells
8100 cells
2700 cells
900 cells
300 cells

Figure 7.

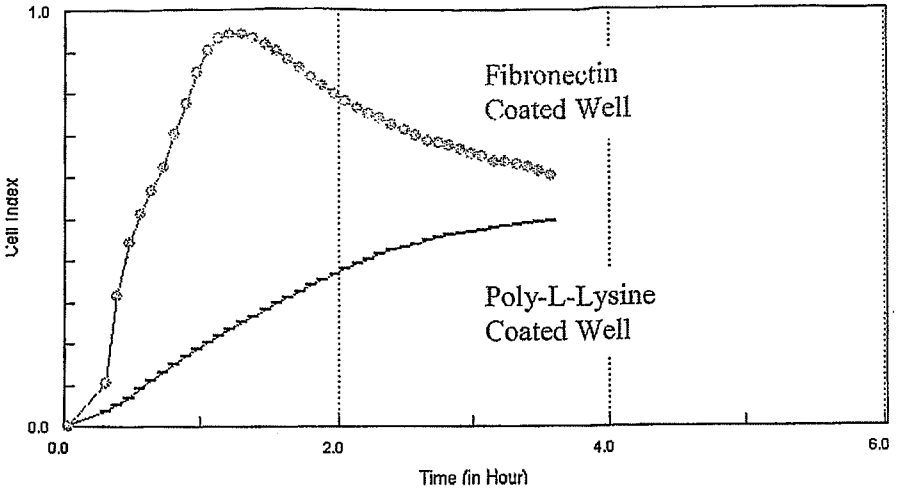

1.0
0.0
Cell Index
0.0
2.0
4.0
6.0
Time (in Hour)
Fibronectin
Coated Well
Poly-L-Lysine
Coated Well

Figure 8.

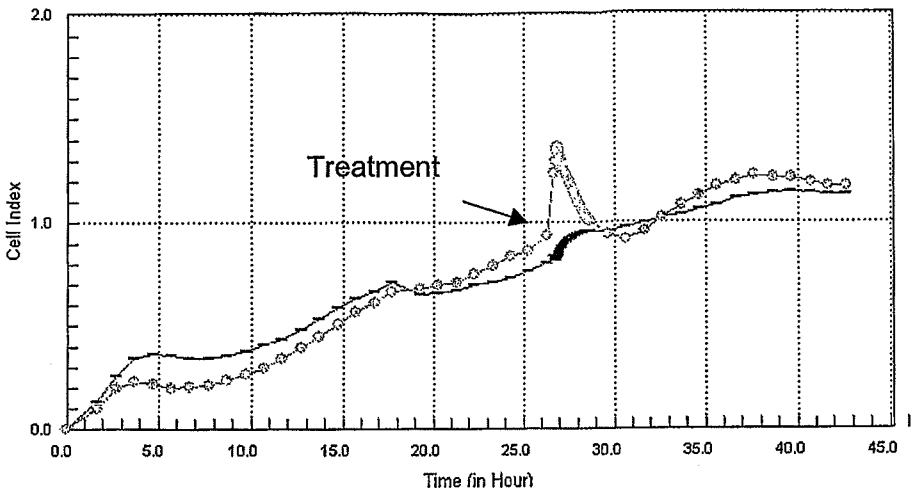

2.0
1.0
0.0
Cell Index
Treatment
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
Time (in Hour)

Figure 9.

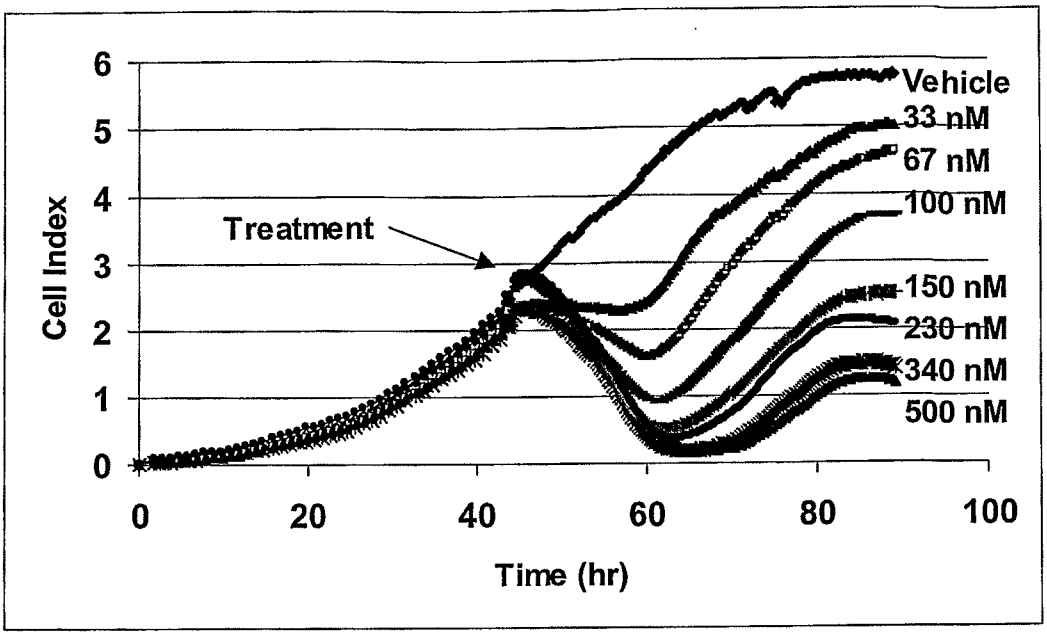

6
5
4
3
2
1
0
Cell Index
Treatment
Vehicle
33 nM
67 nM
100 nM
150 nM
230 nM
340 nM
500 nM
0
20
40
60
80
100
Time (hr)

Figure 10.

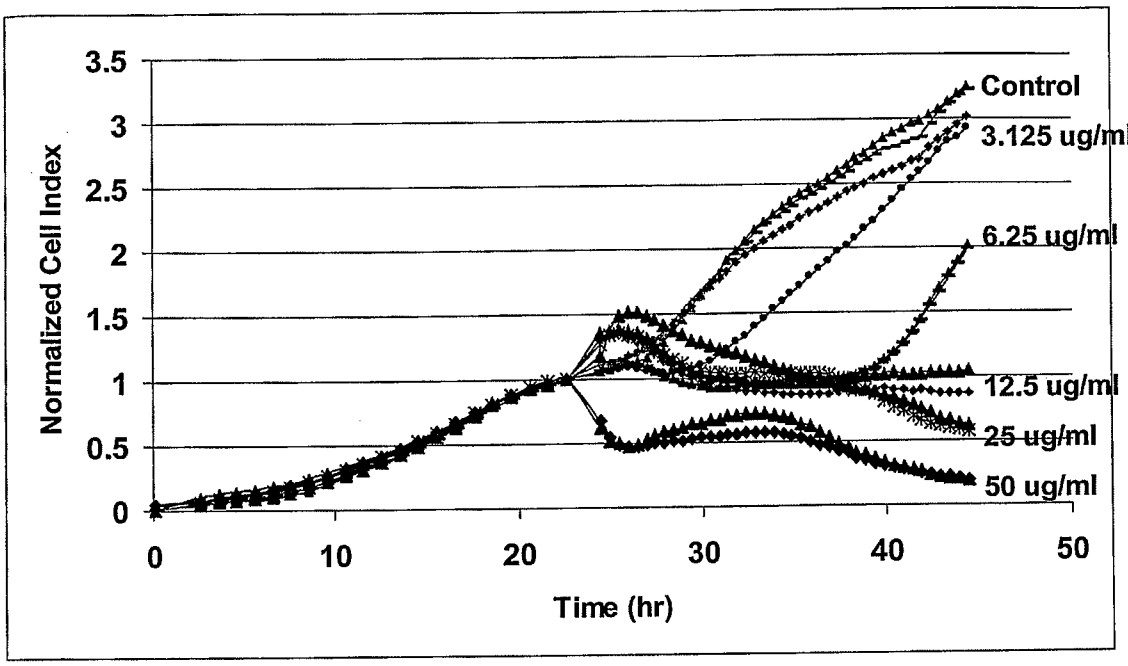

Normalized Cell Index
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
Time (hr)
Control
3.125 ug/ml
6.25 ug/ml
12.5 ug/ml
25 ug/ml
50 ug/ml

Figure 11.

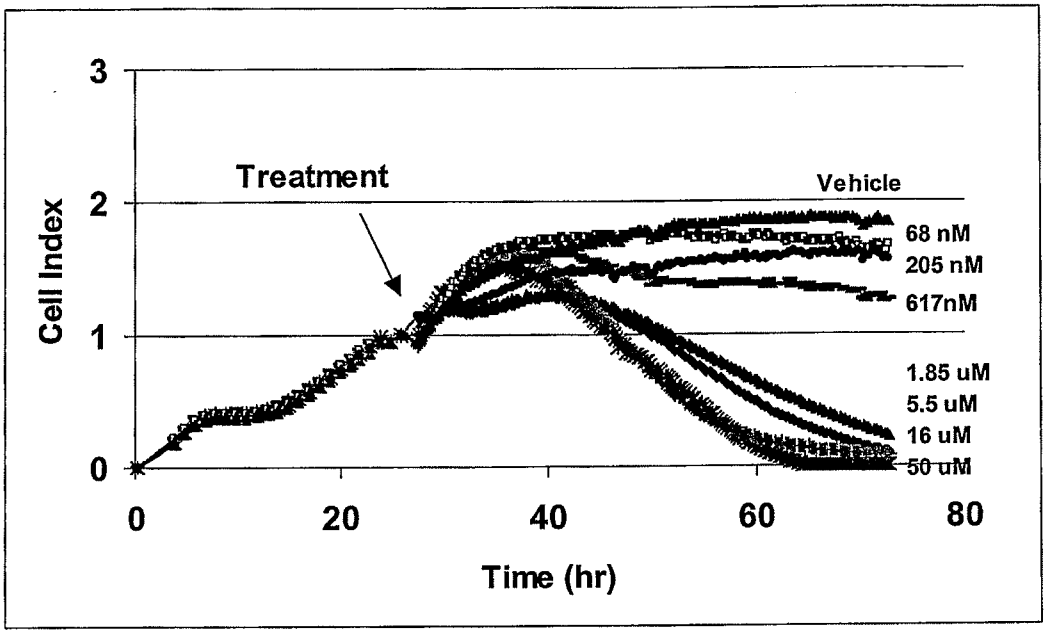
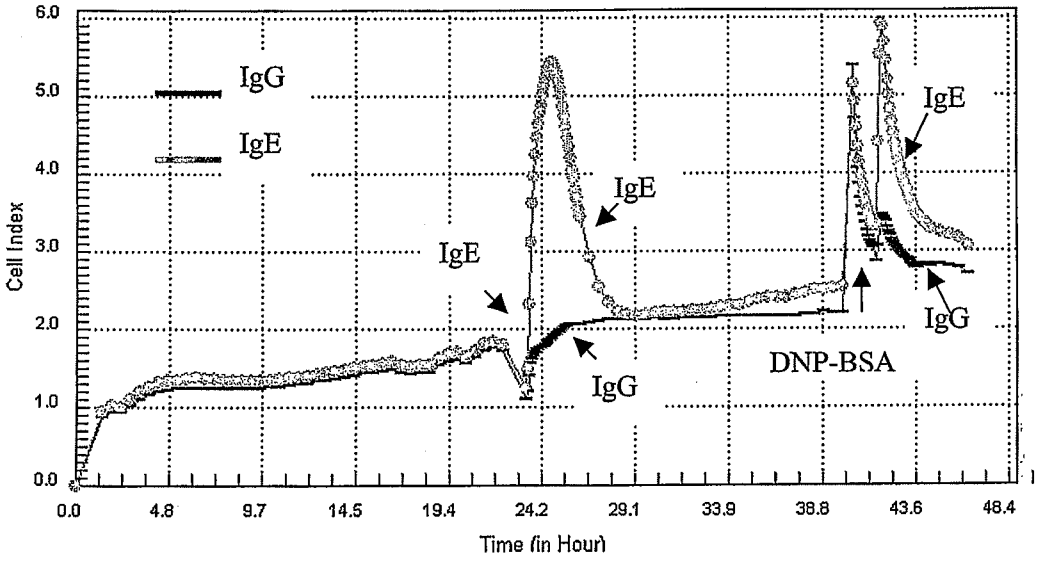

3
2
1
0
Cell Index
Treatment
Vehicle
68 nM
205 nM
617nM
1.85 uM
5.5 uM
16 uM
50 uM
0
20
40
60
80
Time (hr)

Figure 12.

Figure 13.


6.0
5.0
4.0
3.0
2.0
1.0
0.0
Cell Index
0.0
4.8
9.7
14.5
19.4
24.2
29.1
33.9
38.8
43.6
48.4
Time (in Hour)
IgG
IgE
IgE
IgE
IgG
DNP-BSA
IgE
IgG

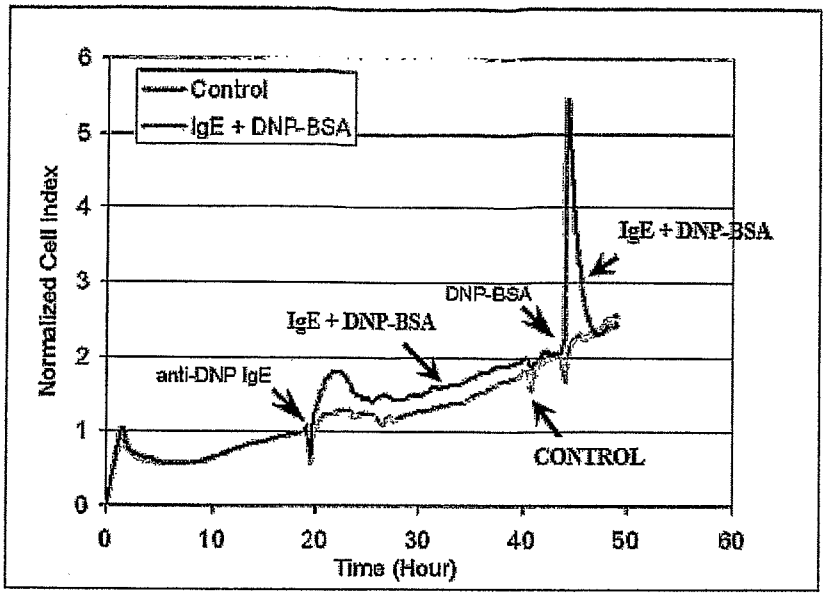

Control
IgE + DNP-BSA
Normalized Cell Index
Time (Hour)
anti-DNP IgE
IgE + DNP-BSA
DNP-BSA
IgE + DNP-BSA
CONTROL
0
1
2
3
4
5
6
0
10
20
30
40
50
60

Figure 14.

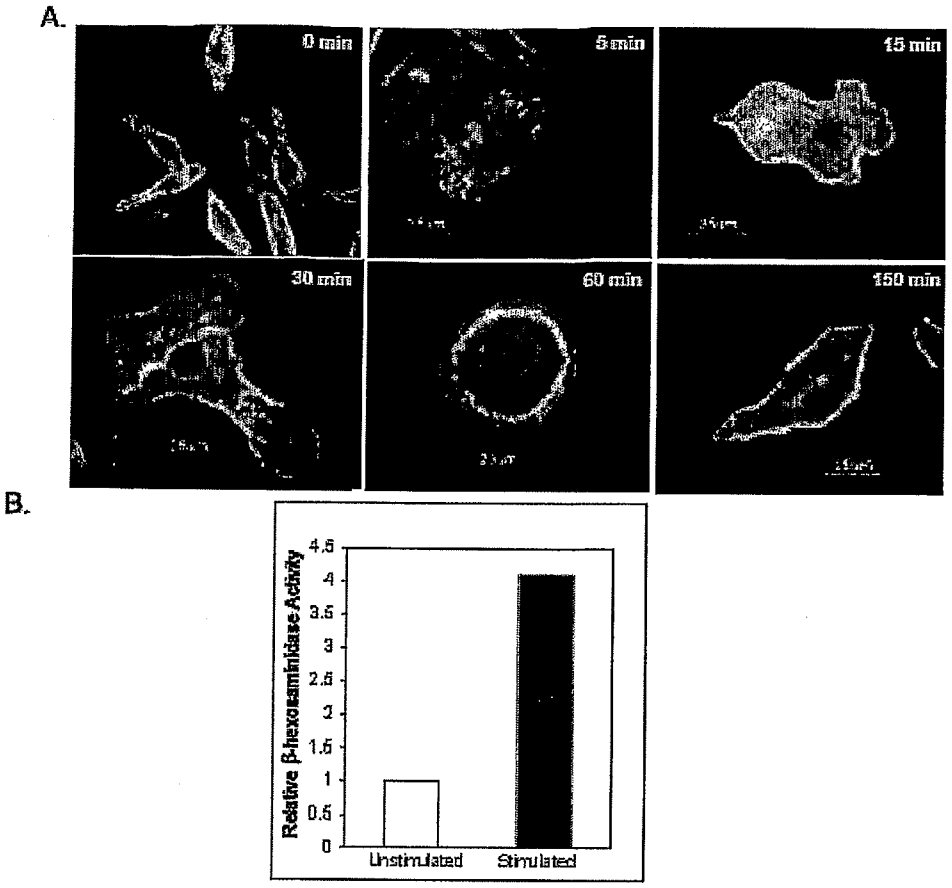

A.
0 min
5 min
15 min
30 min
60 min
150 min
B.
Relative β-hexosaminidase Activity
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Unstimulated
Stimulated

Figure 15.

A.

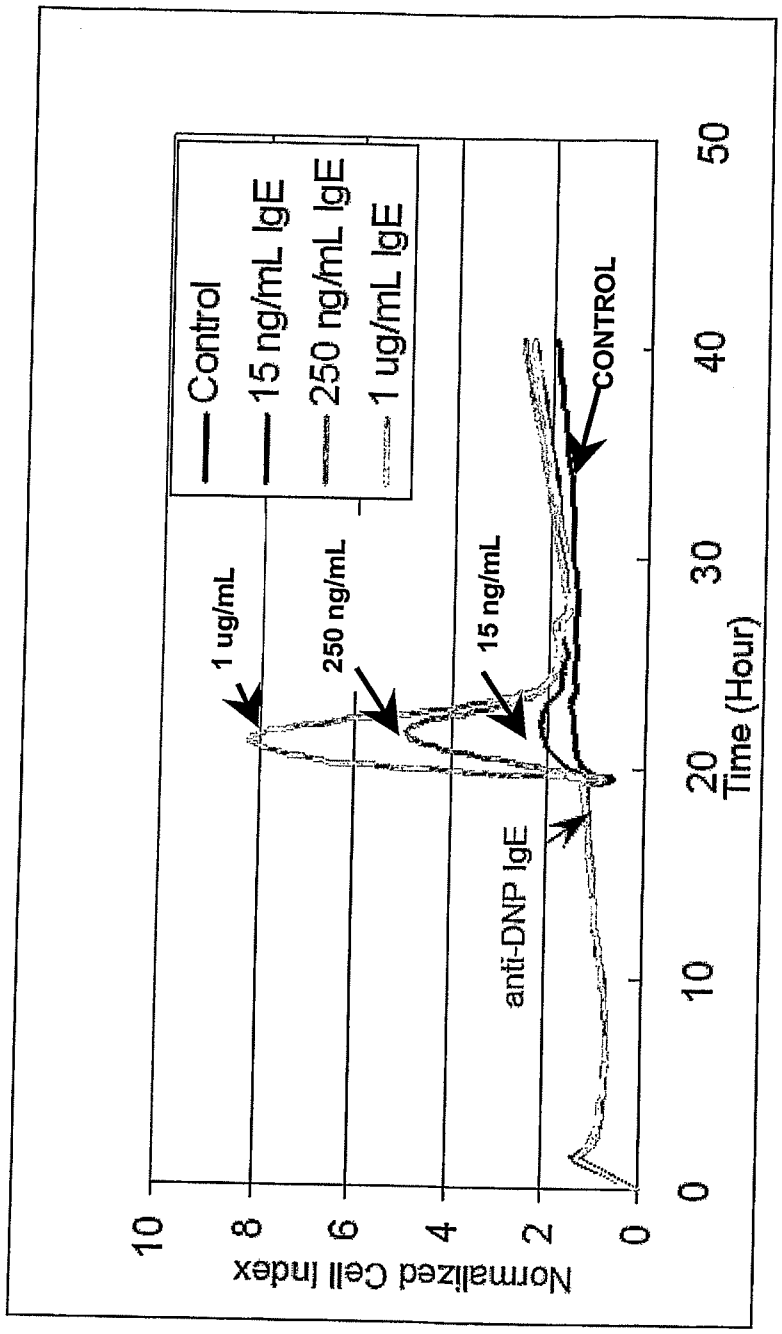

Control
15 ng/mL IgE
250 ng/mL IgE
1 ug/mL IgE
1 ug/mL
250 ng/mL
15 ng/mL
anti-DNP IgE
CONTROL
Normalized Cell Index
Time (Hour)
0
2
4
6
8
10
0
10
20
30
40
50

Figure 16A.

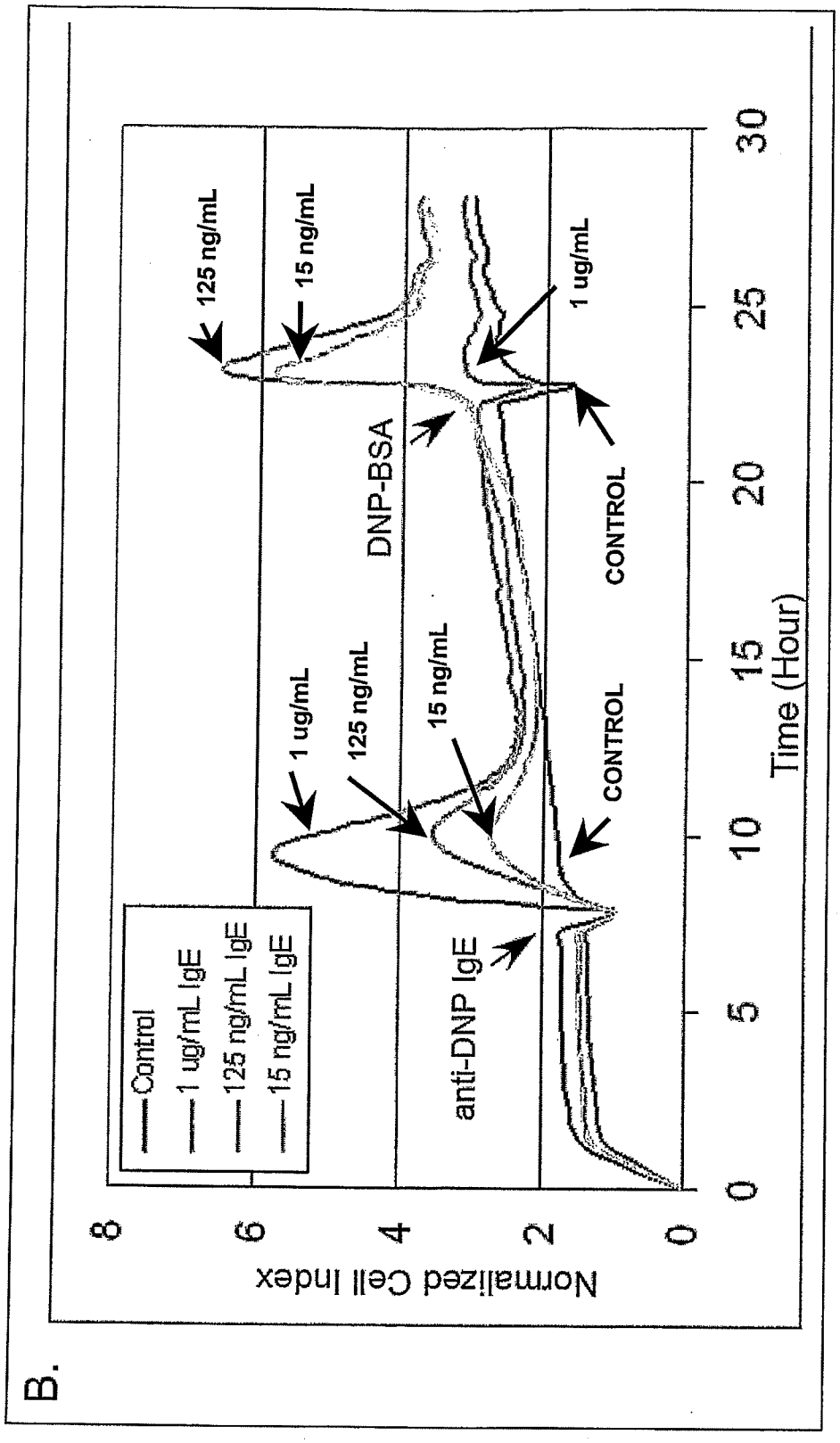

B.
Normalized Cell Index
Time (Hour)
Control
1 ug/mL IgE
125 ng/mL IgE
15 ng/mL IgE
anti-DNP IgE
DNP-BSA
CONTROL
CONTROL
1 ug/mL
125 ng/mL
15 ng/mL
1 ug/mL
125 ng/mL
15 ng/mL

Figure 16B.

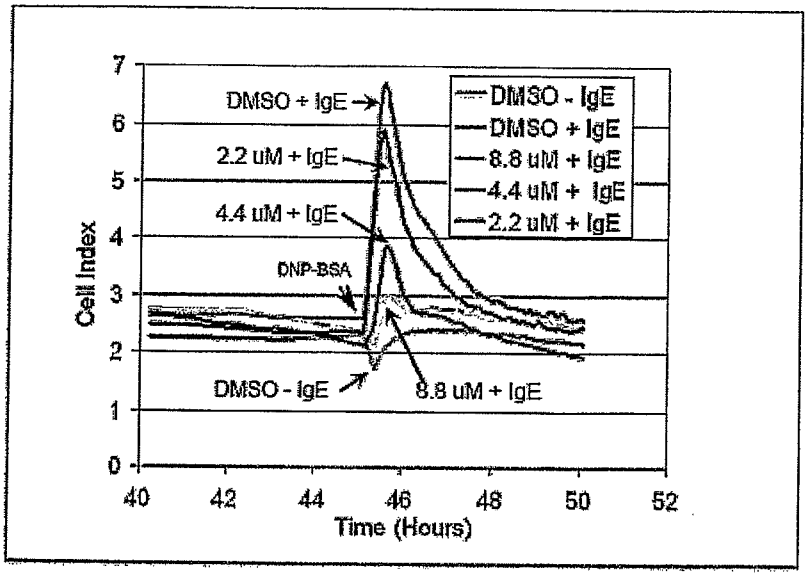

DMSO - IgE
DMSO + IgE
8.8 uM + IgE
4.4 uM + IgE
2.2 uM + IgE
DMSO + IgE
2.2 uM + IgE
4.4 uM + IgE
DNP-BSA
DMSO - IgE
8.8 uM + IgE
Cell Index
Time (Hours)
0
1
2
3
4
5
6
7
40
42
44
46
48
50
52

Figure 17.

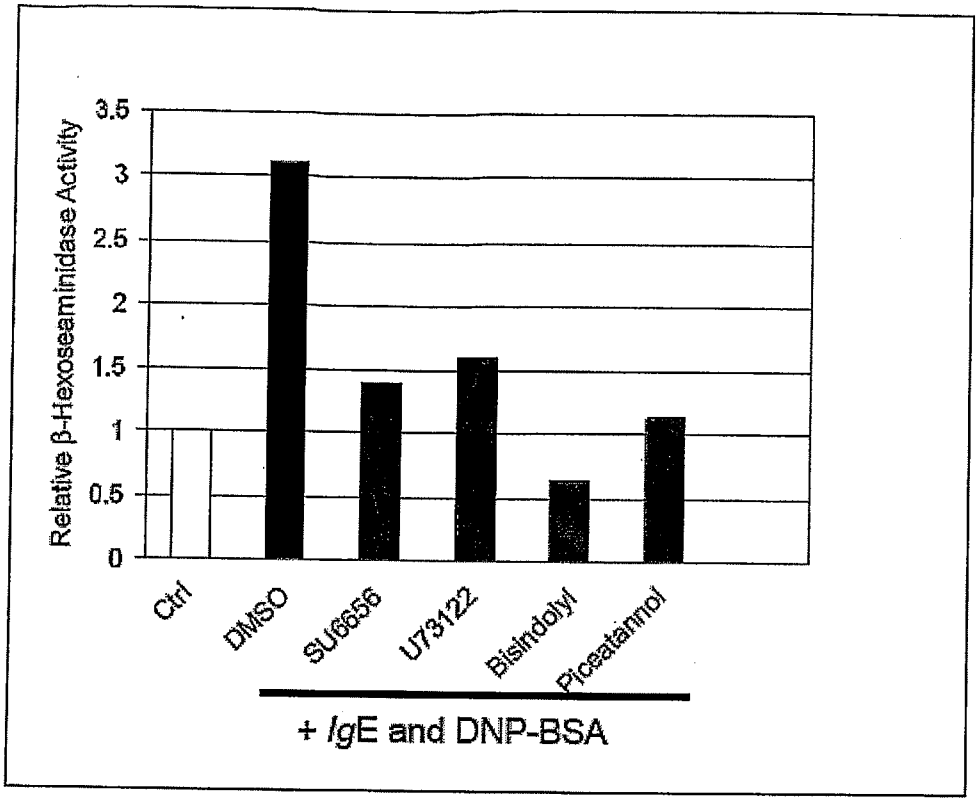

Relative β-Hexoseaminidase Activity
3.5
3
2.5
2
1.5
1
0.5
0
Ctrl
DMSO
SU6656
U73122
Bisindolyl
Piceatannol
+ IgE and DNP-BSA

Figure 18.