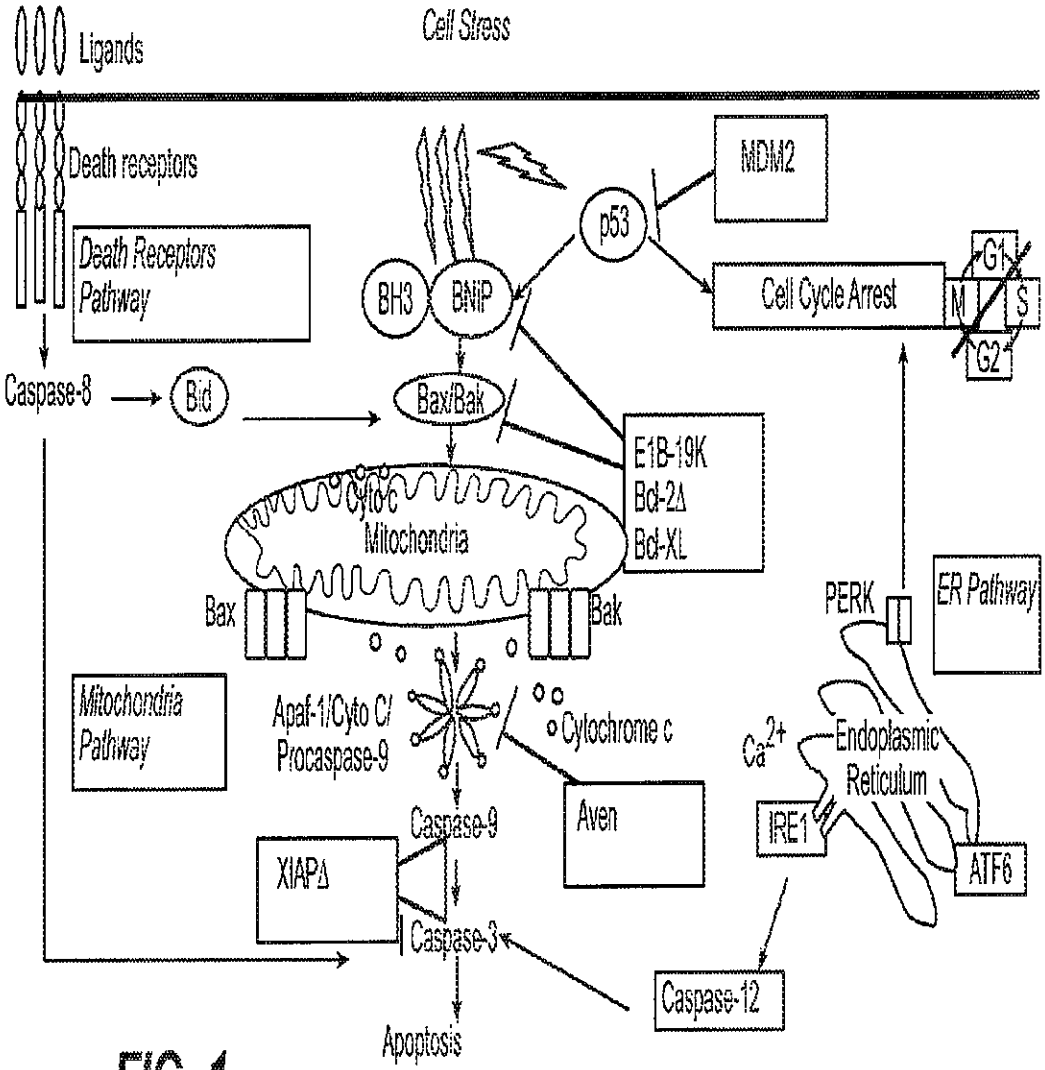
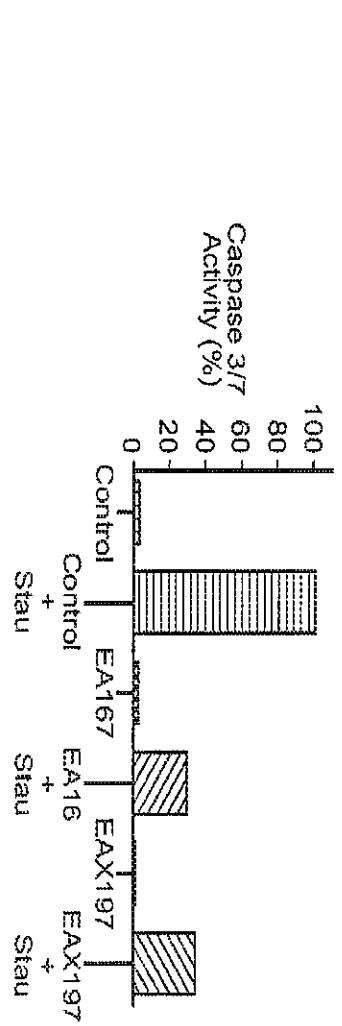

Ligands
Cell Stress
Death receptors
Death Receptors Pathway
Caspase-8
Bid
BH3
BNIP
p53
MDM2
Cell Cycle Arrest
G1
M
S
G2
Bax/Bak
E1B-19K
Bcl-2Δ
Bcl-XL
Cyto C
Mitochondria
Bax
Bak
PERK
ER Pathway
Mitochondria Pathway
Apaf-1/Cyto C/
Procaspase-9
Cytochrome c
Ca2+
Endoplasmic Reticulum
Caspase-9
Aven
IRE1
ATF6
XIAPΔ
Caspase-3
Caspase-12
Apoptosis

FIG. 1



2/19

FIG. 2A

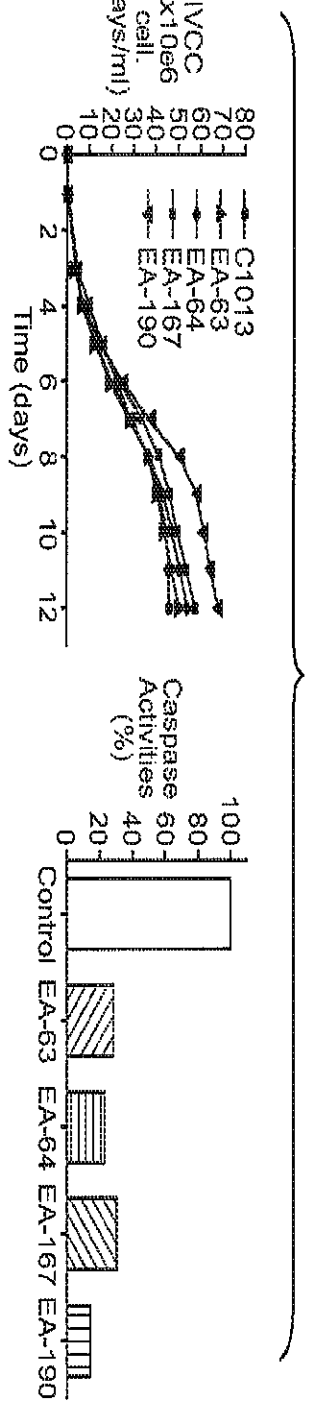


FIG. 2B

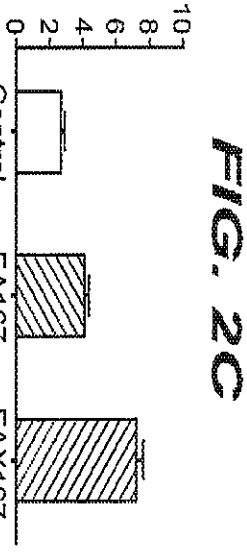


FIG. 2C

FIG. 3A

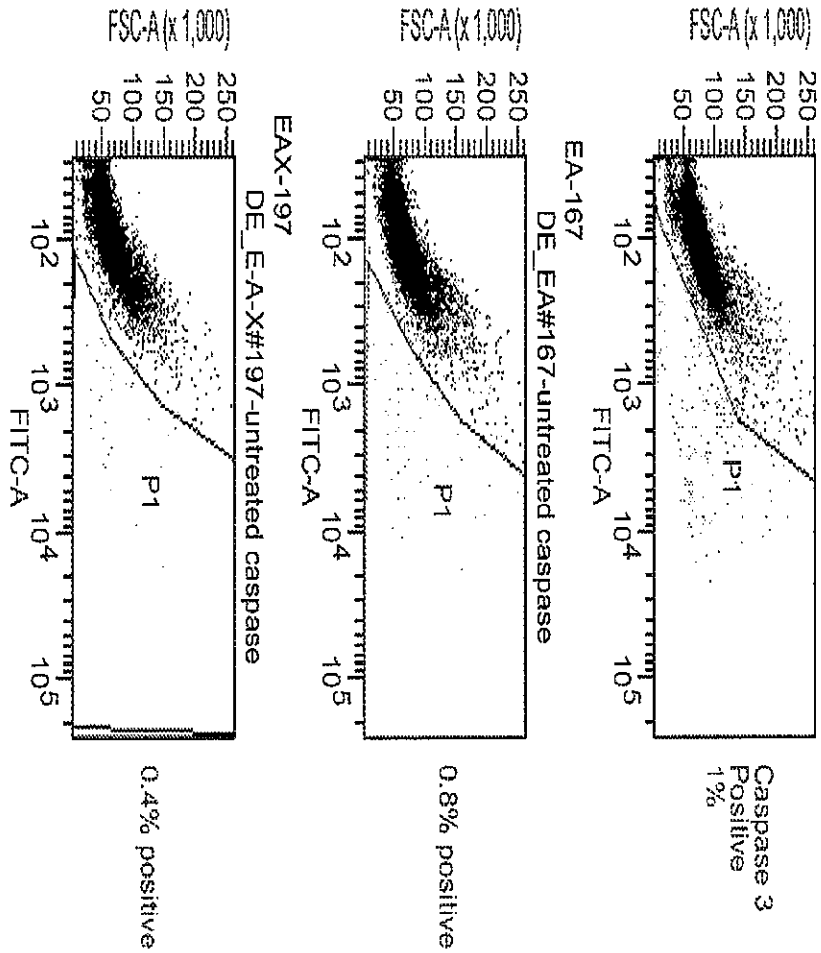

Untreated
C1013
DEC1013-untreated caspase 3
FSC-A (x 1,000)
250
200
150
100
50
P1
10^2
10^3
10^4
10^5
FITC-A
Caspase 3 Positive 1%
EA-167
DE_EA#167-untreated caspase
FSC-A (x 1,000)
P1
FITC-A
0.8% positive
EAX-197
DE_E-A-X#197-untreated caspase
FSC-A (x 1,000)
P1
FITC-A
0.4% positive

FIG. 3B

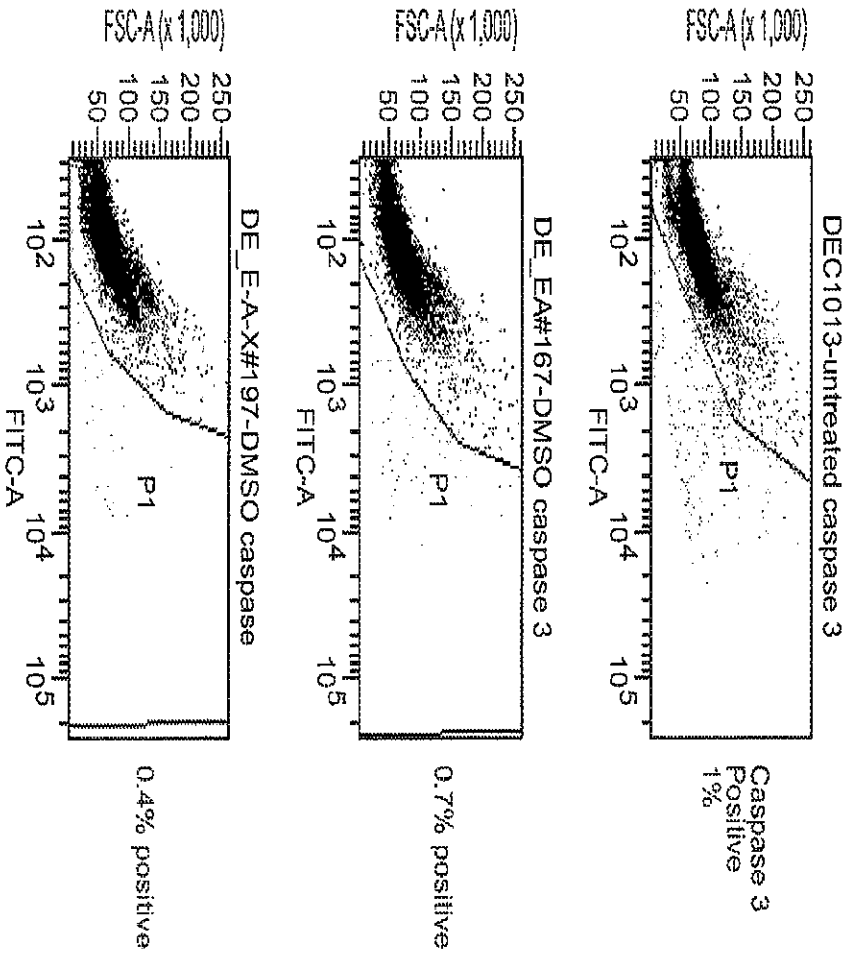
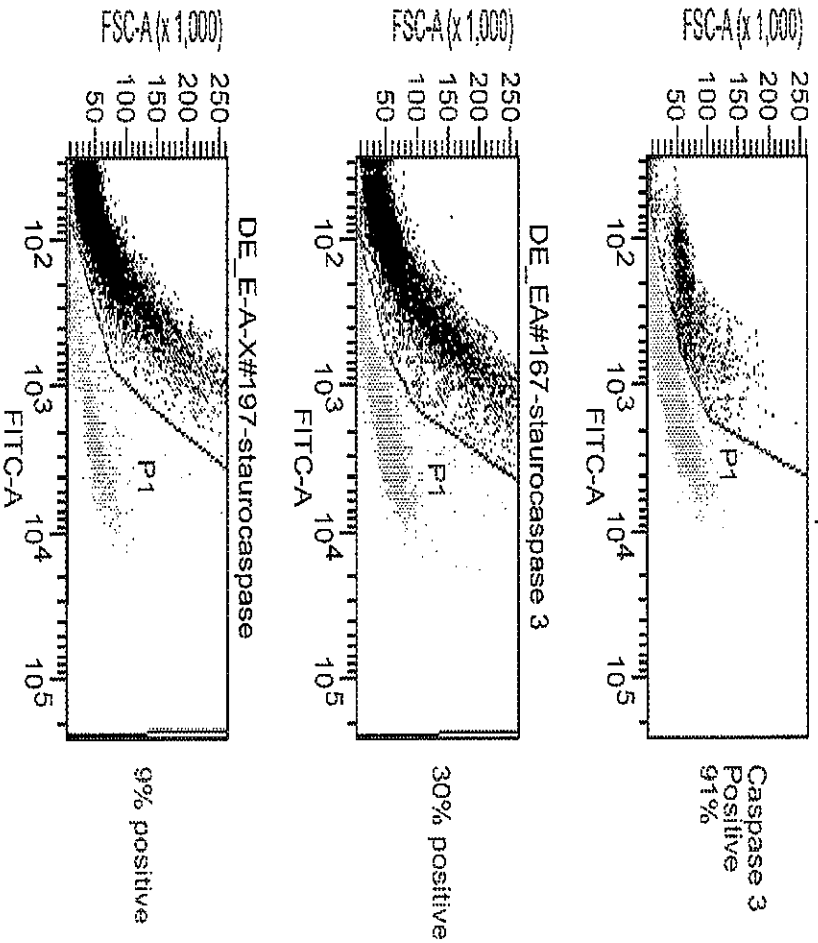

DMSO (Vehicle) treated
DEC1013-untreated caspase 3
FSC-A (x 1,000)
FITC-A
P1
Caspase 3 Positive 1%
DE_EA#167-DMSO caspase 3
0.7% positive
DE_E-A-X#197-DMSO caspase
0.4% positive

FIG. 3C


Staurosporine treated
DEC1013-staurocaspase 3
FSC-A (x 1,000)
250
200
150
100
50
P1
10^2
10^3
10^4
10^5
FITC-A
Caspase 3 Positive 91%
DE_EA#167-staurocaspase 3
30% positive
DE_E-A-X#197-staurocaspase
9% positive

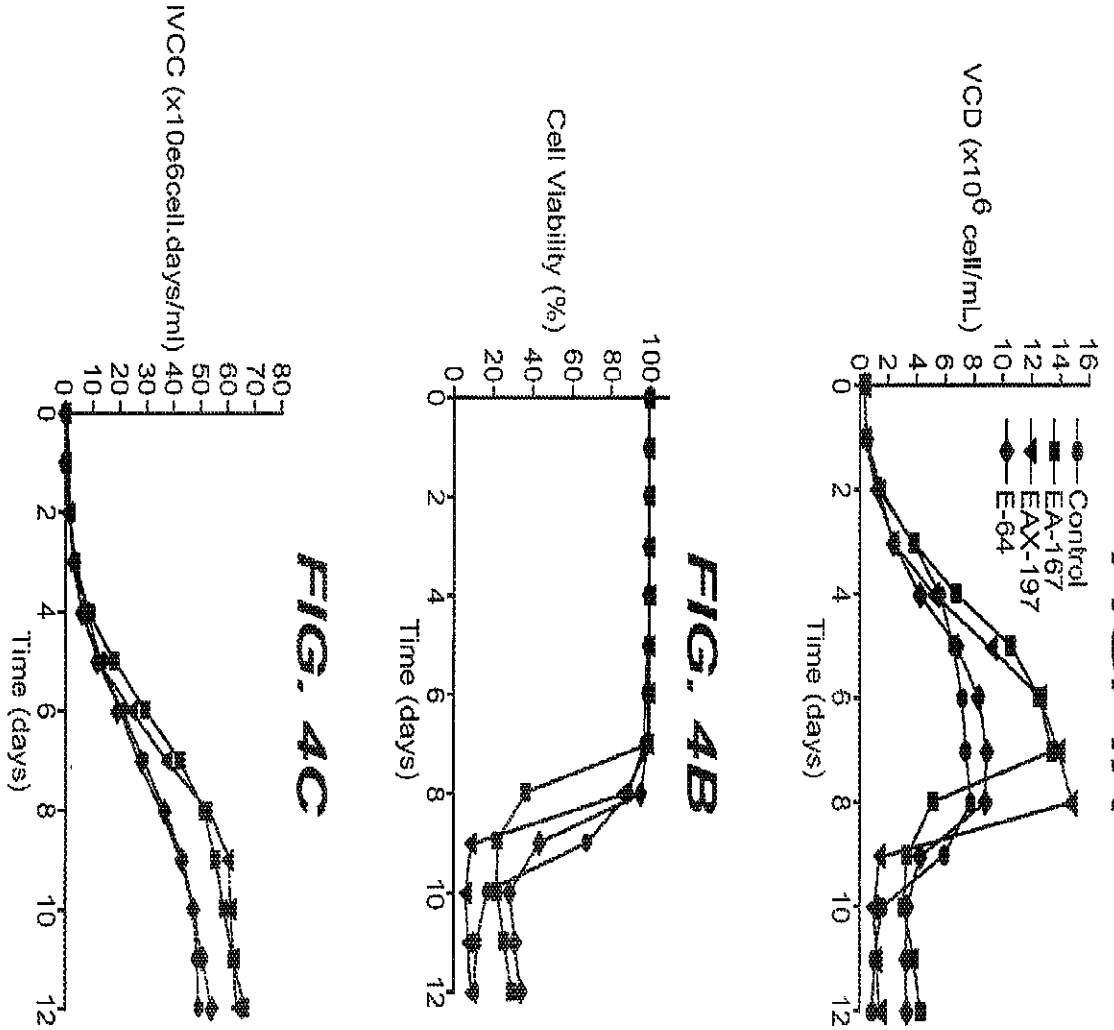


FIG. 4A

FIG. 4B

FIG. 4C

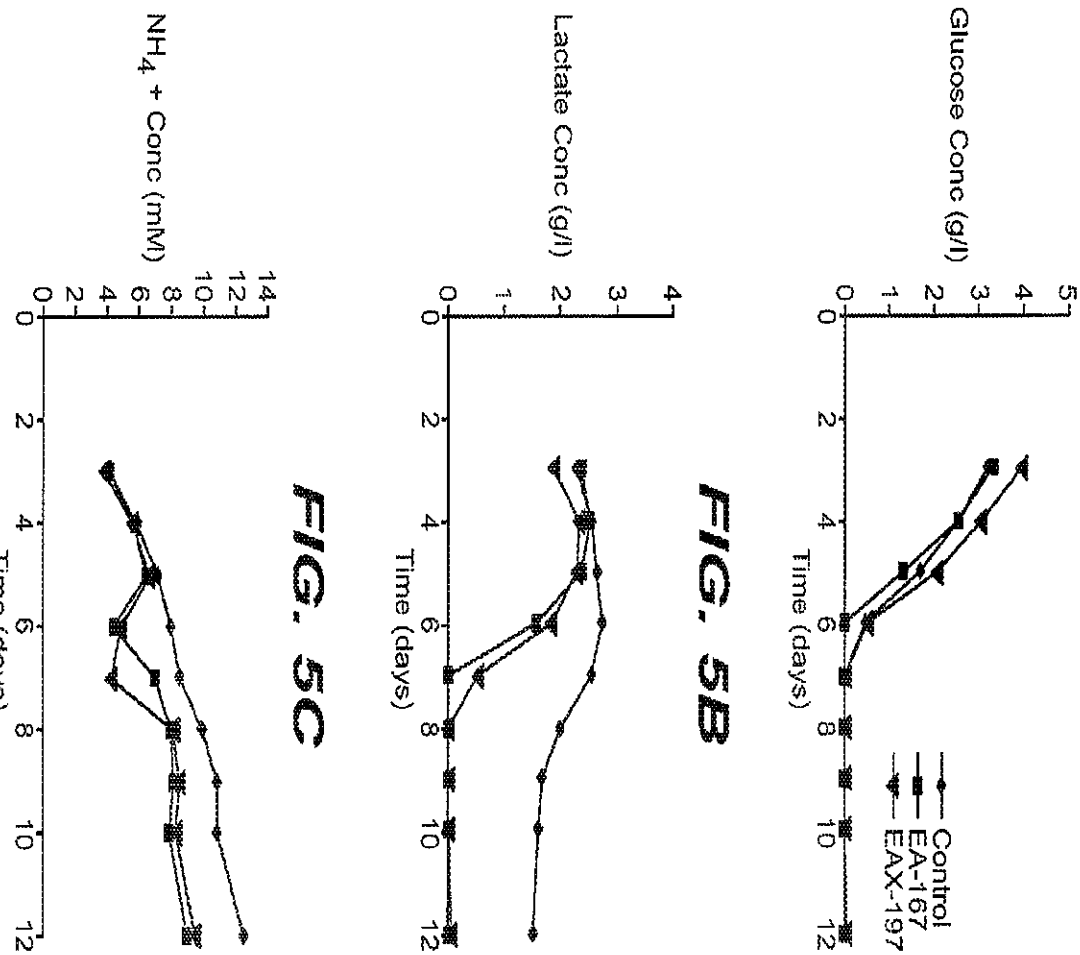


FIG. 5A

FIG. 5B

FIG. 5C

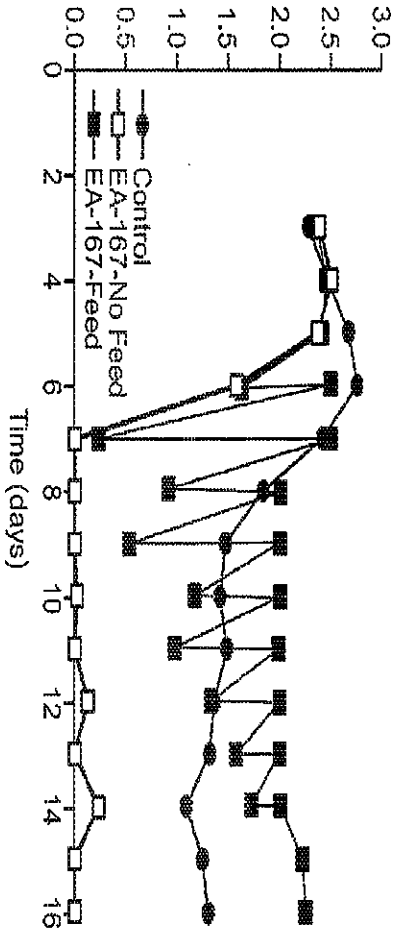

Lactate Conc (g/l)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
Time (days)
Control
EA-167-No Feed
EA-167-Feed

FIG. 6A

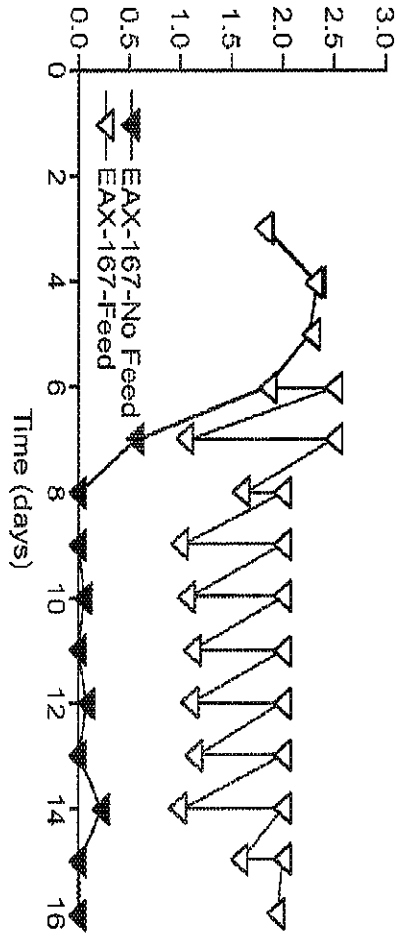

Lactate Conc (g/l)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
Time (days)
EAX-167-No Feed
EAX-167-Feed

FIG. 6B

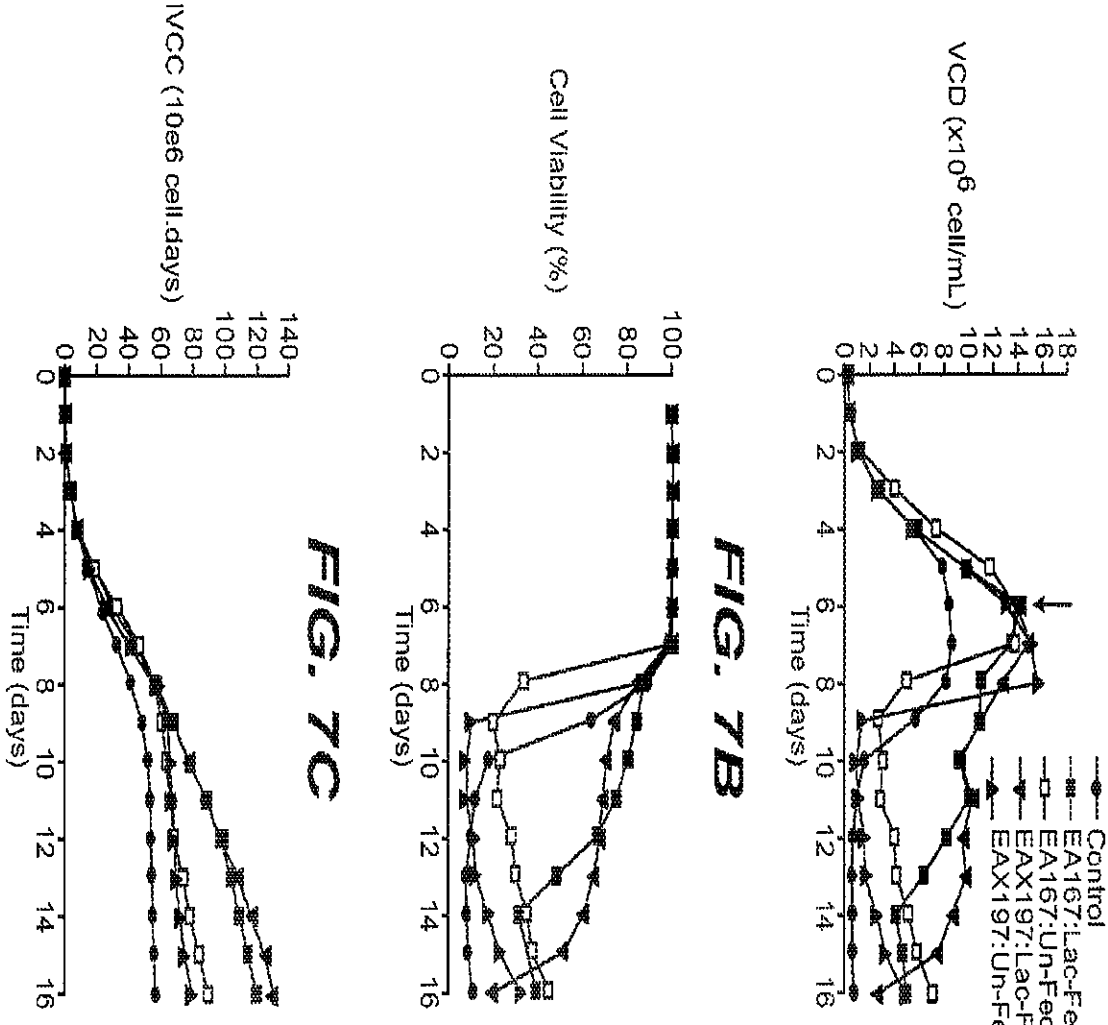


FIG. 7A

FIG. 7B

FIG. 7C

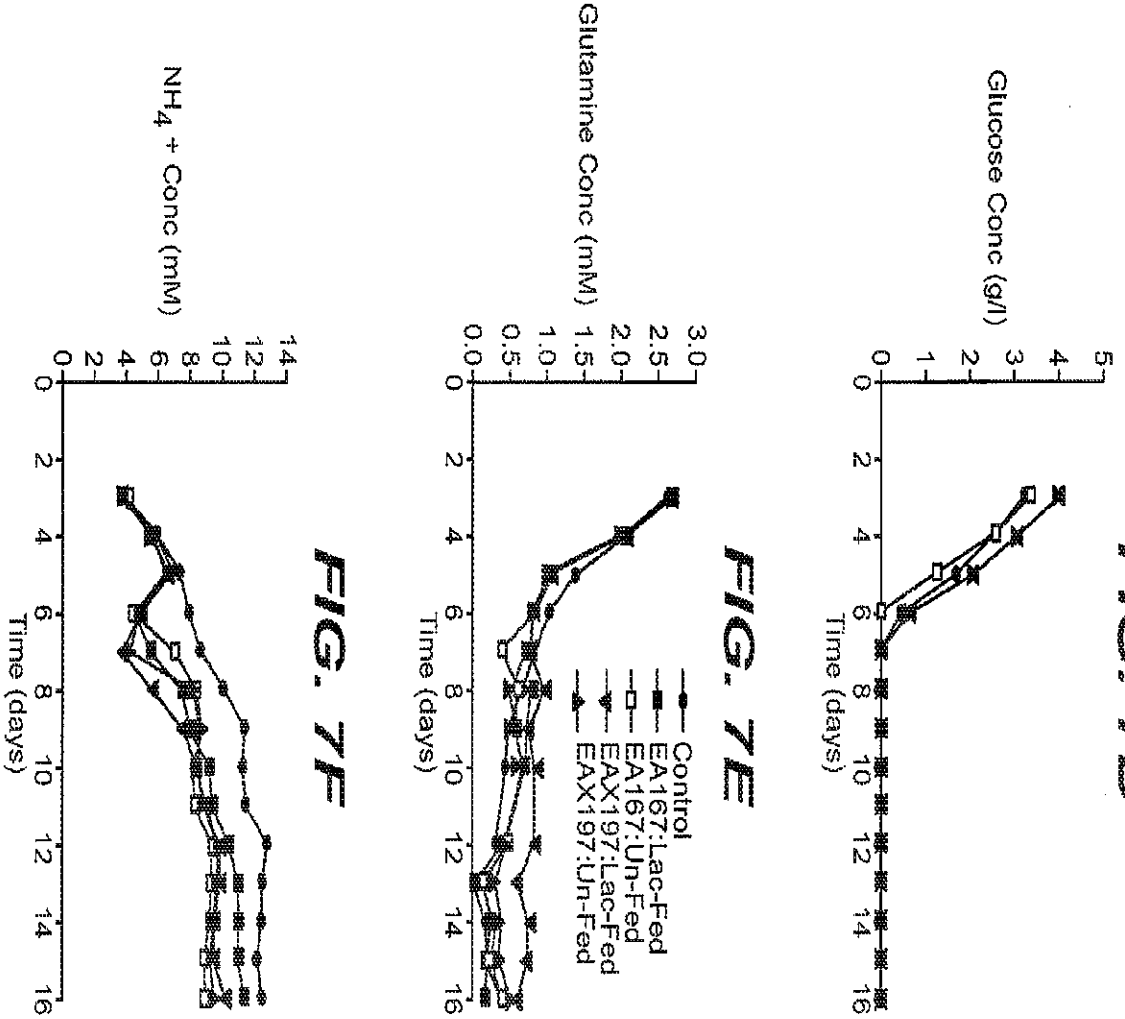


FIG. 7D

FIG. 7E

FIG. 7F

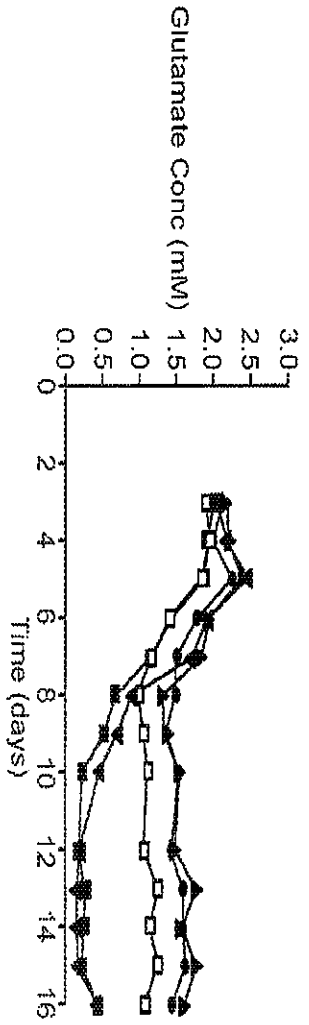

Glutamate Conc (mM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
14
16
Time (days)

FIG. 7G

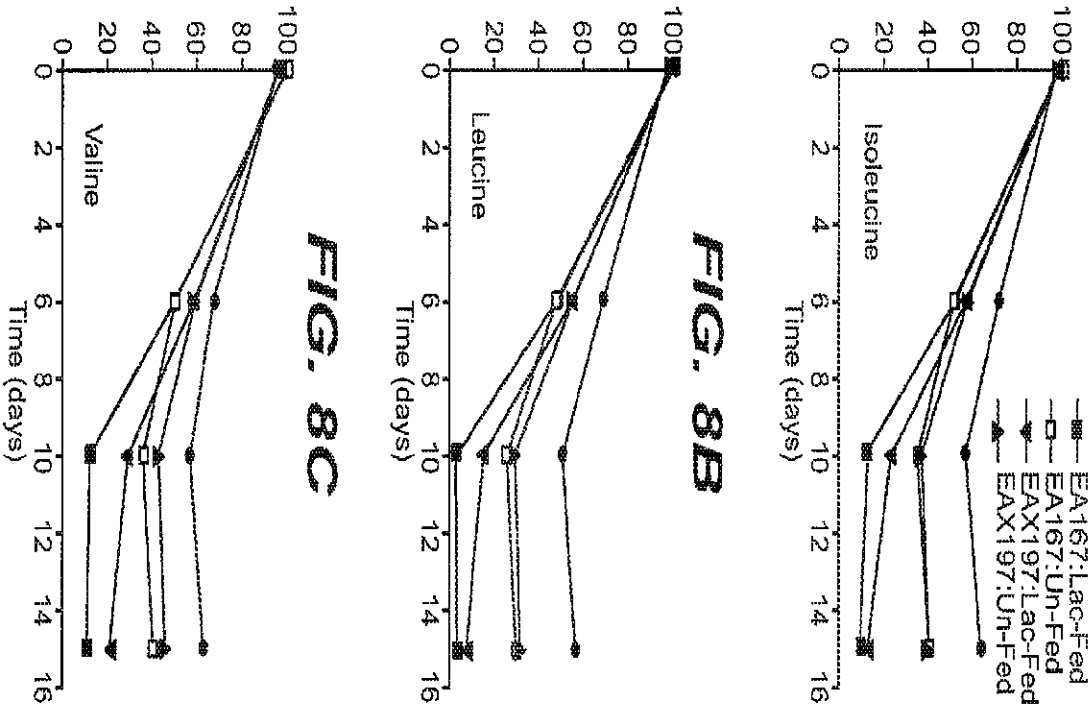


FIG. 8A

FIG. 8B

FIG. 8C

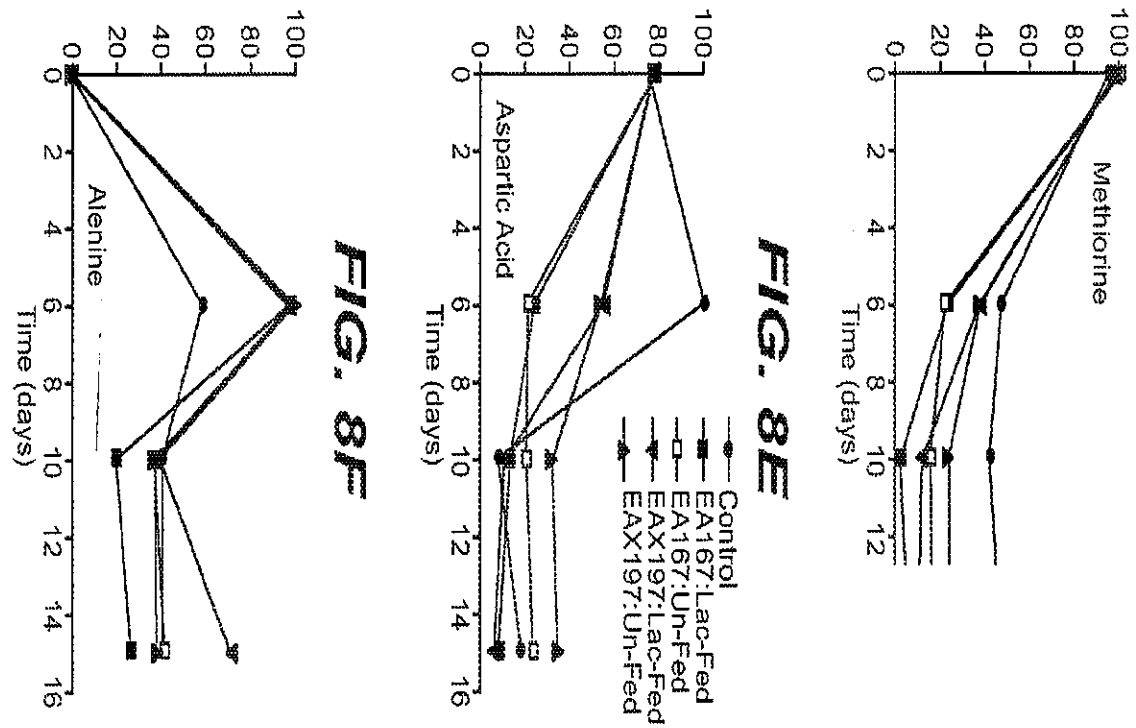


FIG. 8D

FIG. 8E

FIG. 8F

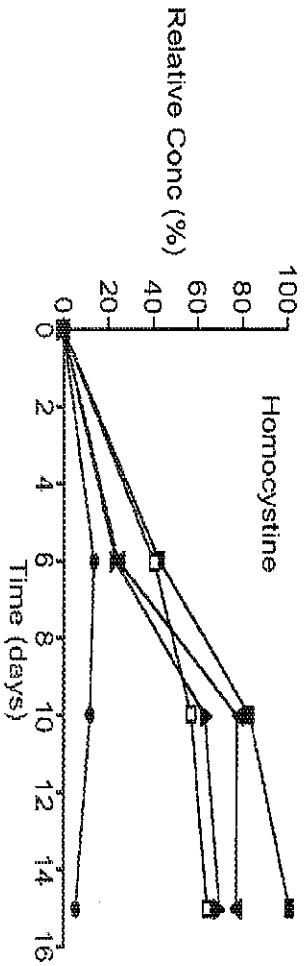

Relative Conc (%)
0
20
40
60
80
100
Homocystine
0
2
4
6
8
10
12
14
16
Time (days)

FIG. 8G

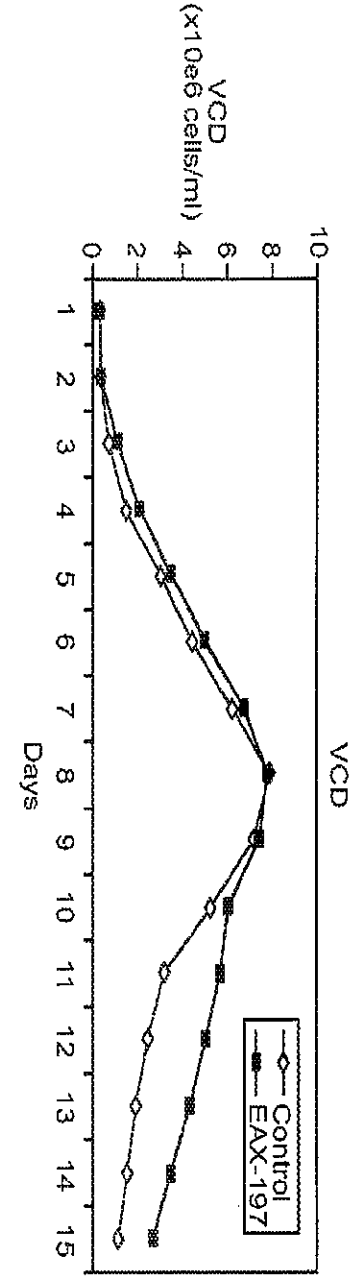

VCD
VCD (x10a6 cells/ml)
Days
Control
EAX-197

FIG. 9A

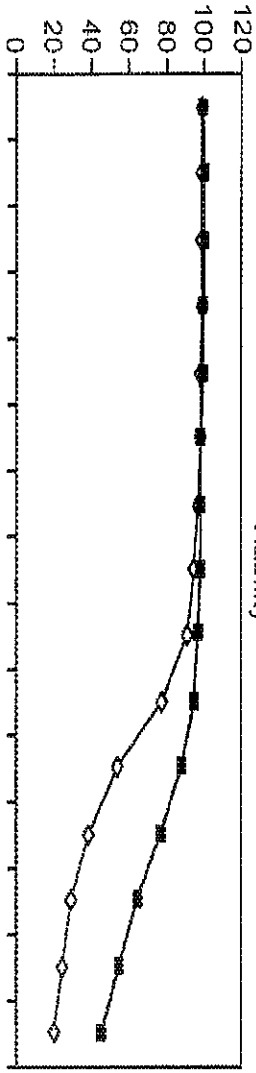

Viability
% Viable
Days

FIG. 9B

~~16/19~~

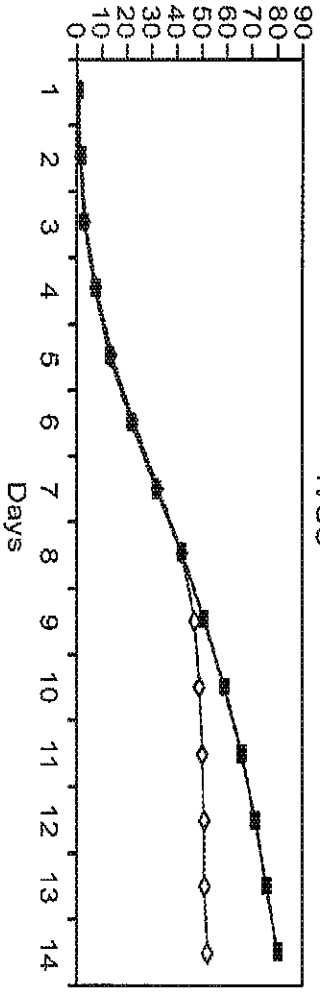


FIG. 9C

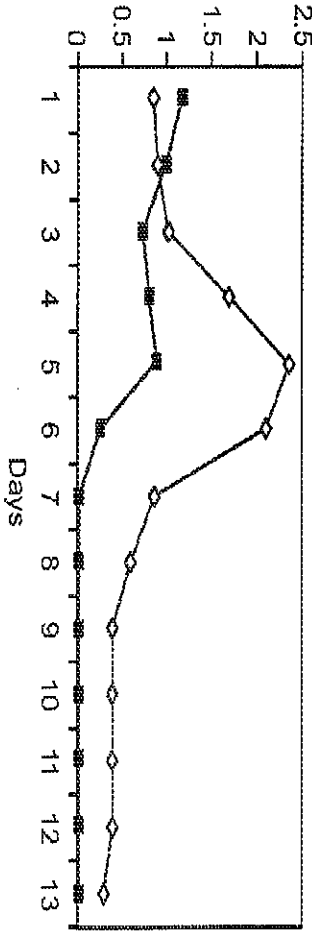


FIG. 9D

FIG. 10A

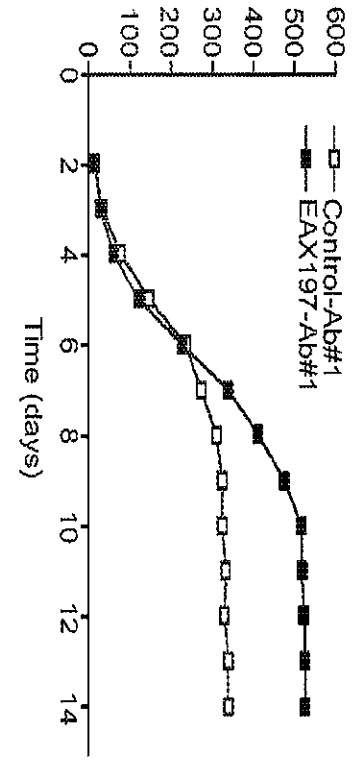


FIG. 10B

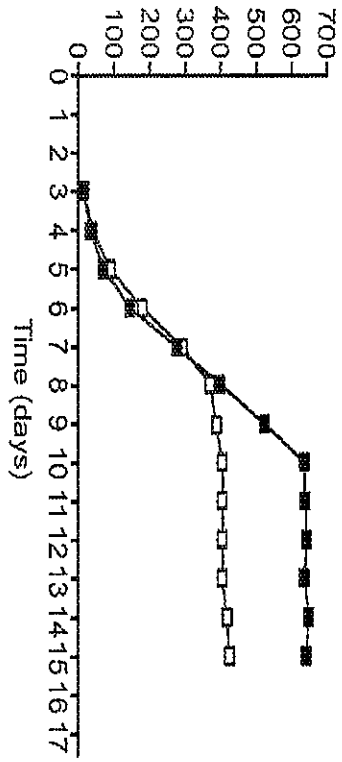


FIG. 11A

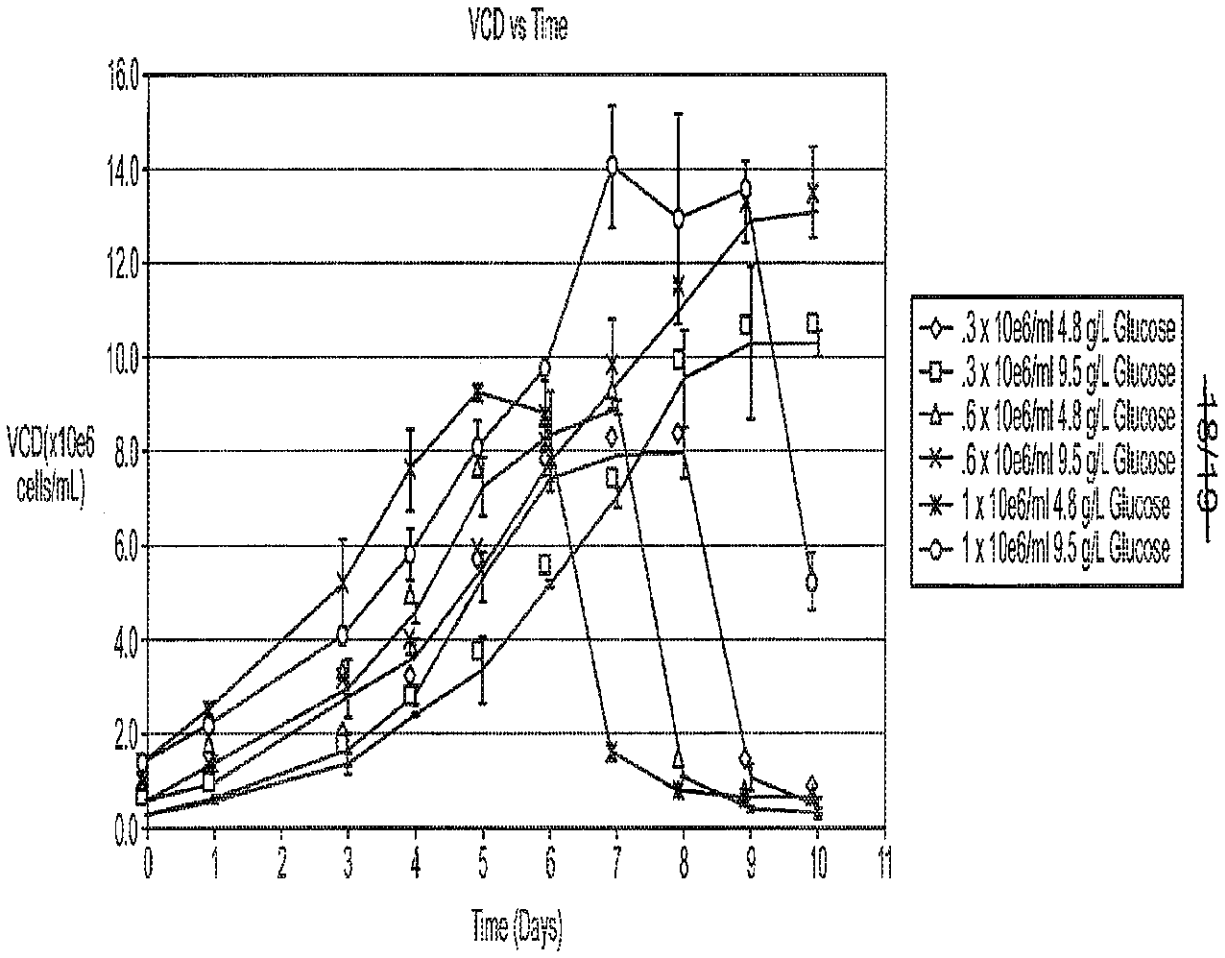
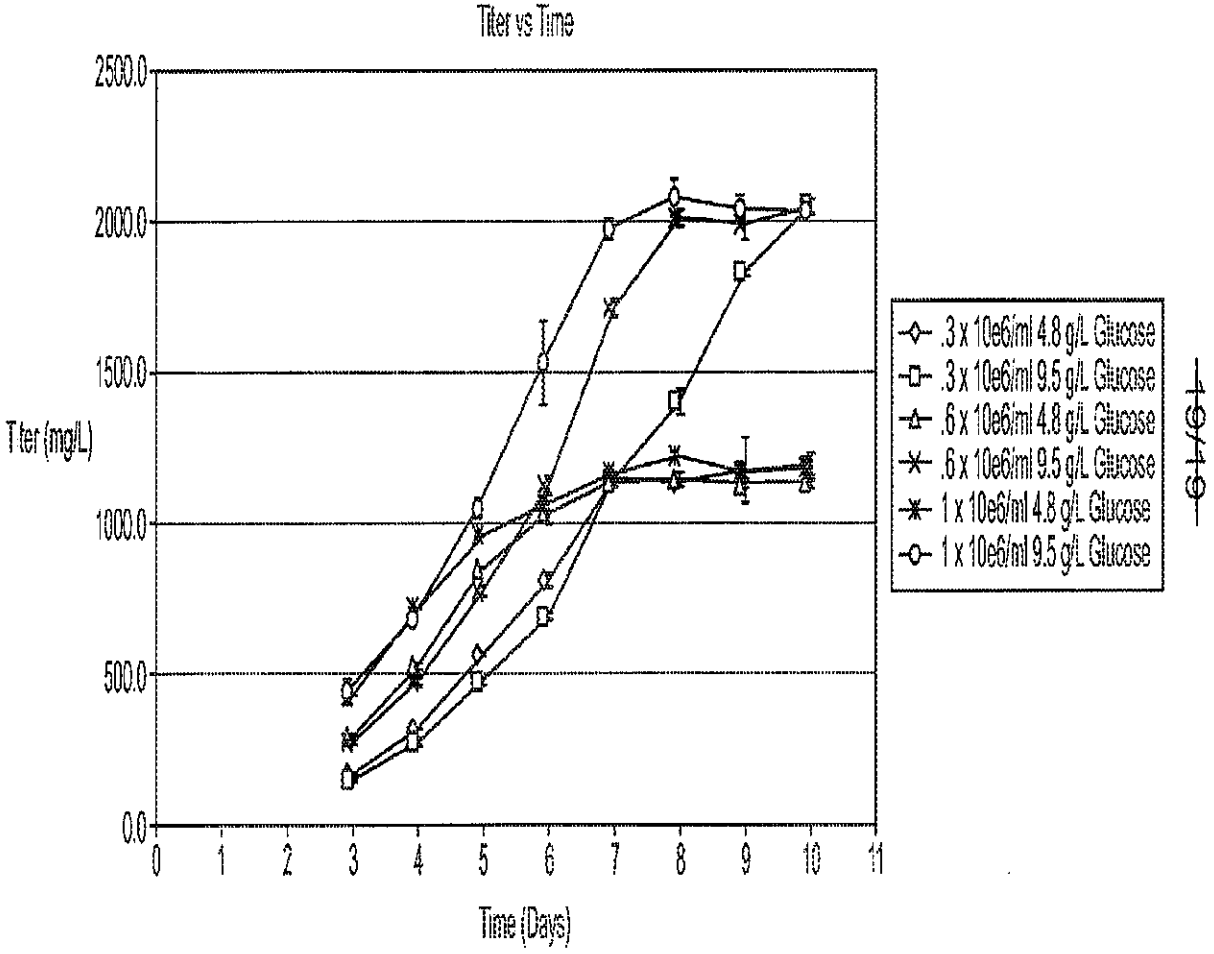

VCD vs Time
VCD(x10e6 cells/mL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
Time (Days)
.3 x 10e6/ml 4.8 g/L Glucose
.3 x 10e6/ml 9.5 g/L Glucose
.6 x 10e6/ml 4.8 g/L Glucose
.6 x 10e6/ml 9.5 g/L Glucose
1 x 10e6/ml 4.8 g/L Glucose
1 x 10e6/ml 9.5 g/L Glucose

FIG. 11B


Titer vs Time
Titer (mg/L)
2500.0
2000.0
1500.0
1000.0
500.0
0.0
0 1 2 3 4 5 6 7 8 9 10 11
Time (Days)
.3 x 10e6/ml 4.8 g/L Glucose
.3 x 10e6/ml 9.5 g/L Glucose
.6 x 10e6/ml 4.8 g/L Glucose
.6 x 10e6/ml 9.5 g/L Glucose
1 x 10e6/ml 4.8 g/L Glucose
1 x 10e6/ml 9.5 g/L Glucose
10/19

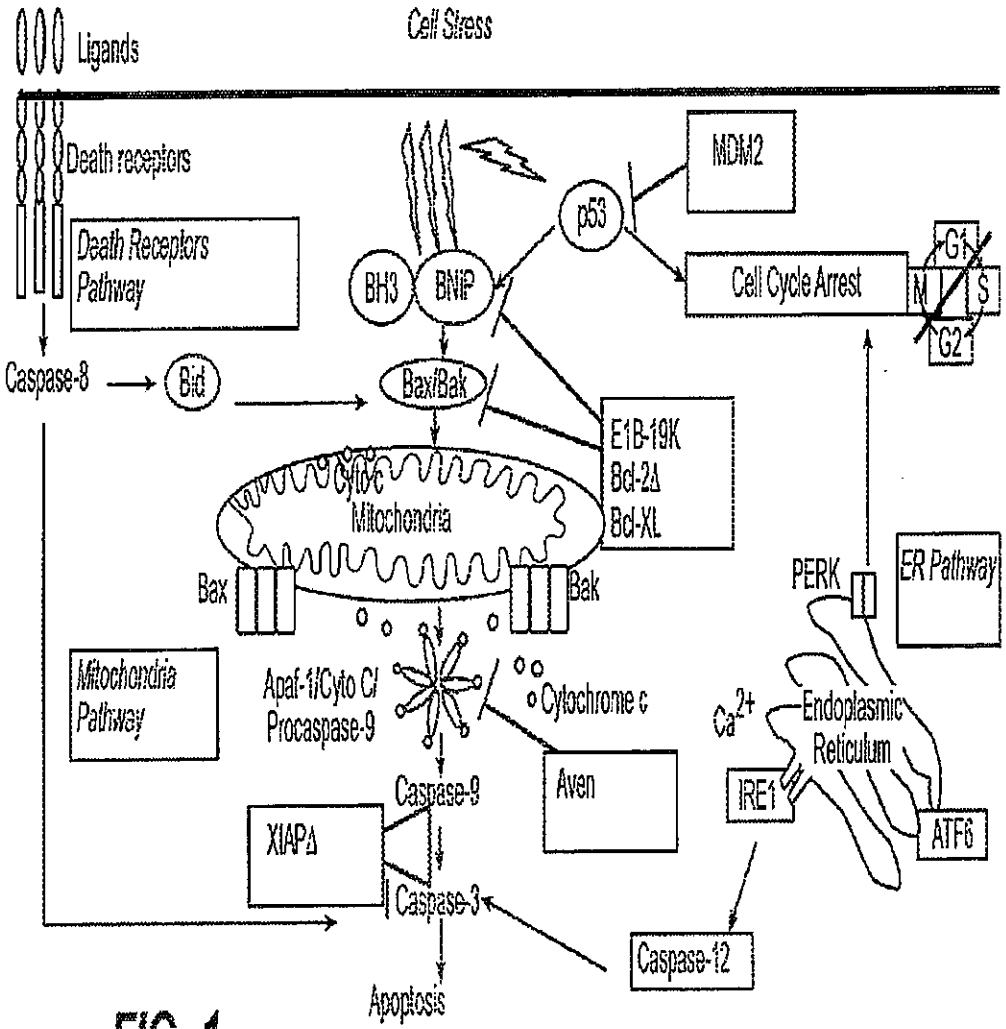

Ligands
Cell Stress
Death receptors
Death Receptors Pathway
Caspase-8
Bid
BH3
BNiP
p53
MDM2
Cell Cycle Arrest
G1
S
G2
M
Bax/Bak
E1B-19K
Bcl-2Δ
Bcl-XL
Cyto C
Mitochondria
Bax
Bak
PERK
ER Pathway
Mitochondria Pathway
Apaf-1/Cyto C/
Procaspase-9
Cytochrome c
Ca2+
Endoplasmic Reticulum
IRE1
ATF6
Caspase-9
Aven
XIAPΔ
Caspase-3
Caspase-12
Apoptosis

FIG. 1

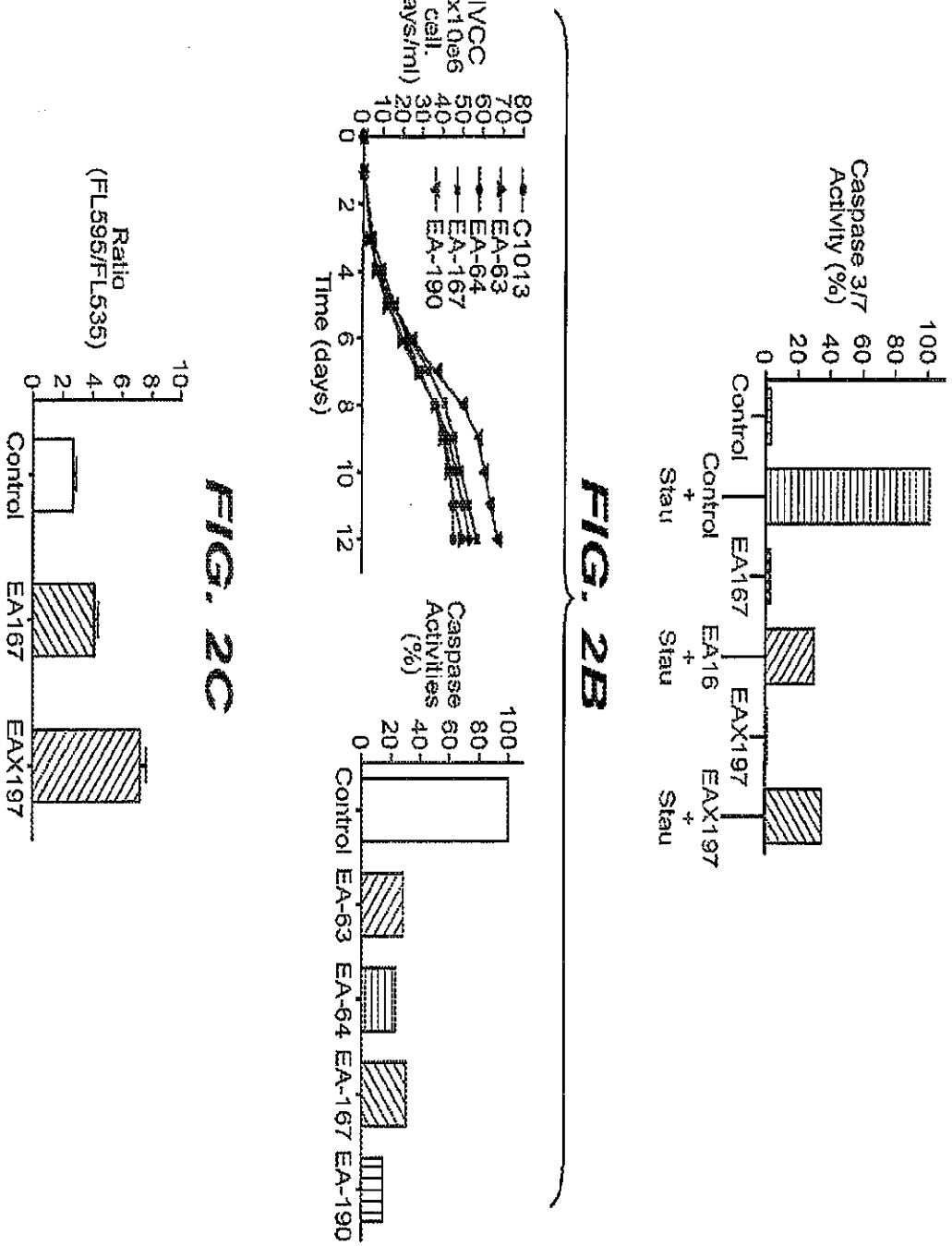


FIG. 2A

FIG. 2B

FIG. 2C

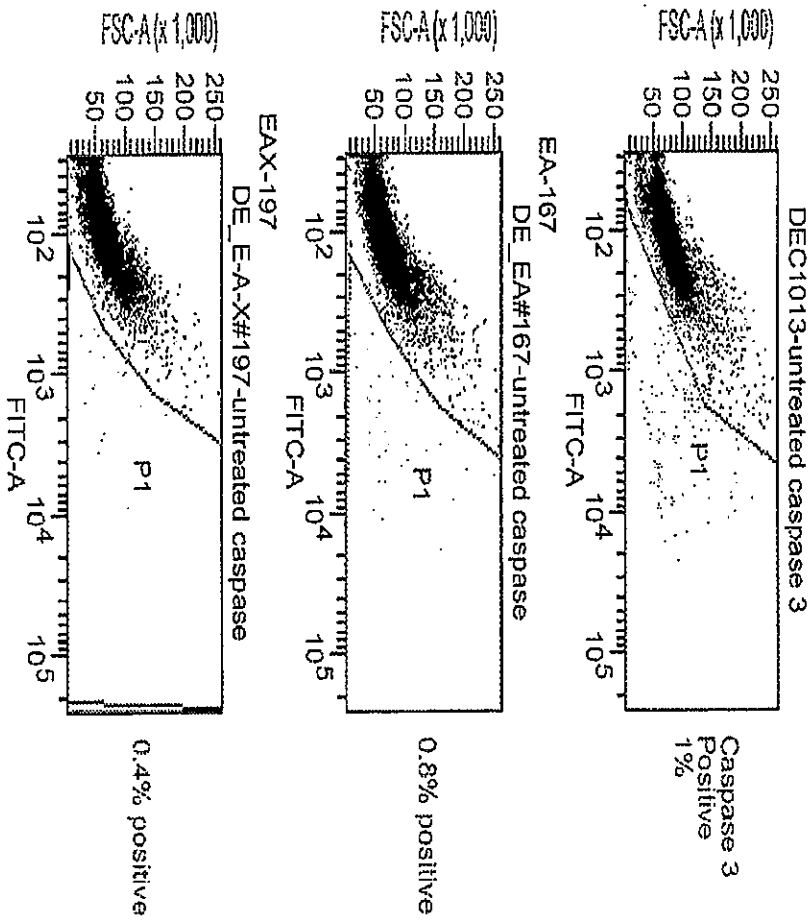

Untreated
C1013
DEC1013-untreated caspase 3
FSC-A (x 1,000)
250
200
150
100
50
10^2
10^3
10^4
10^5
FITC-A
P1
Caspase 3 Positive 1%
EA-167
DE_EA#167-untreated caspase
0.8% positive
EAX-197
DE_E-A-X#197-untreated caspase
0.4% positive

FIG. 3A

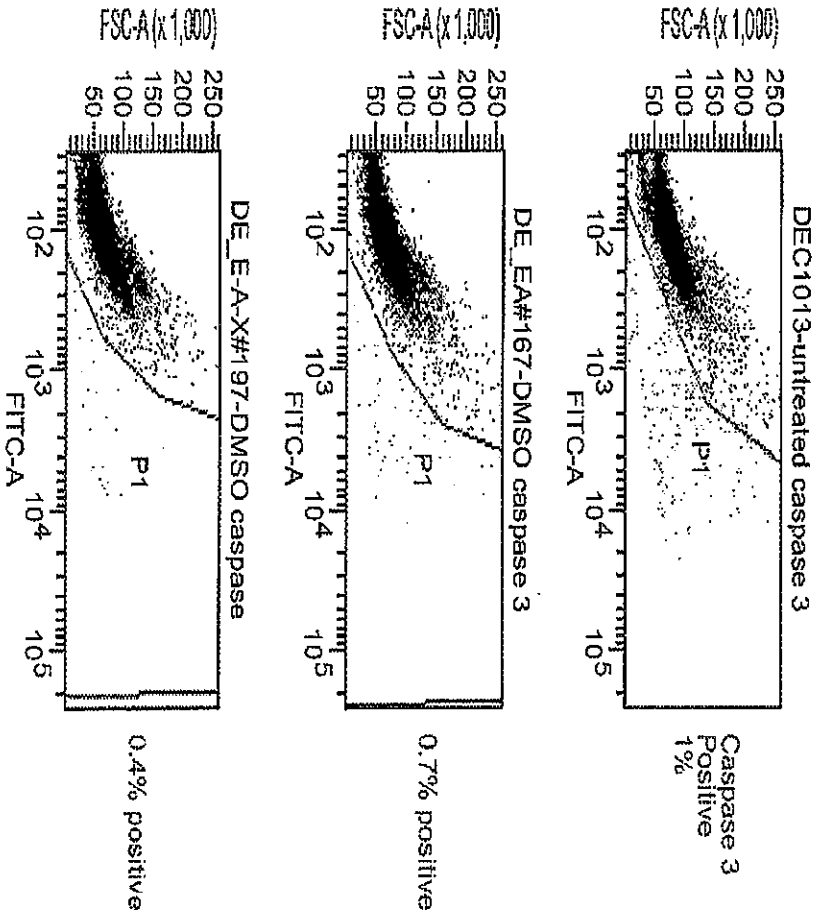
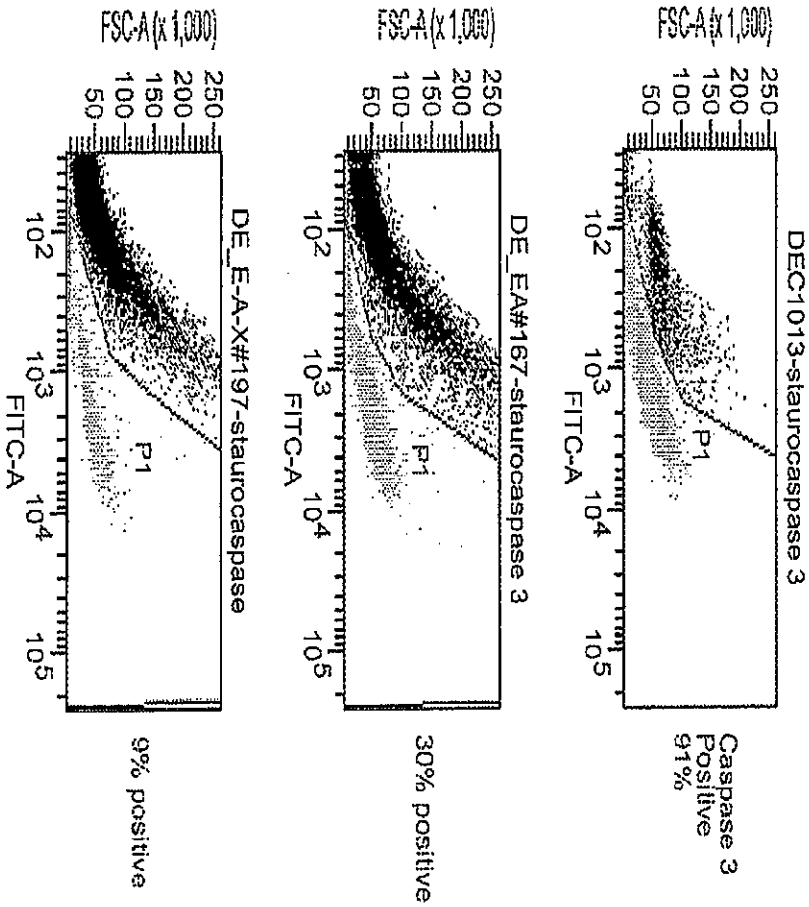

DMSO (Vehicle) treated
DEC1013-untreated caspase 3
FSC-A (x 1,000)
FITC-A
P1
Caspase 3 Positive 1%
DE_EA#167-DMSO caspase 3
0.7% positive
DE_E-A-X#197-DMSO caspase
0.4% positive

FIG. 3B

FIG. 3C


Staurosporine treated
DEC1013-staurocaspase 3
FSC-A (x 1,000)
FITC-A
P1
Caspase 3 Positive 91%
DE_EA#167-staurocaspase 3
30% positive
DE_E-A-X#197-staurocaspase
9% positive

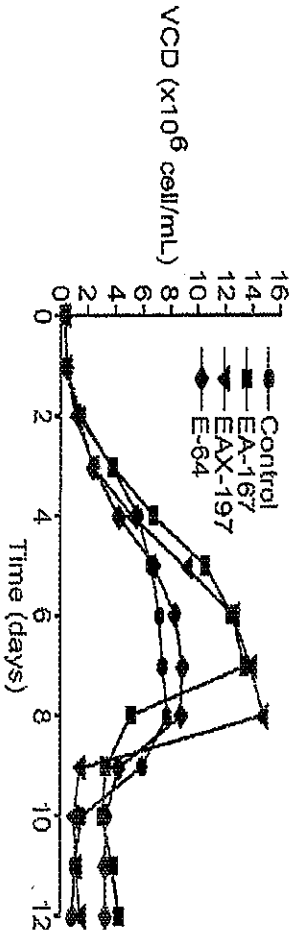


FIG. 4A

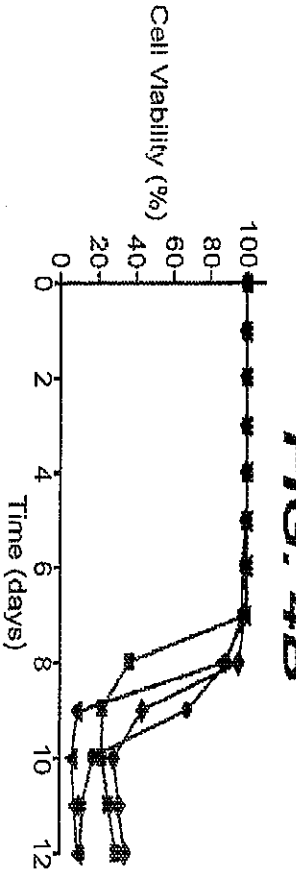


FIG. 4B

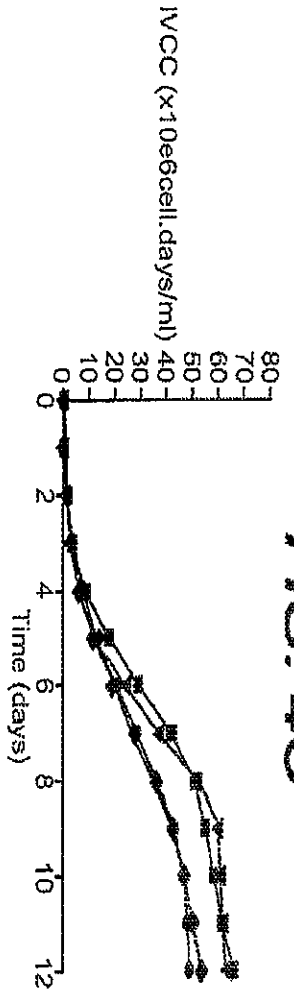


FIG. 4C

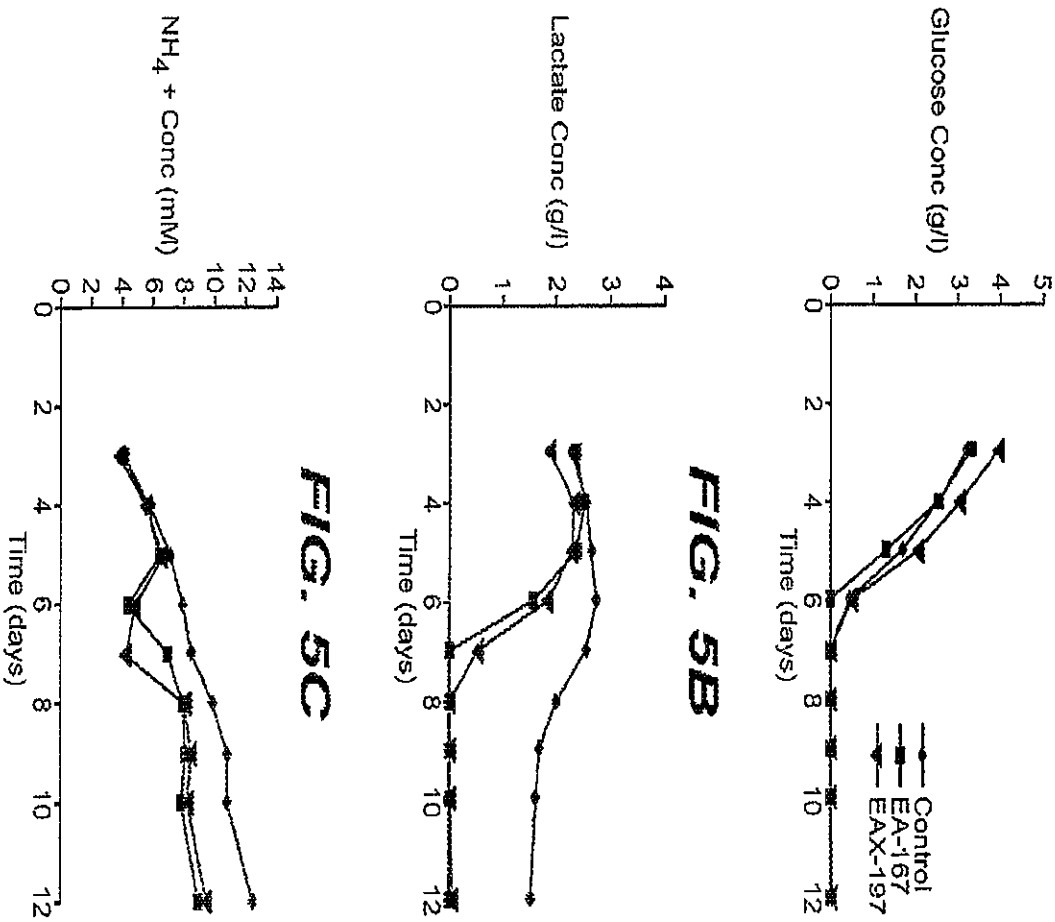


FIG. 5A

FIG. 5B

FIG. 5C

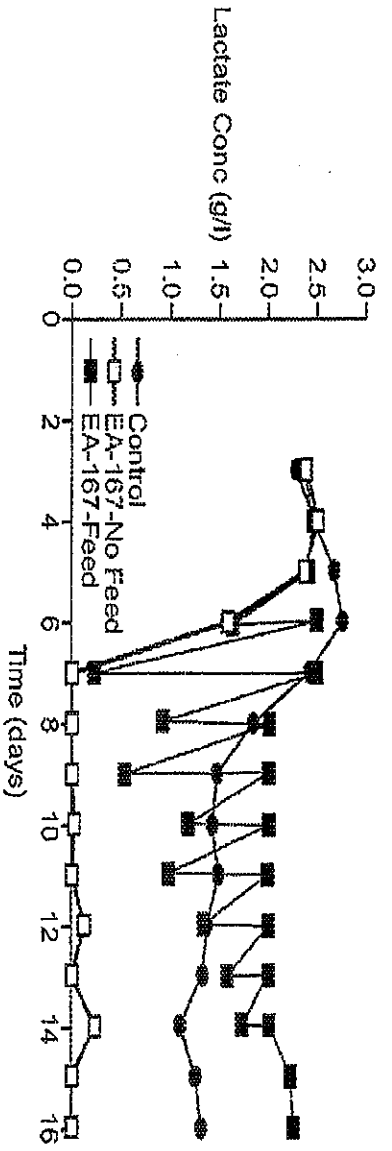

Lactate Conc (g/l)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Control
EA-167-No Feed
EA-167-Feed
Time (days)
0
2
4
6
8
10
12
14
16

FIG. 6A

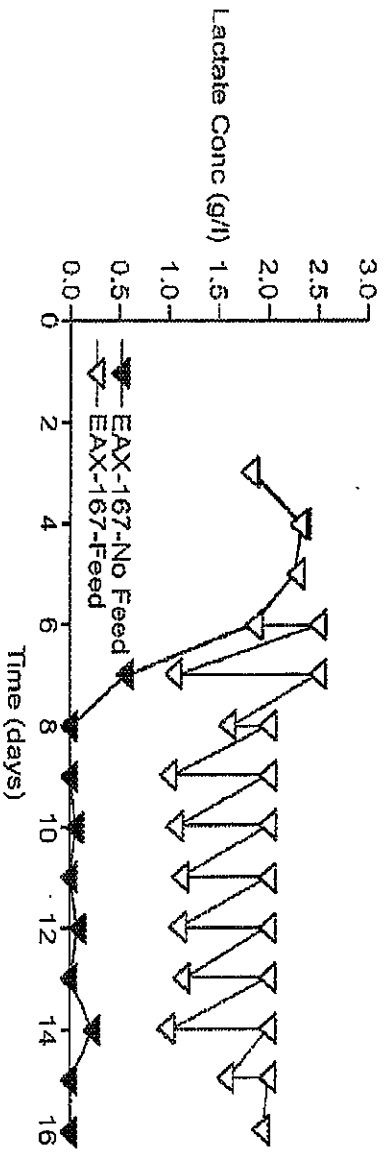

Lactate Conc (g/l)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
EAX-167-No Feed
EAX-167-Feed
Time (days)
0
2
4
6
8
10
12
14
16

FIG. 6B

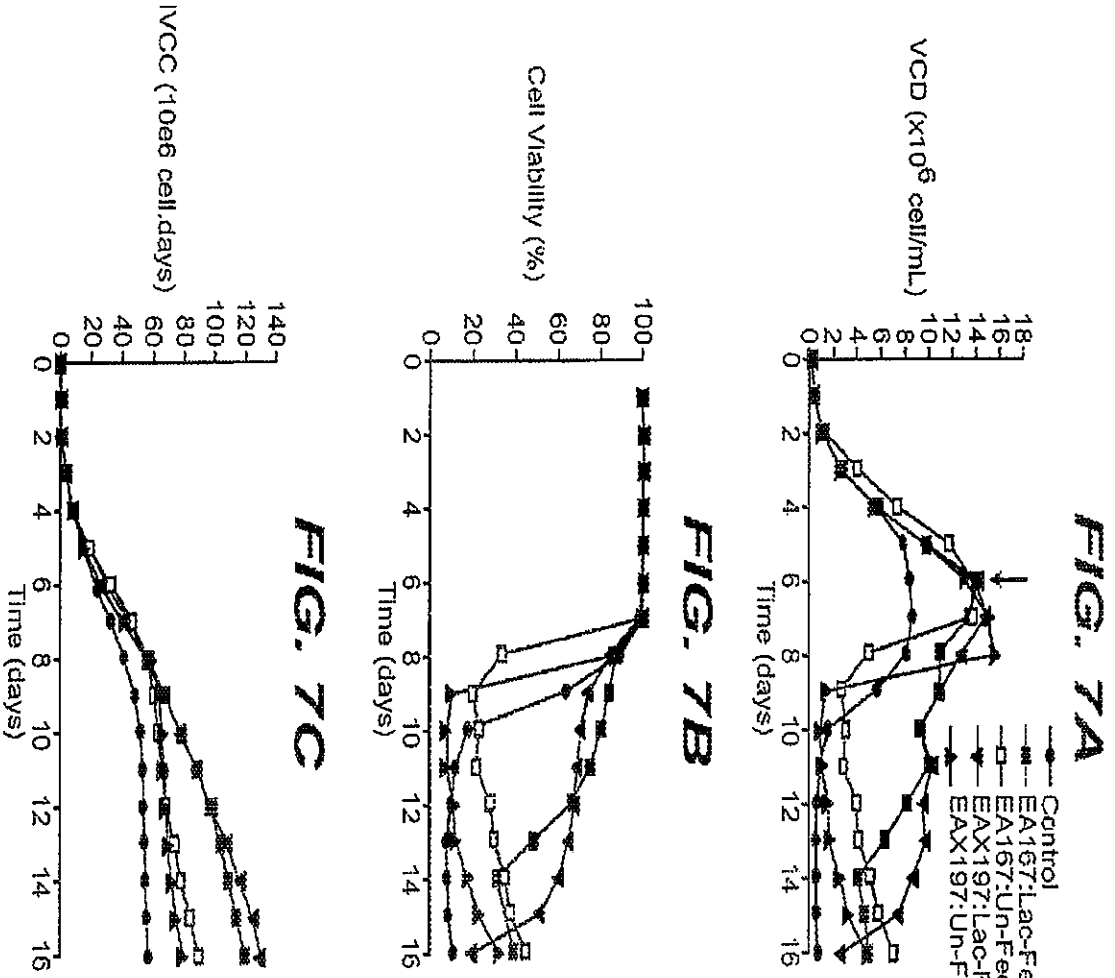


FIG. 7A

FIG. 7B

FIG. 7C

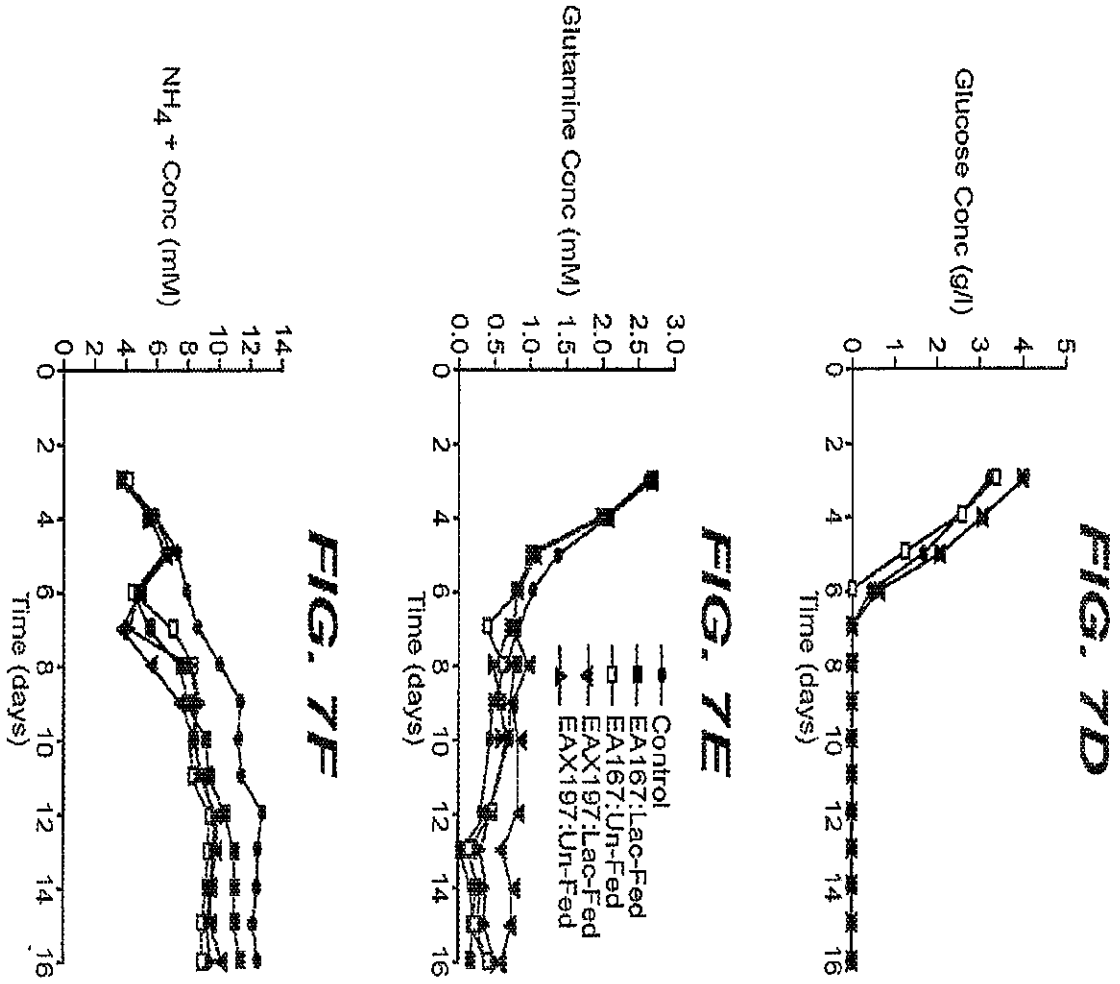


FIG. 7D

FIG. 7E

FIG. 7F

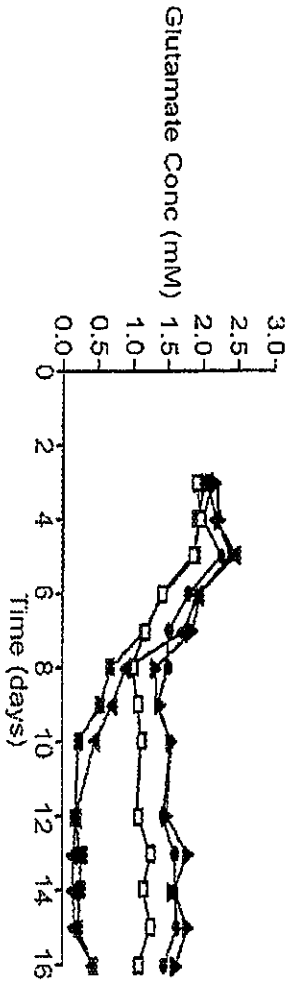

Glutamate Conc (mM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time (days)
0
2
4
6
8
10
12
14
16

FIG. 7G

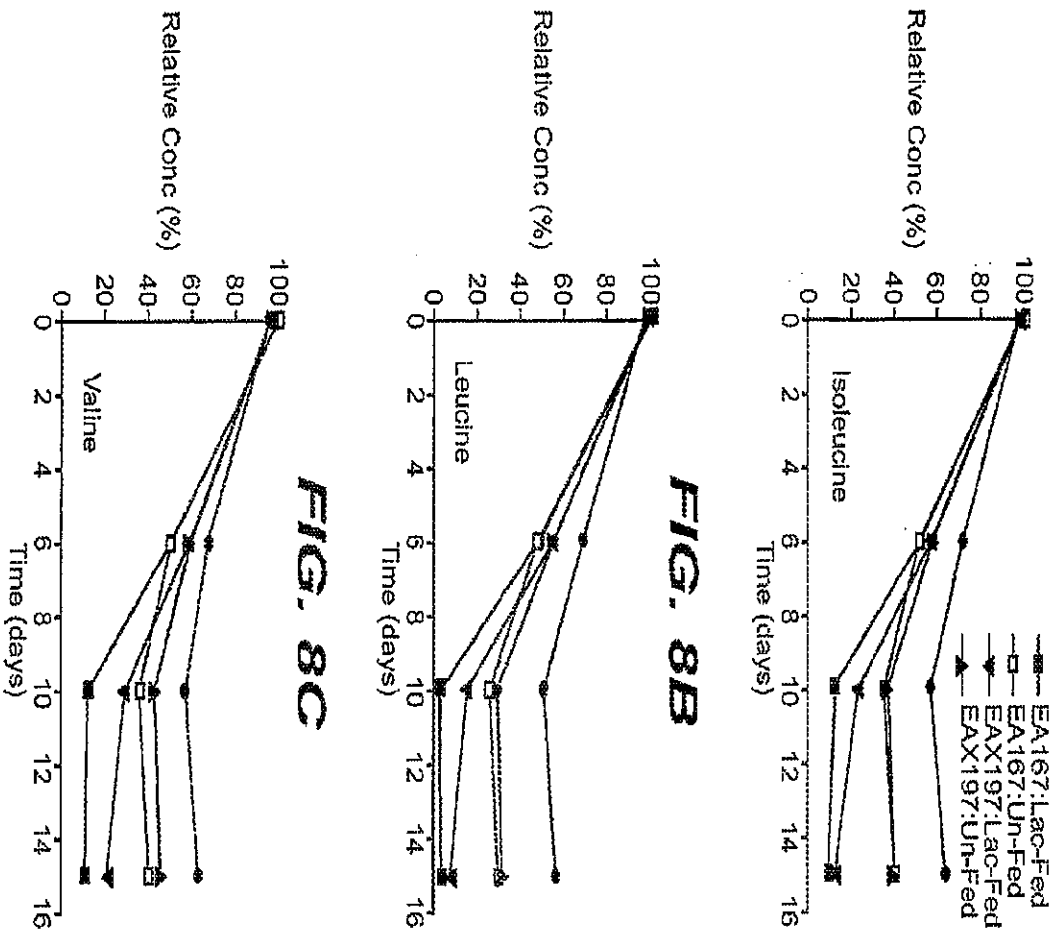


FIG. 8A

FIG. 8B

FIG. 8C

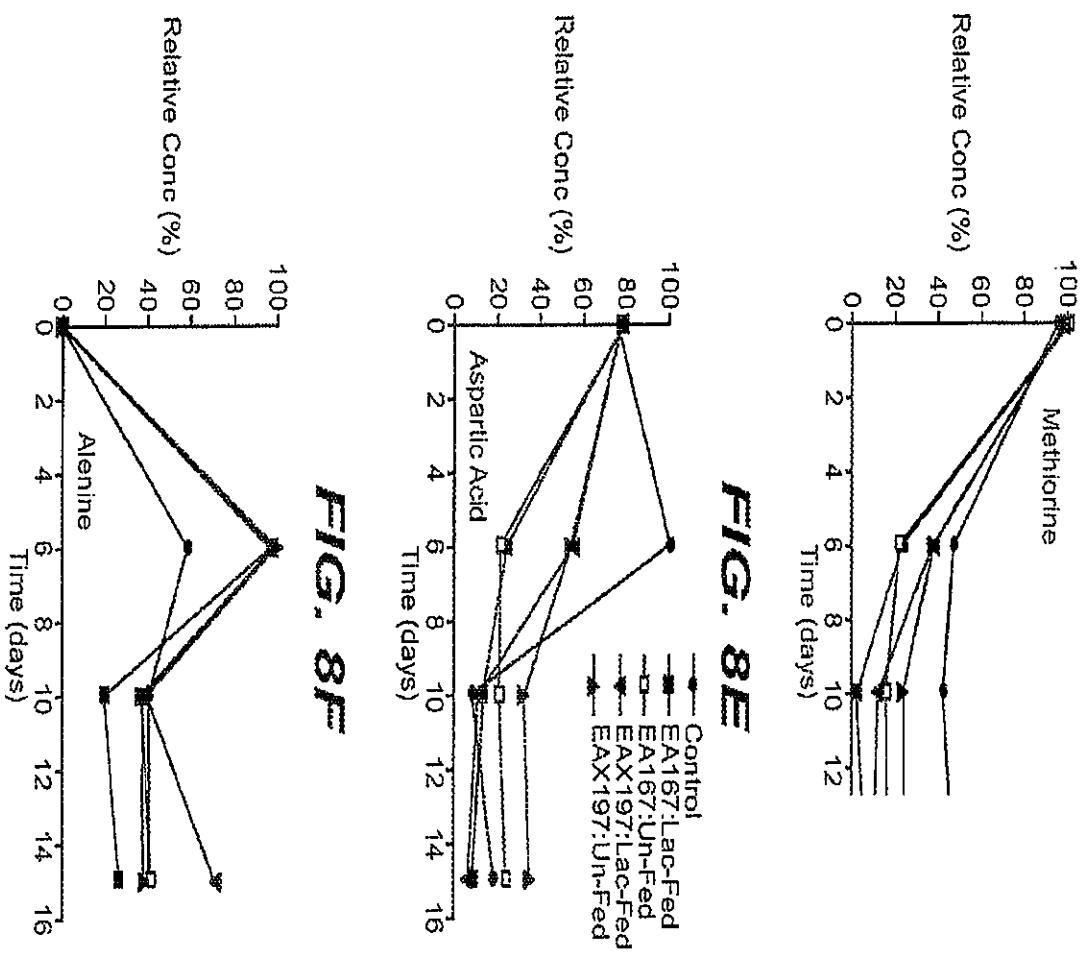


FIG. 8D

FIG. 8E

FIG. 8F

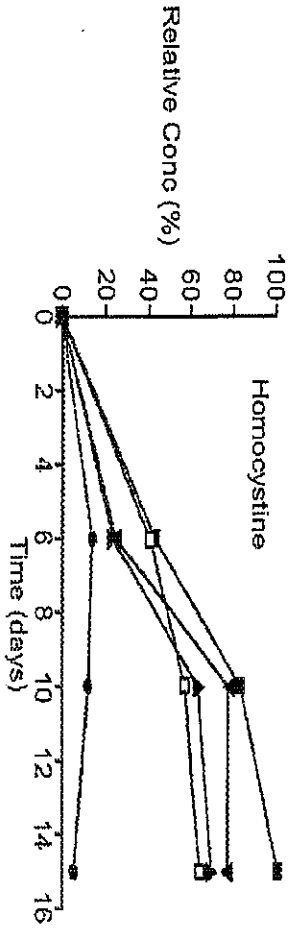

Relative Conc (%)
0
20
40
60
80
100
Homocystine
Time (days)
0
2
4
6
8
10
12
14
16

FIG. 8G

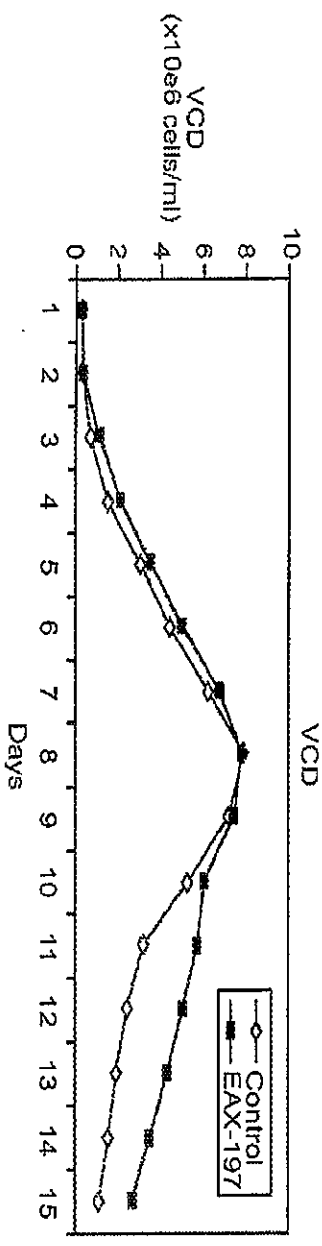

VCD
(x10e6 cells/ml)
0
2
4
6
8
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days
Control
EAX-197

FIG. 9A

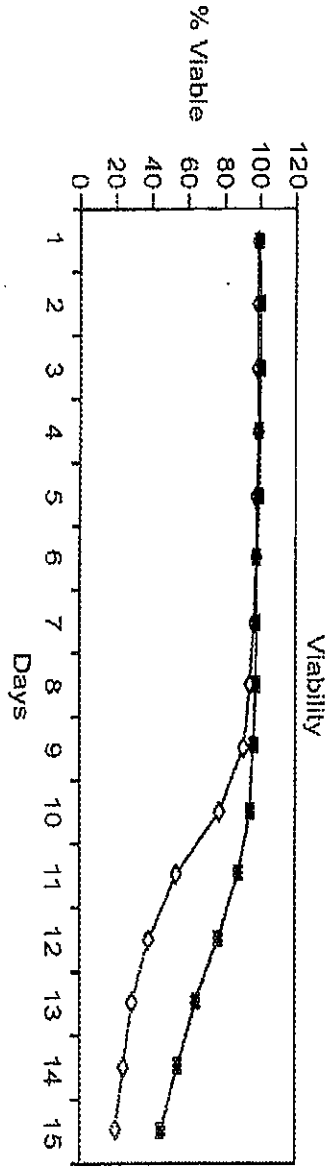

Viability
% Viable
0
20
40
60
80
100
120
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days

FIG. 9B

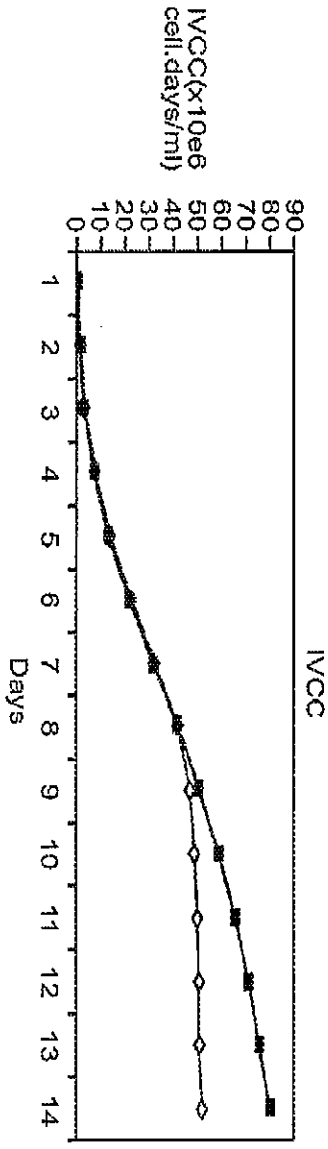

IVCC
IVCC(x10e6 cell.days/ml)
0
10
20
30
40
50
60
70
80
90
Days
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIG. 9C

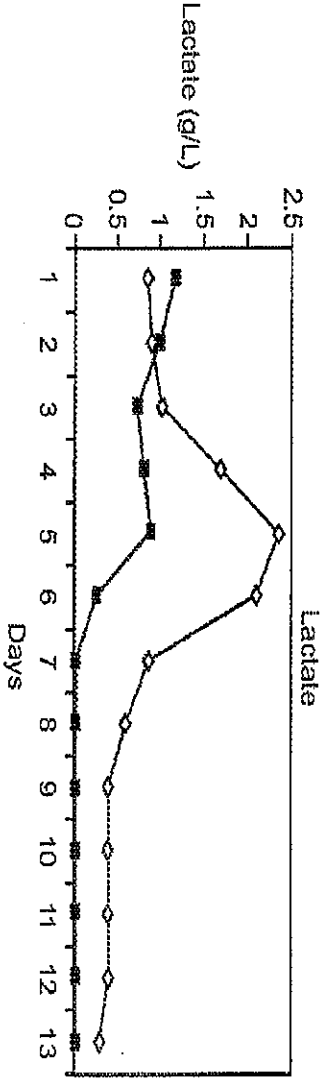

Lactate
Lactate (g/L)
0
0.5
1
1.5
2
2.5
Days
1
2
3
4
5
6
7
8
9
10
11
12
13

FIG. 9D

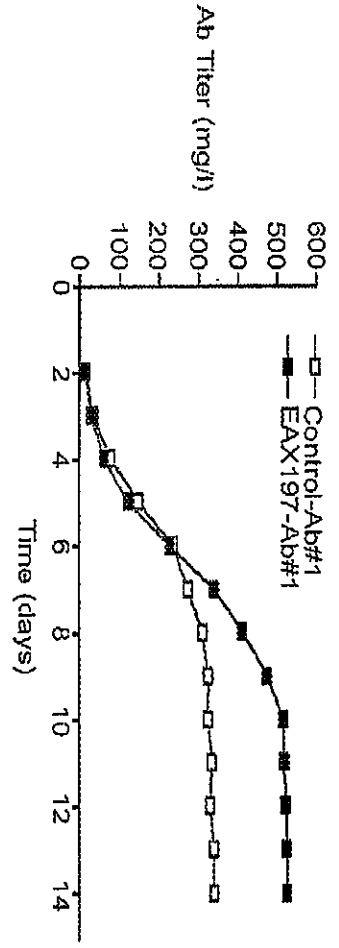


FIG. 10A

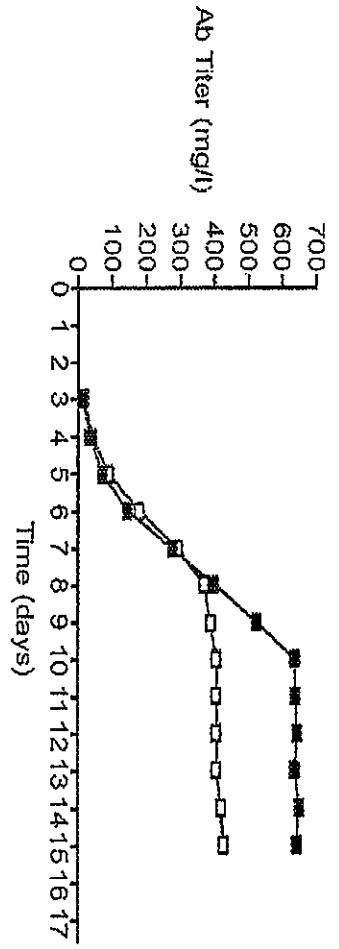


FIG. 10B

FIG. 11A

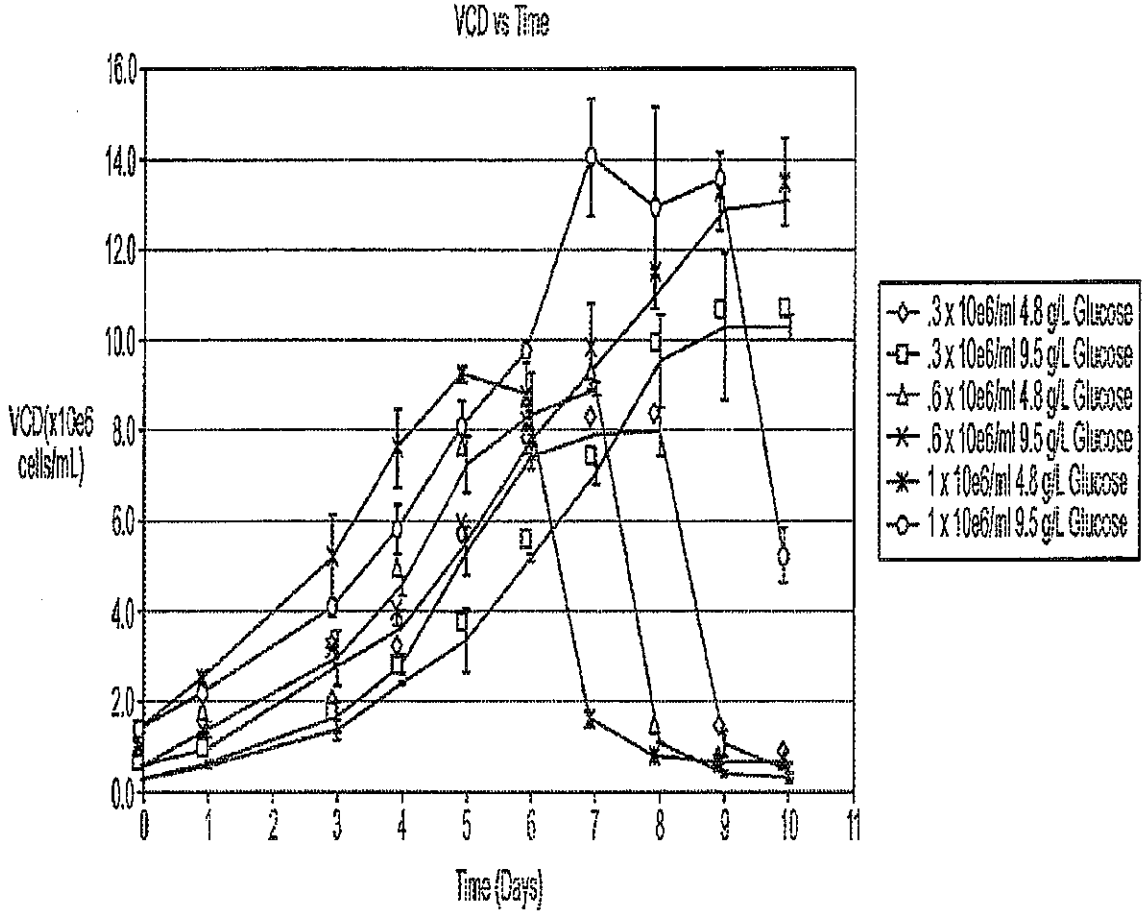
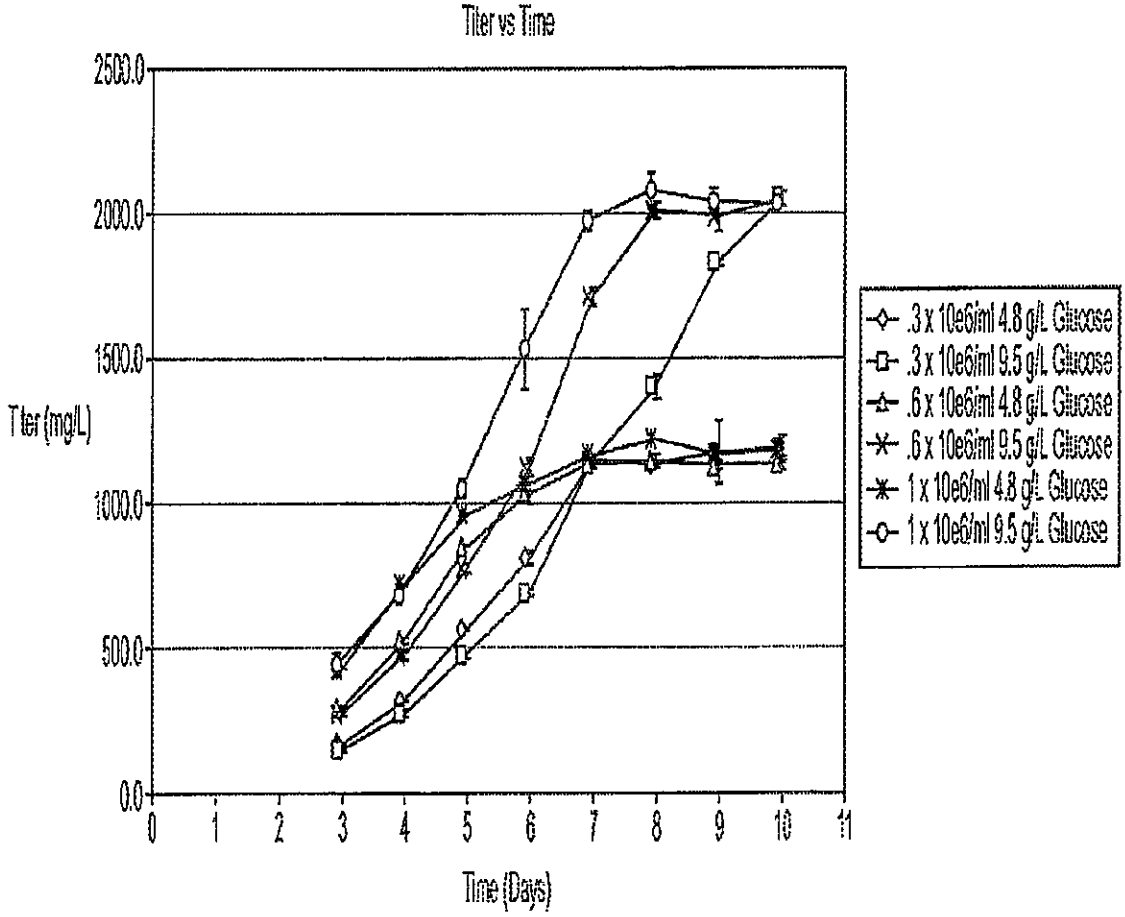

VCD vs Time
VCD(x10e6 cells/mL)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
Time (Days)
.3 x 10e6/ml 4.8 g/L Glucose
.3 x 10e6/ml 9.5 g/L Glucose
.6 x 10e6/ml 4.8 g/L Glucose
.6 x 10e6/ml 9.5 g/L Glucose
1 x 10e6/ml 4.8 g/L Glucose
1 x 10e6/ml 9.5 g/L Glucose

FIG. 11B


Titer vs Time
2500.0
2000.0
1500.0
1000.0
500.0
0.0
Titer (mg/L)
0
1
2
3
4
5
6
7
8
9
10
11
Time (Days)
.3 x 10e6/ml 4.8 g/L Glucose
.3 x 10e6/ml 9.5 g/L Glucose
.6 x 10e6/ml 4.8 g/L Glucose
.6 x 10e6/ml 9.5 g/L Glucose
1 x 10e6/ml 4.8 g/L Glucose
1 x 10e6/ml 9.5 g/L Glucose