FIG. 1

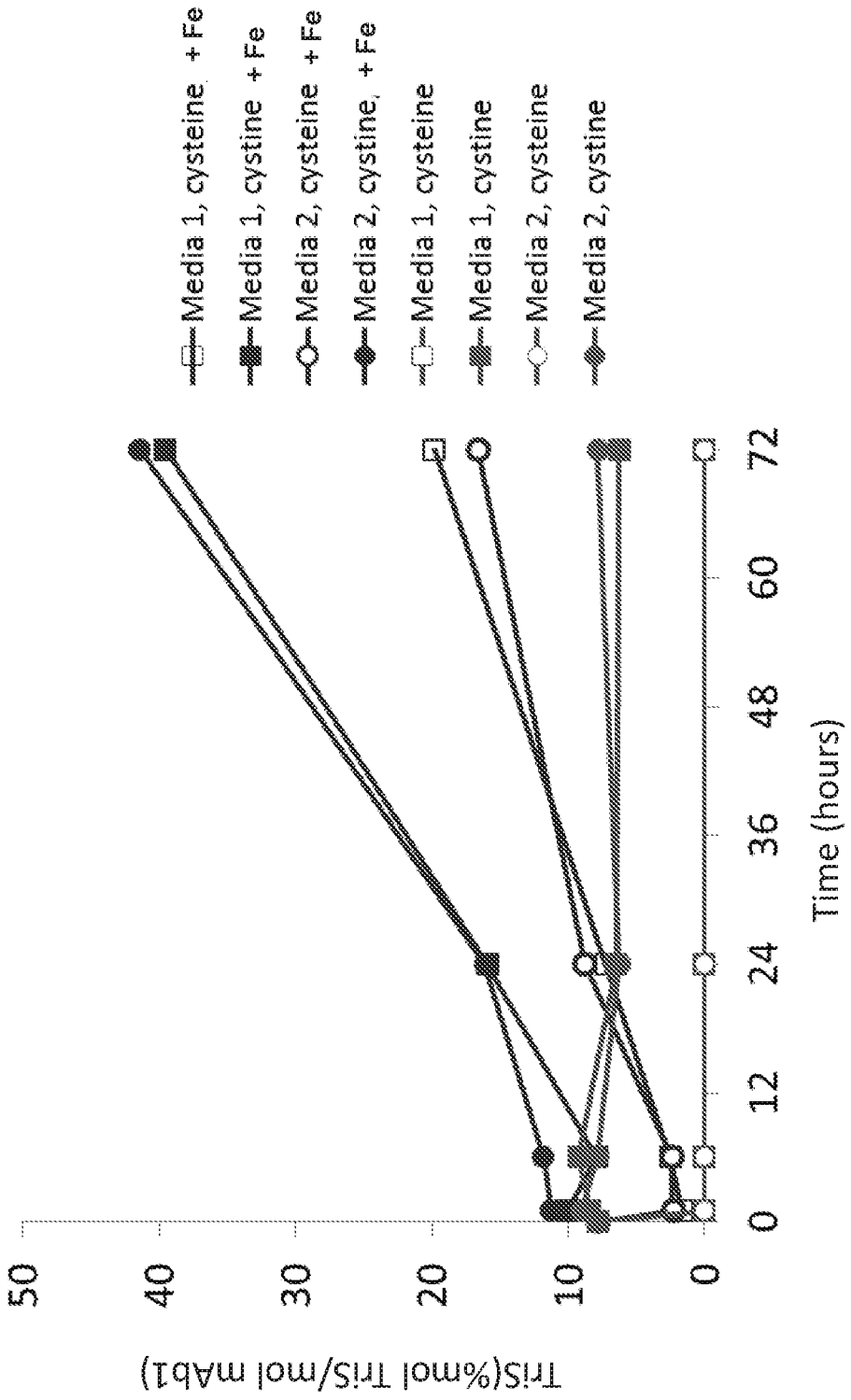

Media 1, cysteine + Fe
Media 1, cystine + Fe
Media 2, cysteine + Fe
Media 2, cystine + Fe
Media 1, cysteine
Media 1, cystine
Media 2, cysteine
Media 2, cystine
Tris(%mol Tris/mol mAb1)
0
10
20
30
40
50
0
12
24
36
48
60
72
Time (hours)

FIG. 2A

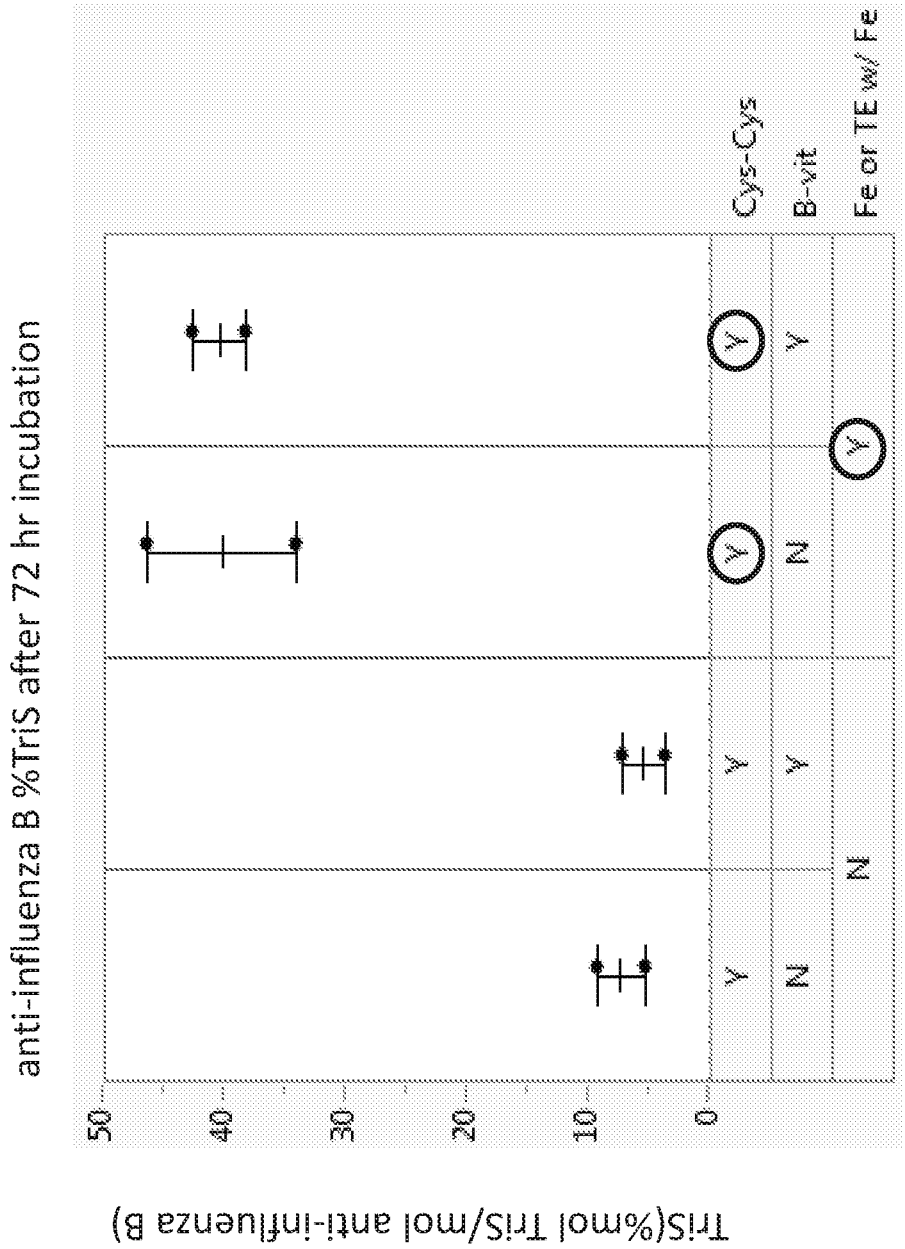

anti-influenza B %TriS after 72 hr incubation
TriS(%mol TriS/mol anti-influenza B)
50
40
30
20
10
0
Y
N
Y
Y
N
Y
Y
N
Y
Cys-Cys
B-vit
Fe or TE w/ Fe

FIG. 2B

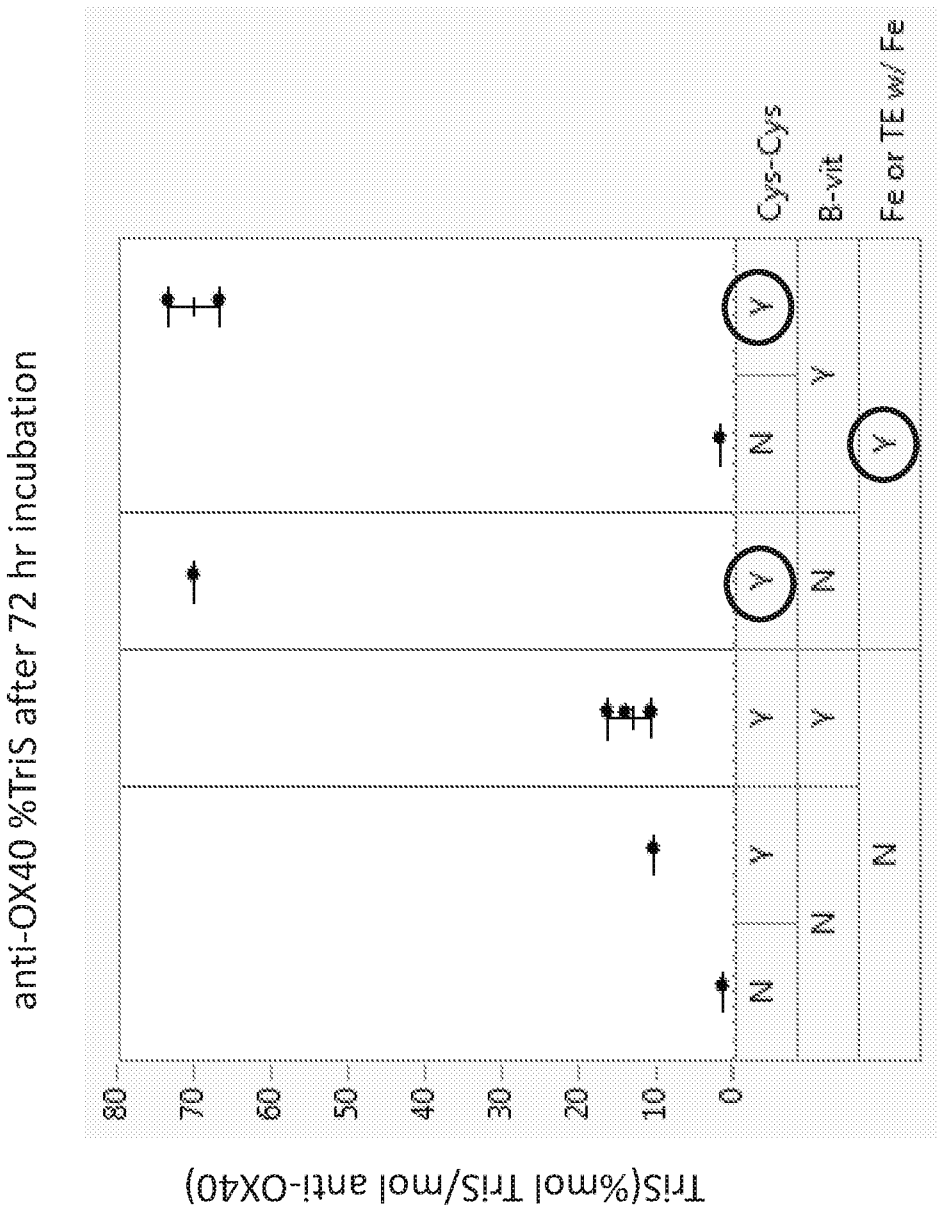

anti-OX40 %TriS after 72 hr incubation
TriS(%mol TriS/mol anti-OX40)
0
10
20
30
40
50
60
70
80
N
Y
N
Y
N
Y
N
Y
Cys-Cys
B-vit
Fe or TE w/ Fe

FIG. 3

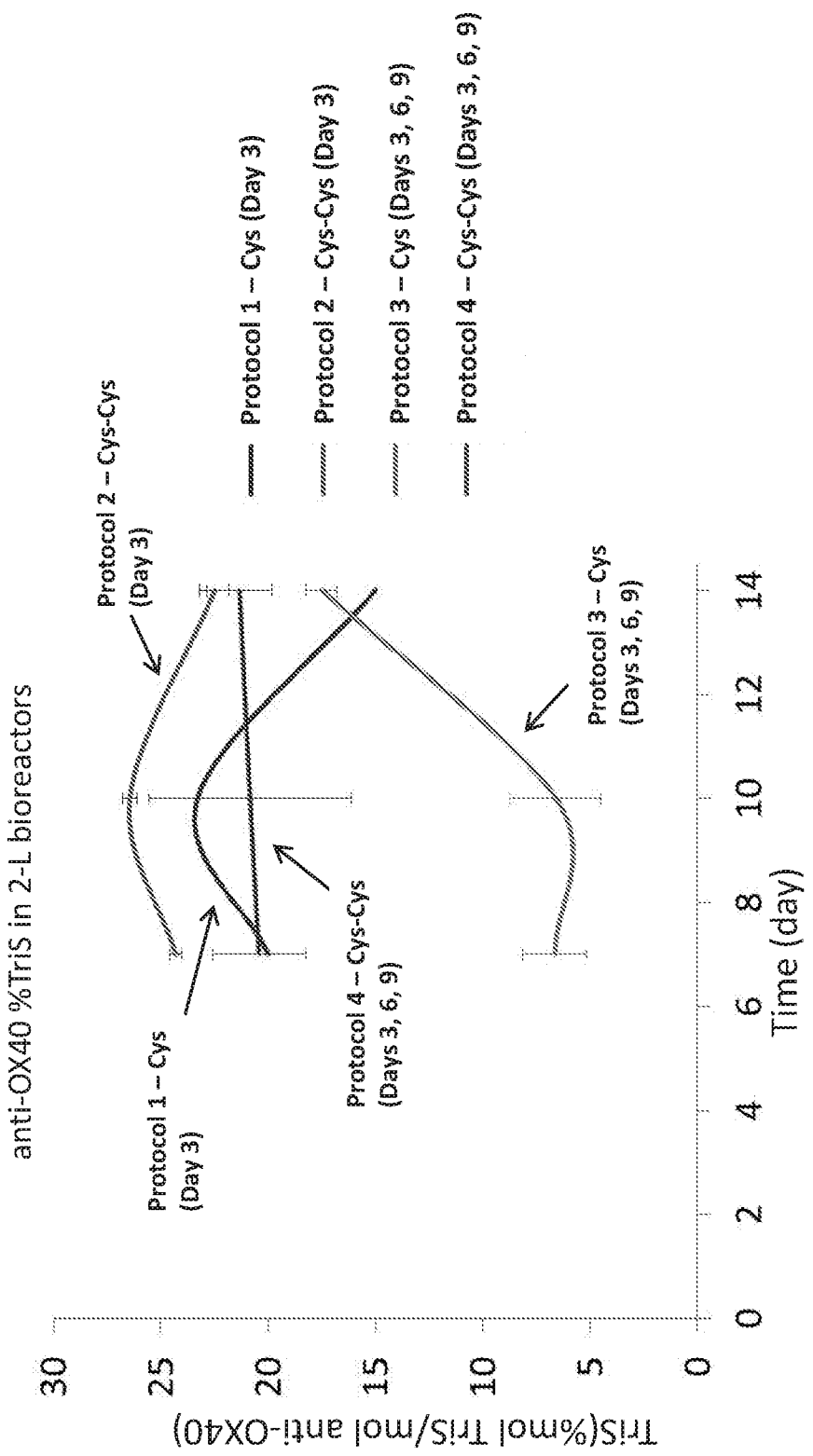

anti-OX40 %TriS in 2-L bioreactors
TriS(%mol TriS/mol anti-OX40)
Time (day)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
Protocol 1 – Cys (Day 3)
Protocol 2 – Cys-Cys (Day 3)
Protocol 3 – Cys (Days 3, 6, 9)
Protocol 4 – Cys-Cys (Days 3, 6, 9)
Protocol 1 – Cys (Day 3)
Protocol 2 – Cys-Cys (Day 3)
Protocol 4 – Cys-Cys (Days 3, 6, 9)
Protocol 3 – Cys (Days 3, 6, 9)

FIG. 4A

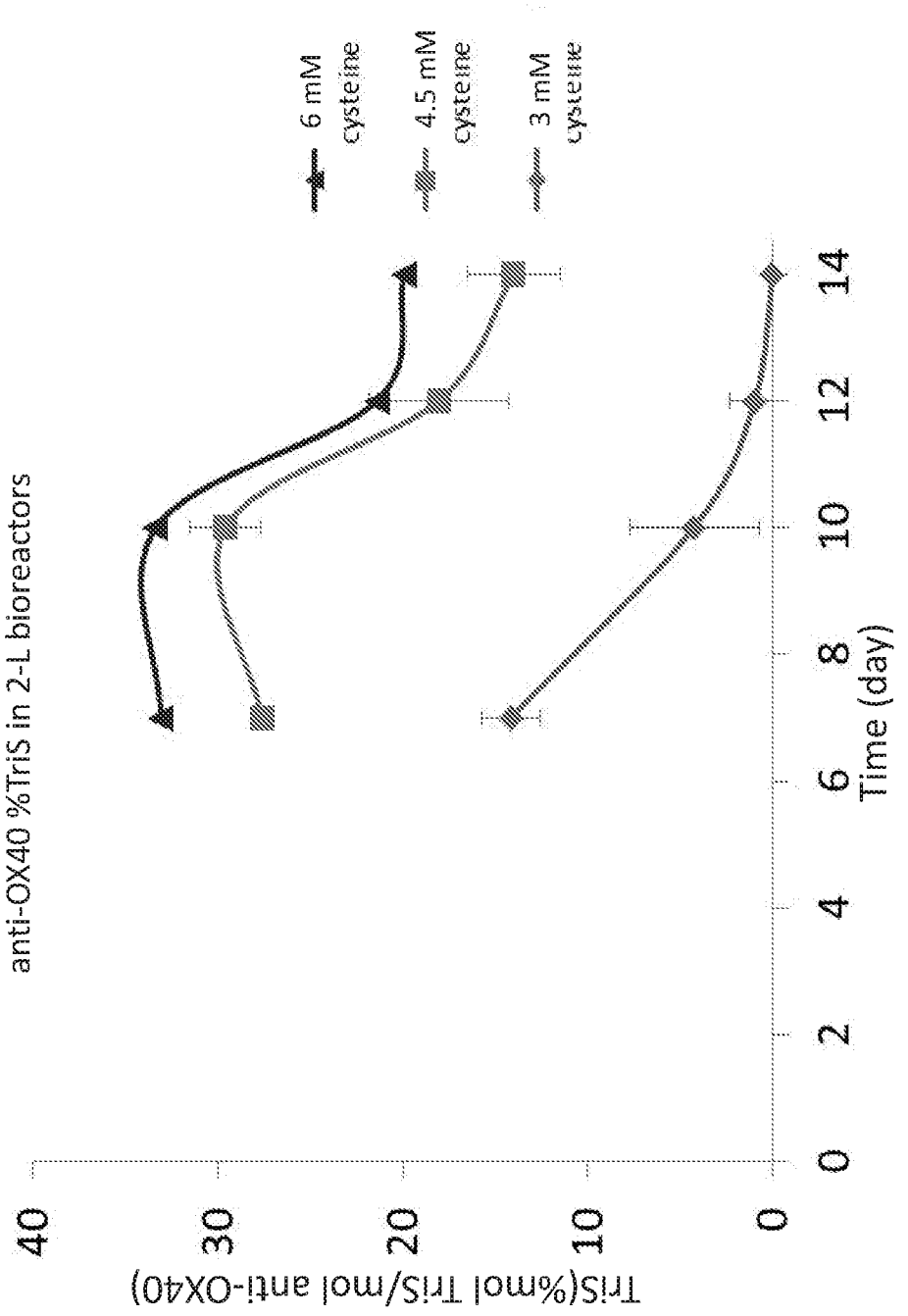

anti-OX40 %TriS in 2-L bioreactors
6 mM cysteine
4.5 mM cysteine
3 mM cysteine
TriS(%mol TriS/mol anti-OX40)
0
10
20
30
40
0
2
4
6
8
10
12
14
Time (day)

FIG. 4B

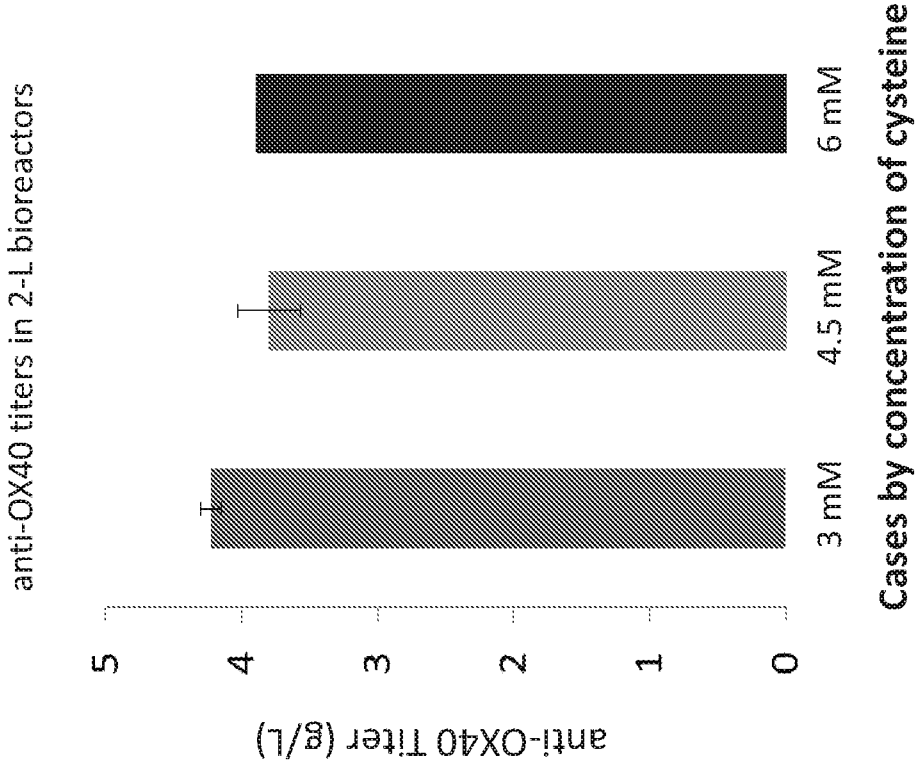

anti-OX40 titers in 2-L bioreactors
anti-OX40 Titer (g/L)
0
1
2
3
4
5
3 mM
4.5 mM
6 mM
Cases by concentration of cysteine

FIG. 5A

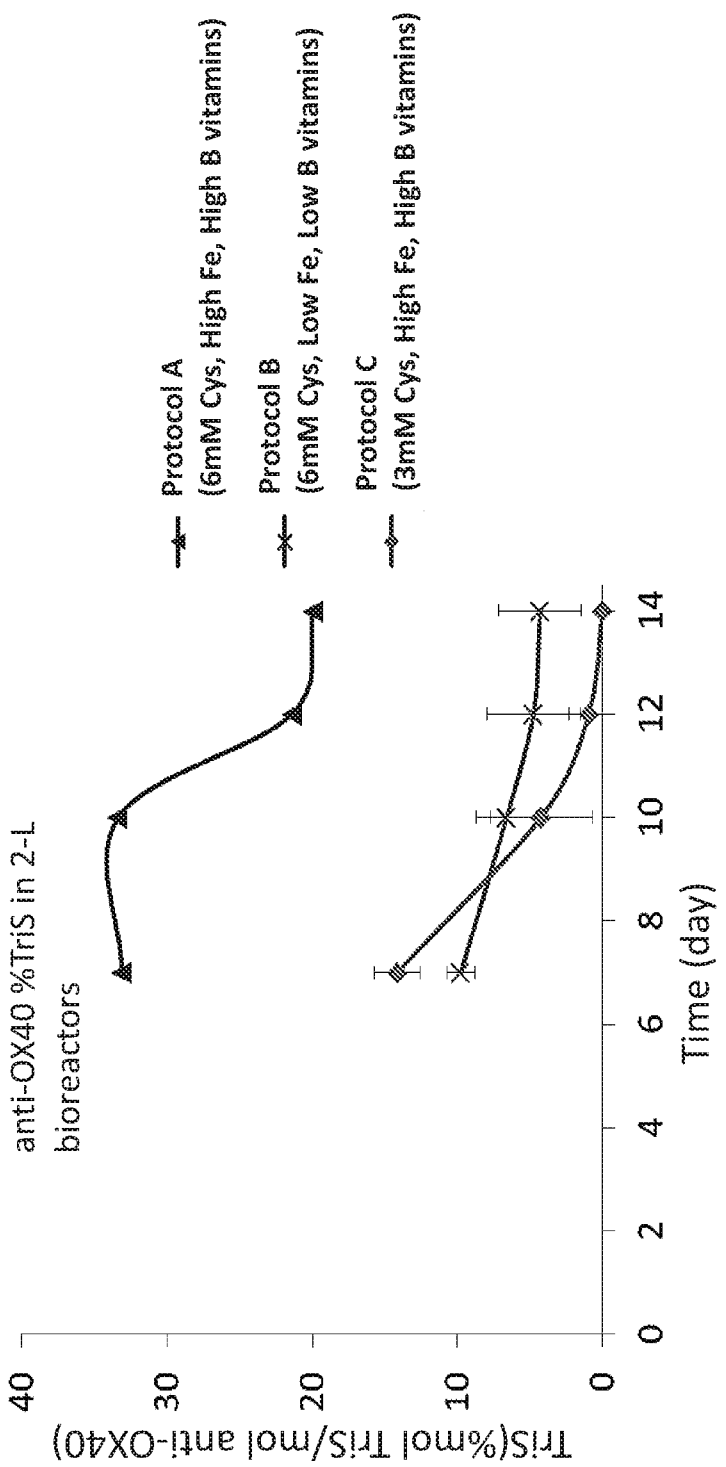

anti-OX40 %TriS in 2-L bioreactors
Protocol A (6mM Cys, High Fe, High B vitamins)
Protocol B (6mM Cys, Low Fe, Low B vitamins)
Protocol C (3mM Cys, High Fe, High B vitamins)
TriS(%mol TriS/mol anti-OX40)
Time (day)
0
10
20
30
40
0
2
4
6
8
10
12
14

FIG. 5B

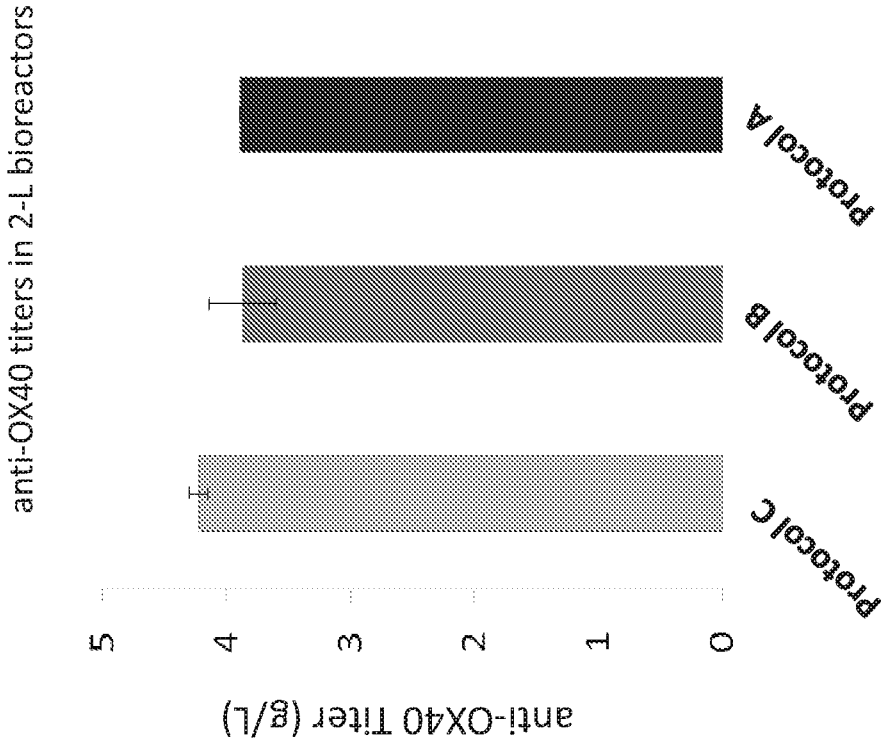

anti-OX40 titers in 2-L bioreactors
anti-OX40 Titer (g/L)
0
1
2
3
4
5
Protocol C
Protocol B
Protocol A

FIG. 5C

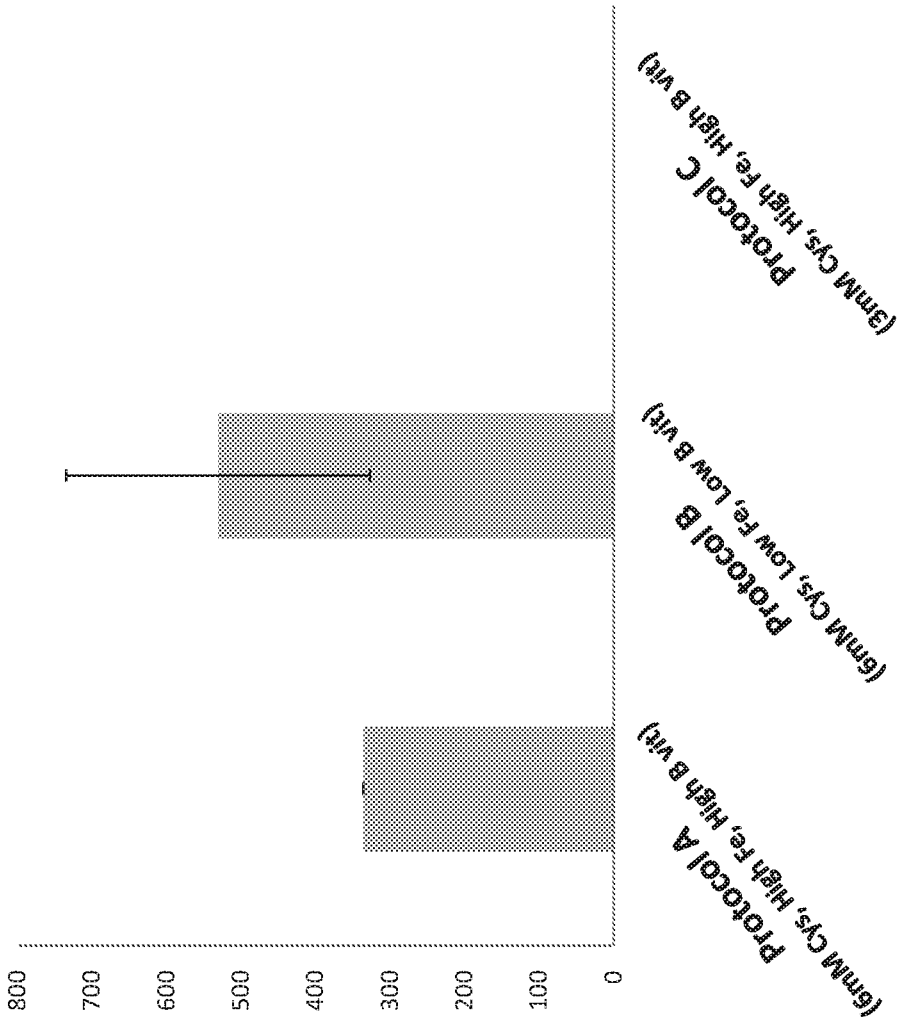

800
700
600
500
400
300
200
100
0
(6mM Cys, High Fe, High B Vit)
Protocol A
(6mM Cys, Low Fe, Low B Vit)
Protocol B
(3mM Cys, High Fe, High B Vit)
Protocol C

FIG. 6

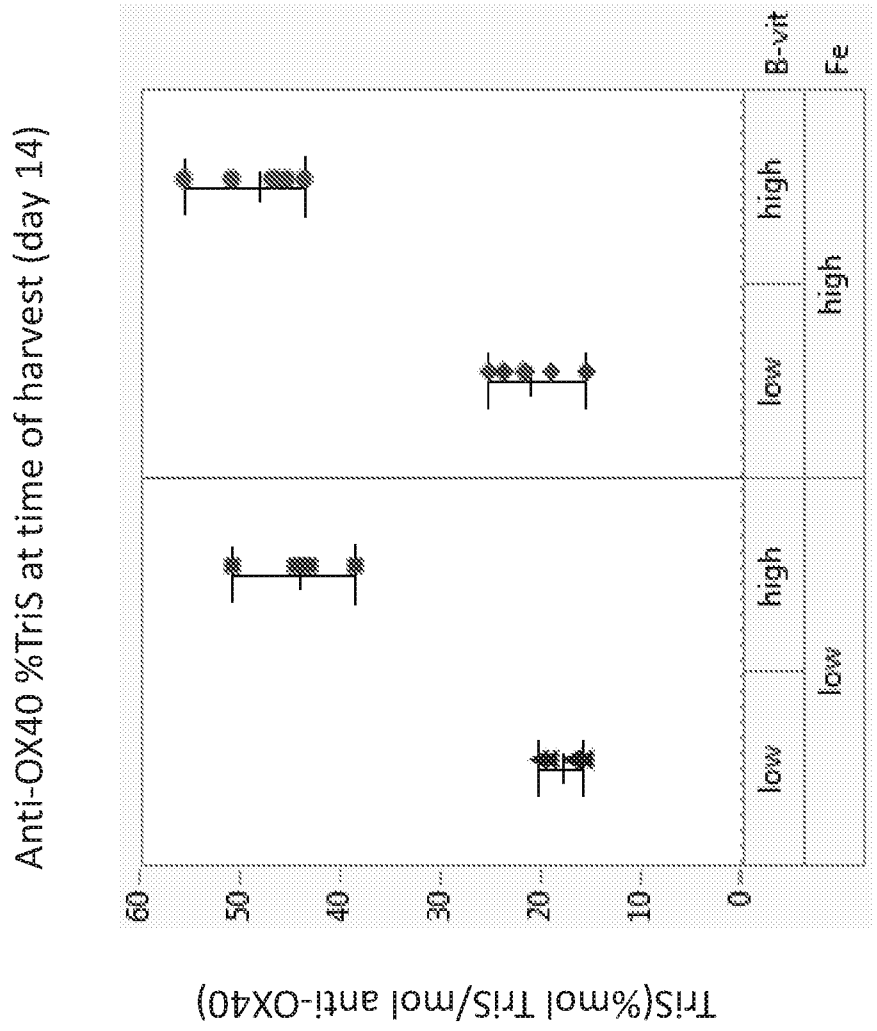

Anti-OX40 %TriS at time of harvest (day 14)
TriS(%mol TriS/mol anti-OX40)
0
10
20
30
40
50
60
Fe
B-vit
low
high
low
high
low
high

FIG. 7A

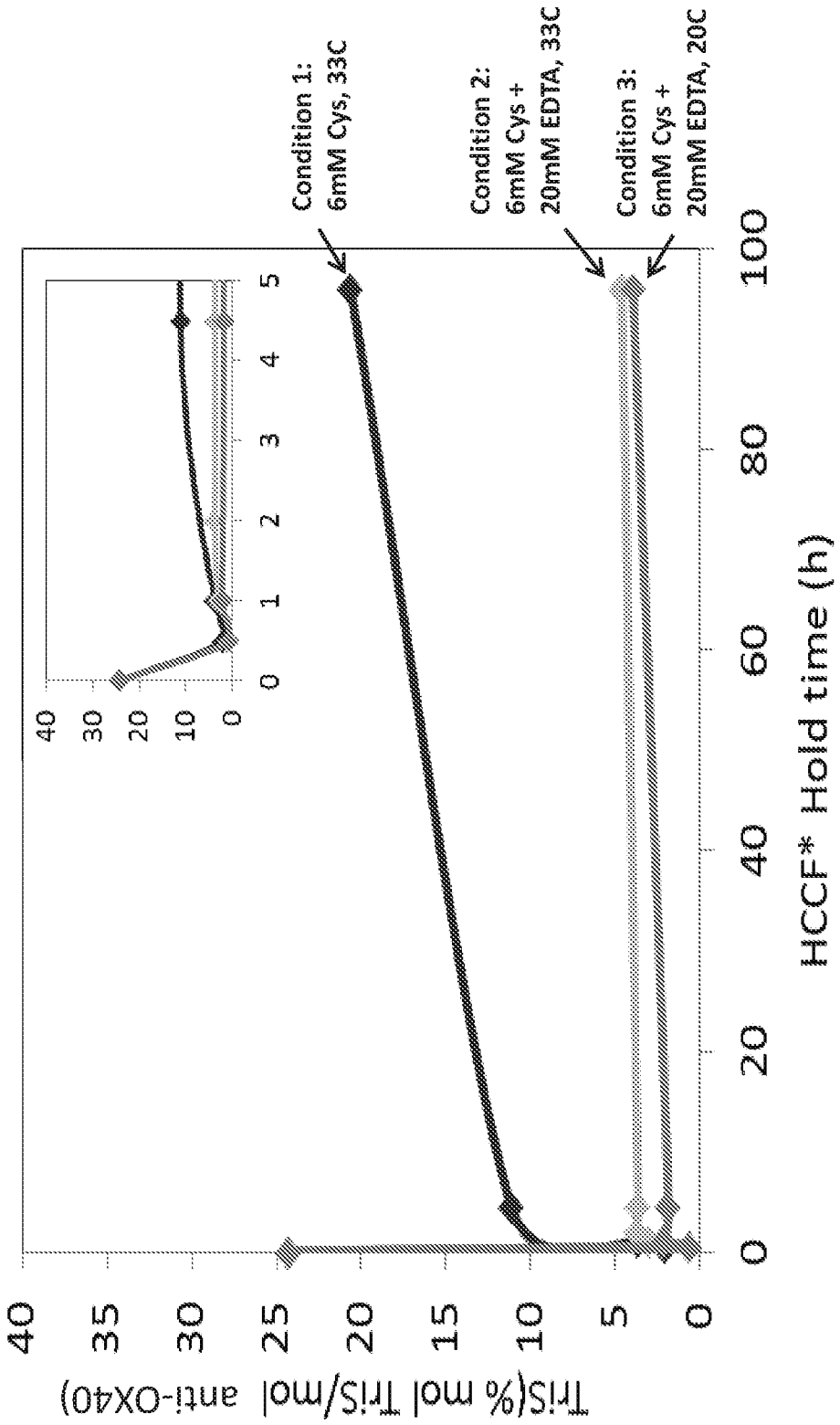

Condition 1:
6mM Cys, 33C
Condition 2:
6mM Cys +
20mM EDTA, 33C
Condition 3:
6mM Cys +
20mM EDTA, 20C
HCCF* Hold time (h)
Tris(% mol Tris/mol anti-OX40)
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40
0
1
2
3
4
5
0
10
20
30
40

FIG. 7B

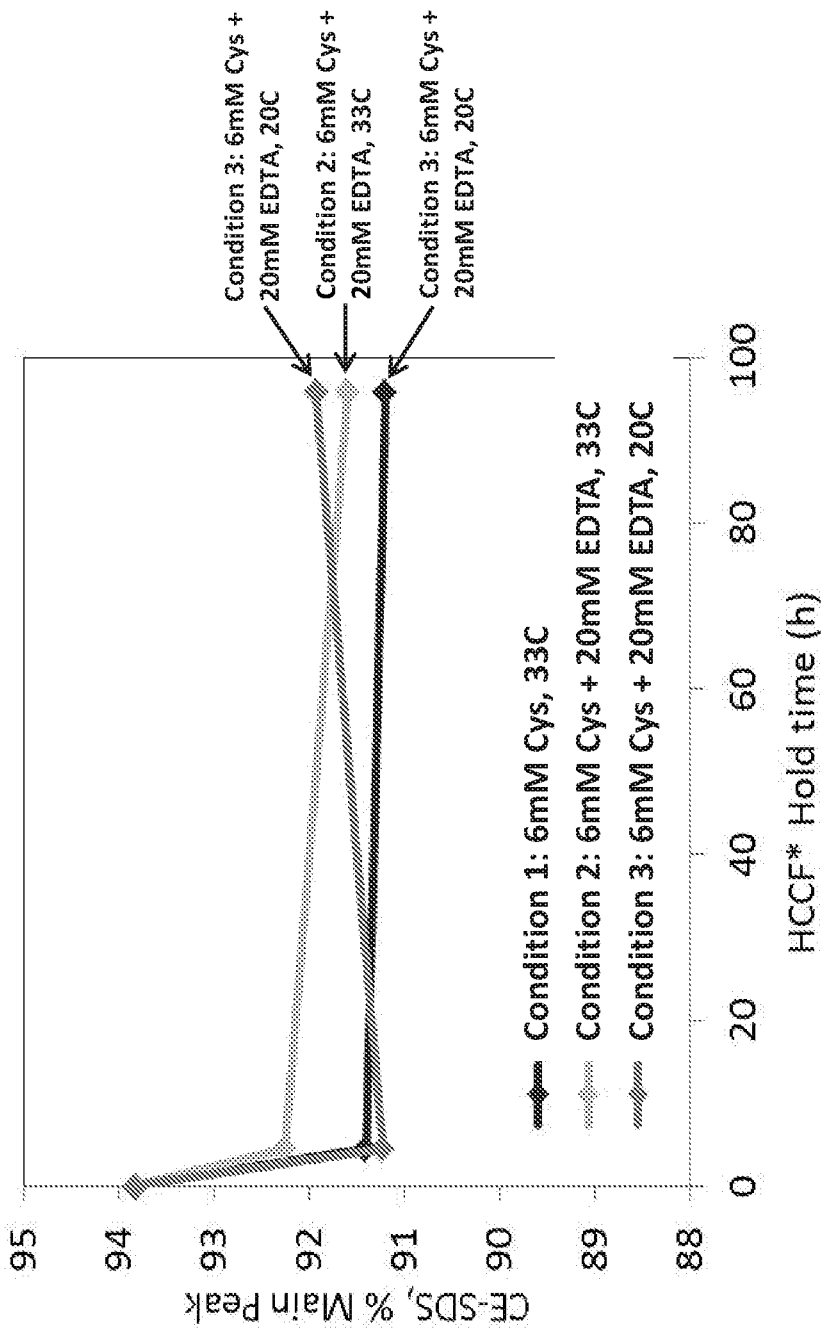

Condition 3: 6mM Cys + 20mM EDTA, 20C
Condition 2: 6mM Cys + 20mM EDTA, 33C
Condition 3: 6mM Cys + 20mM EDTA, 20C
Condition 1: 6mM Cys, 33C
Condition 2: 6mM Cys + 20mM EDTA, 33C
Condition 3: 6mM Cys + 20mM EDTA, 20C
CE-SDS, % Main Peak
95
94
93
92
91
90
89
88
0
20
40
60
80
100
HCCF* Hold time (h)

FIG. 8A

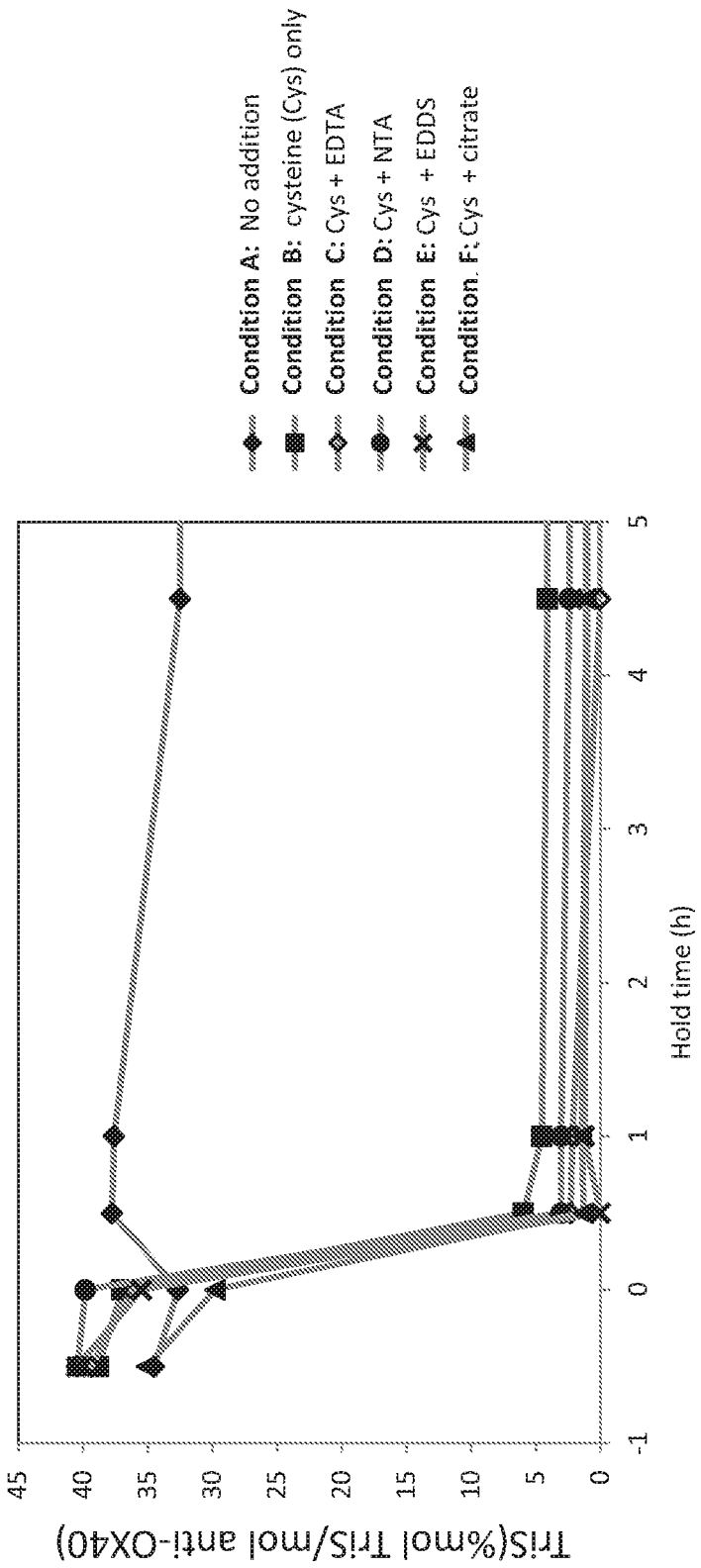

Condition A: No addition
Condition B: cysteine (Cys) only
Condition C: Cys + EDTA
Condition D: Cys + NTA
Condition E: Cys + EDDS
Condition F: Cys + citrate
TriS(%mol TriS/mol anti-OX40)
Hold time (h)
45
40
35
30
25
20
15
10
5
0
-1
0
1
2
3
4
5

FIG. 8B

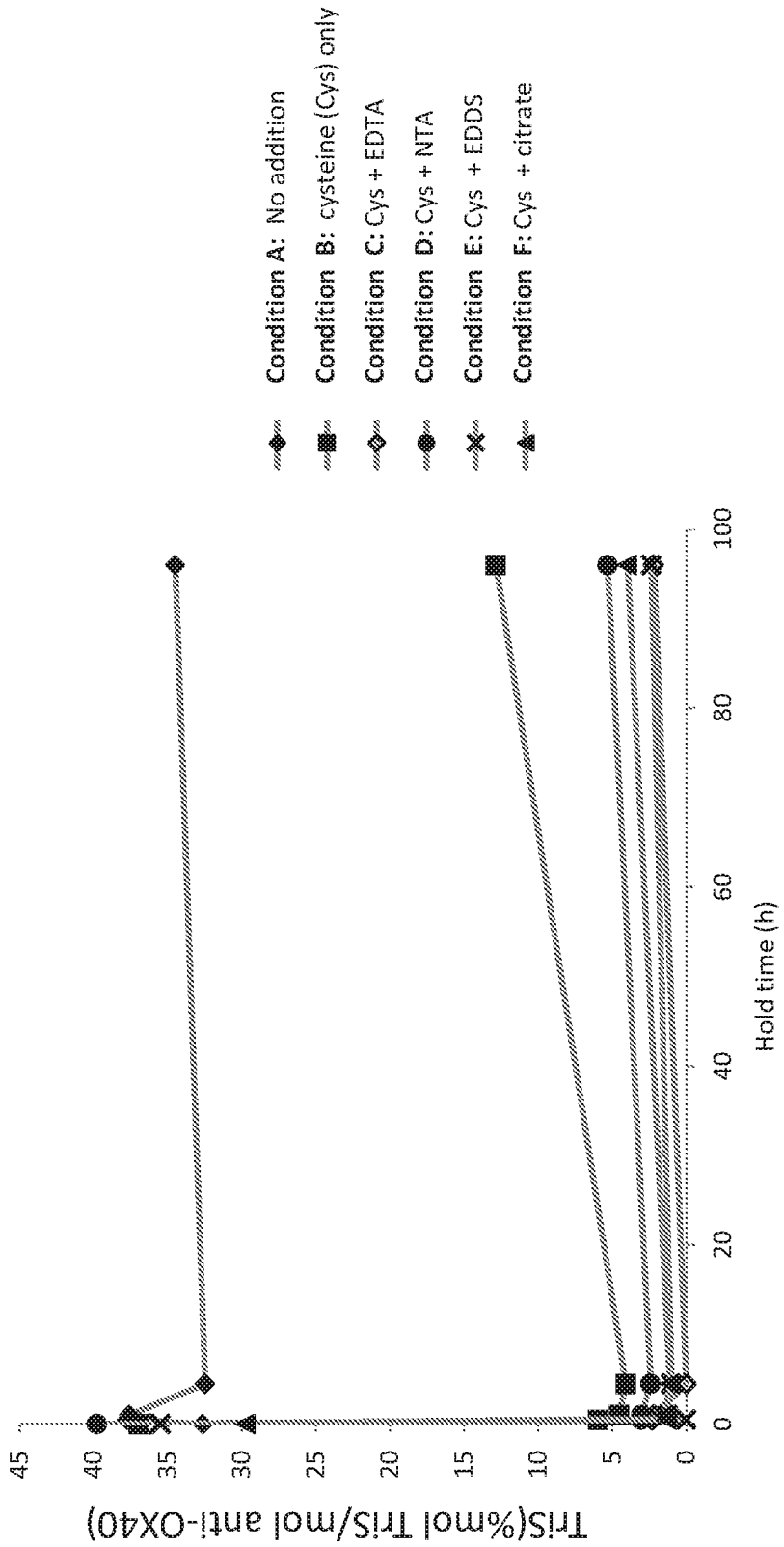

Condition A: No addition
Condition B: cysteine (Cys) only
Condition C: Cys + EDTA
Condition D: Cys + NTA
Condition E: Cys + EDDS
Condition F: Cys + citrate
TriS(%mol TriS/mol anti-OX40)
Hold time (h)
0
5
10
15
20
25
30
35
40
45
0
20
40
60
80
100

FIG. 9

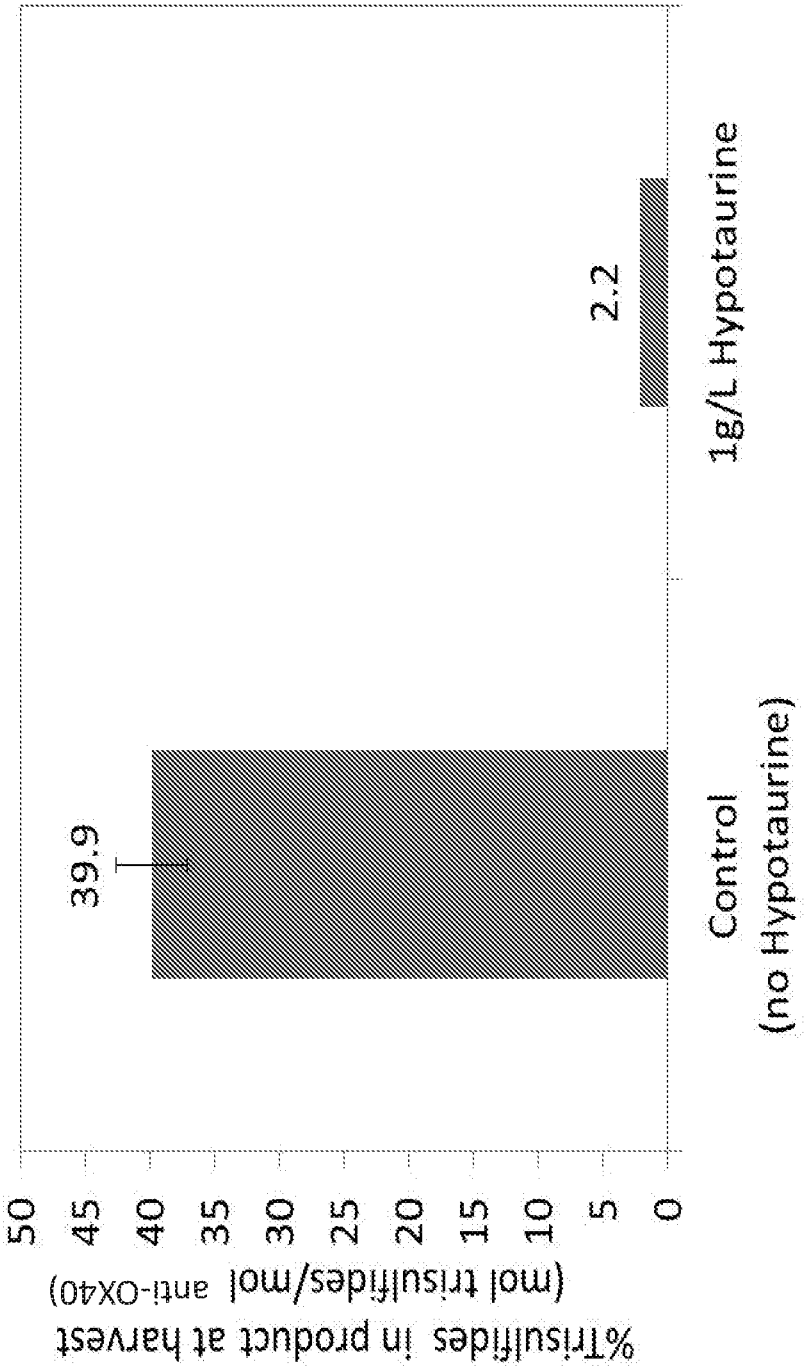

%Trisulfides in product at harvest
(mol trisulfides/mol anti-OX40)
50
45
40
35
30
25
20
15
10
5
0
39.9
2.2
Control
(no Hypotaurine)
1g/L Hypotaurine

FIG. 10

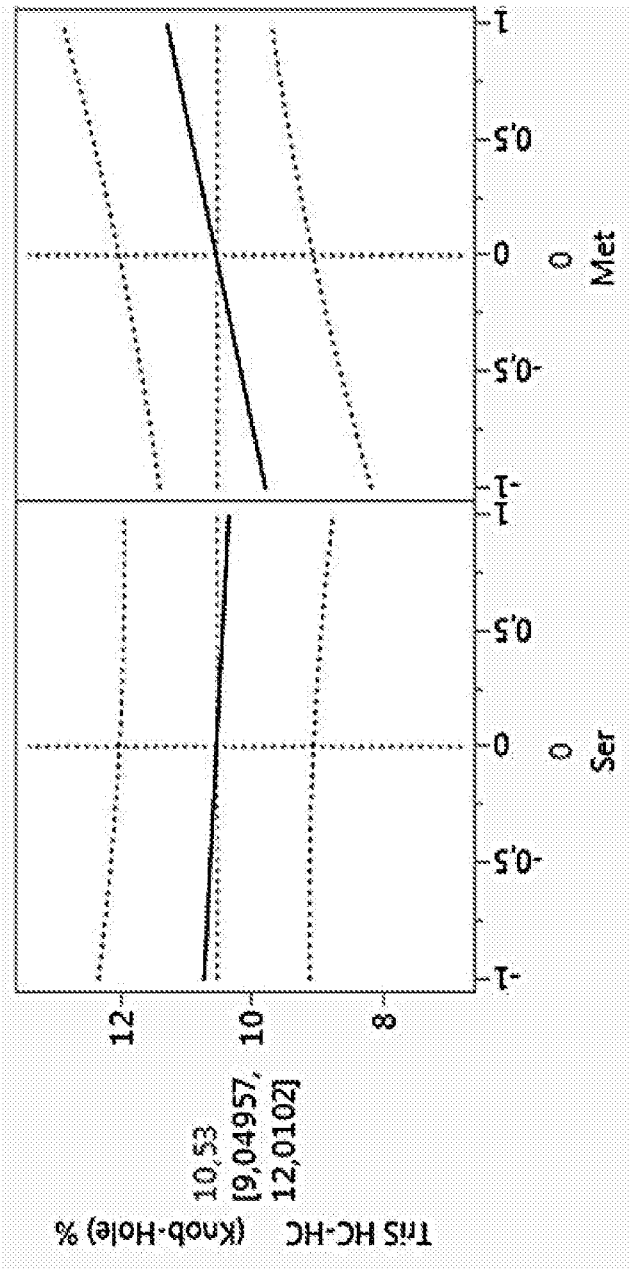

Tris HC-HC (Knob-Hole) %
10,53
[9,04957, 12,0102]
12
10
8
-1
-0,5
0
0,5
1
Ser
Met

FIG. 11

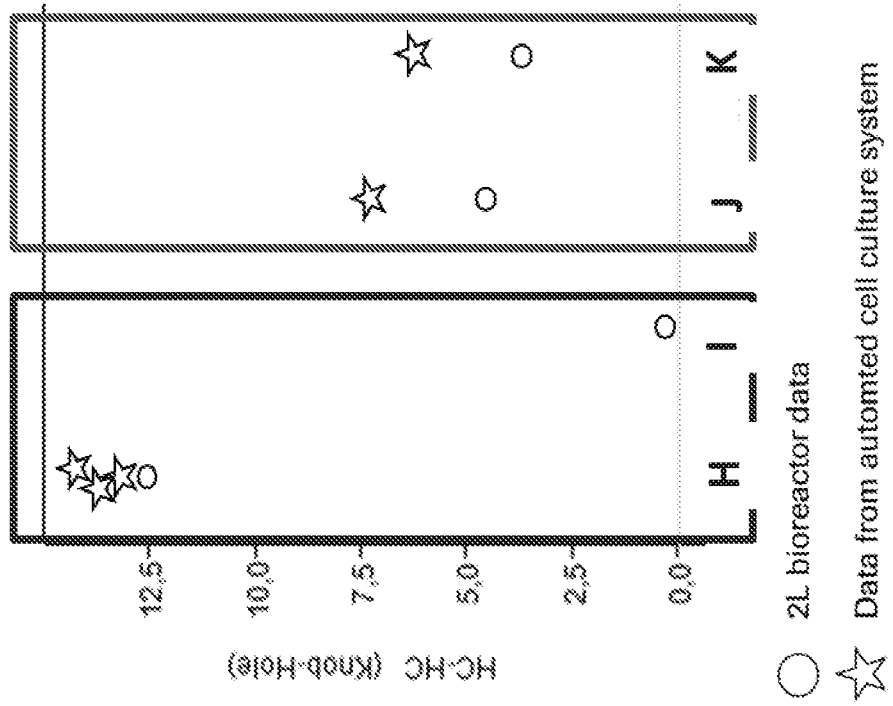
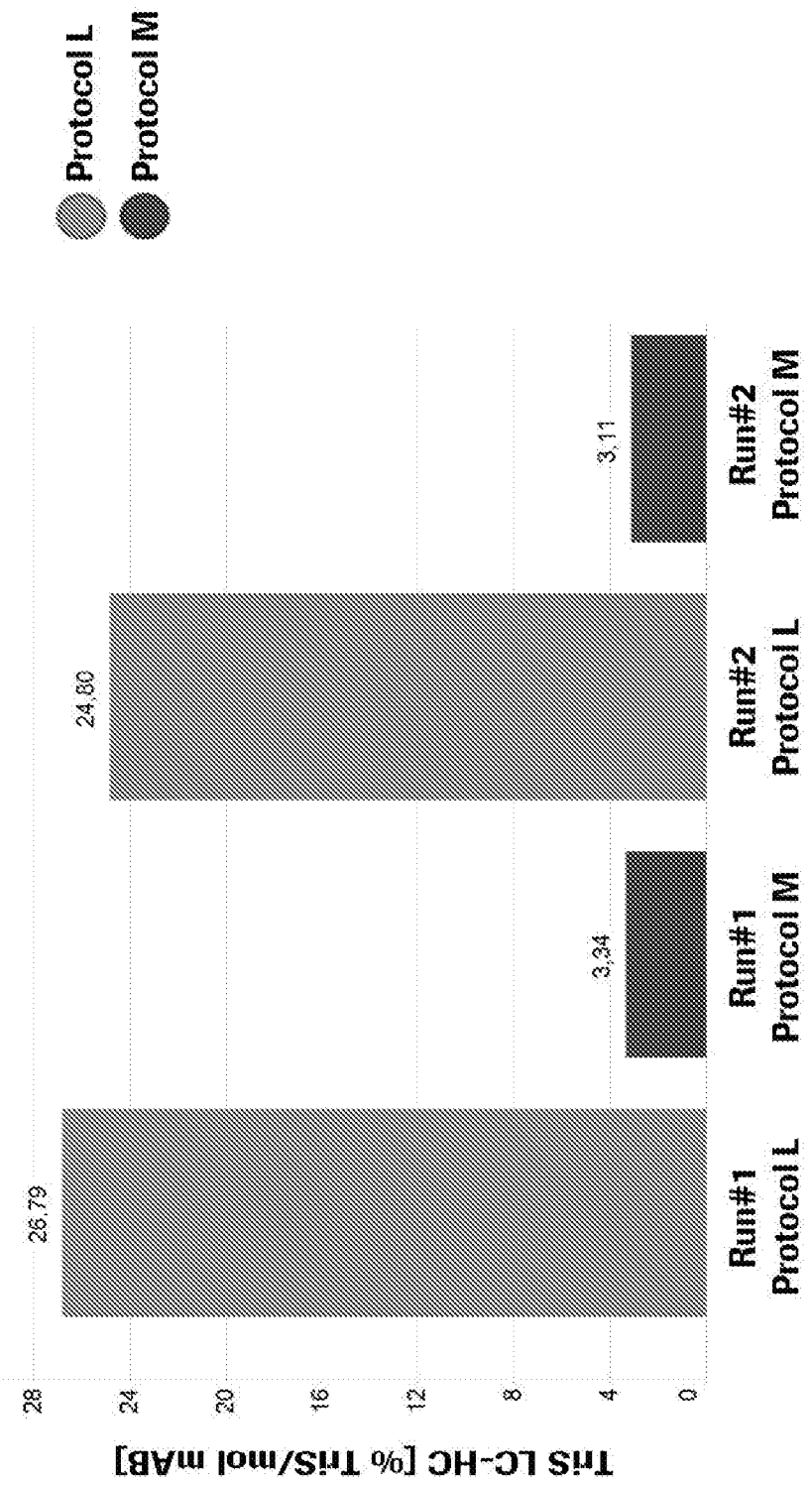

H
I
J
K
12.5
10.0
7.5
5.0
2.5
0.0
HC-HC (Knob-Hole)
2L bioreactor data
Data from automted cell culture system

FIG. 12


Protocol L
Protocol M
TriS LC-HC [% TriS/mol mAB]
28
24
20
16
12
8
4
0
26.79
3.34
24.80
3.11
Run#1 Protocol L
Run#1 Protocol M
Run#2 Protocol L
Run#2 Protocol M