FIG. 1

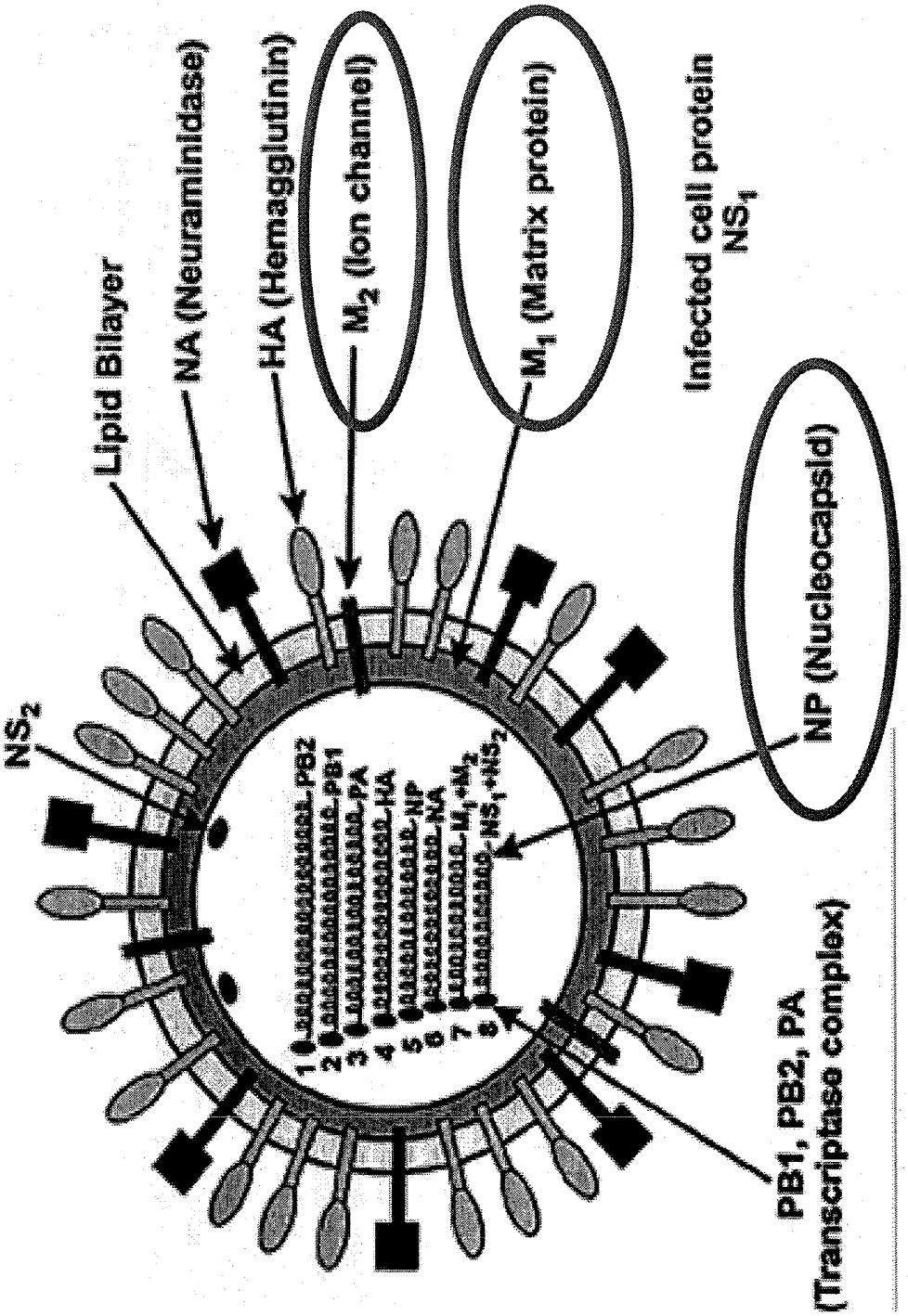

Lipid Bilayer
NA (Neuraminidase)
HA (Hemagglutinin)
M2 (Ion channel)
M1 (Matrix protein)
Infected cell protein
NS1
NS2
NP (Nucleocapsid)
PB1, PB2, PA
(Transcriptase complex)
1
2
3
4
5
6
7
8

FIG. 2

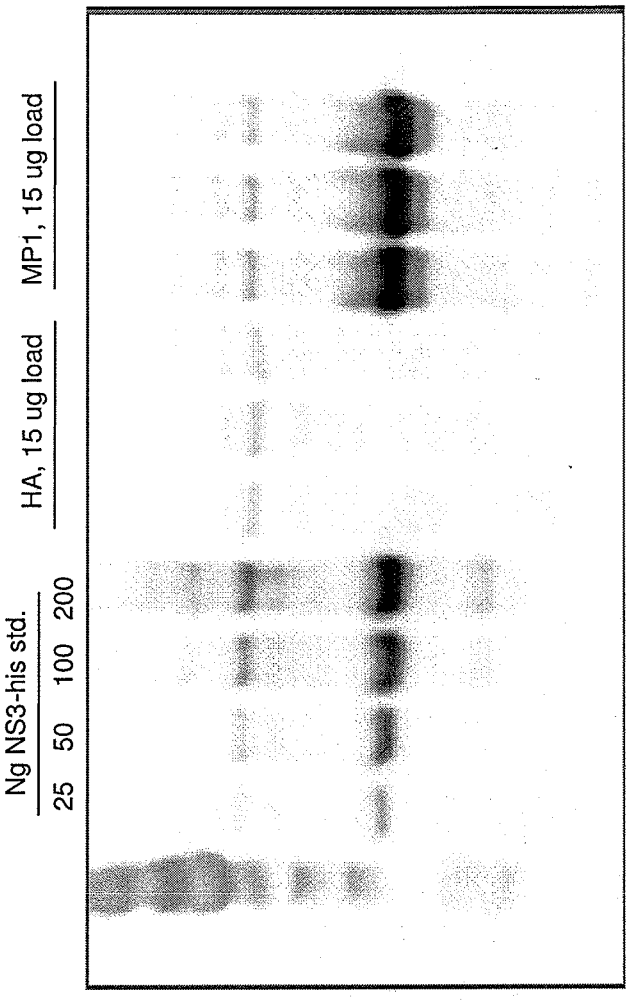


FIG. 3B

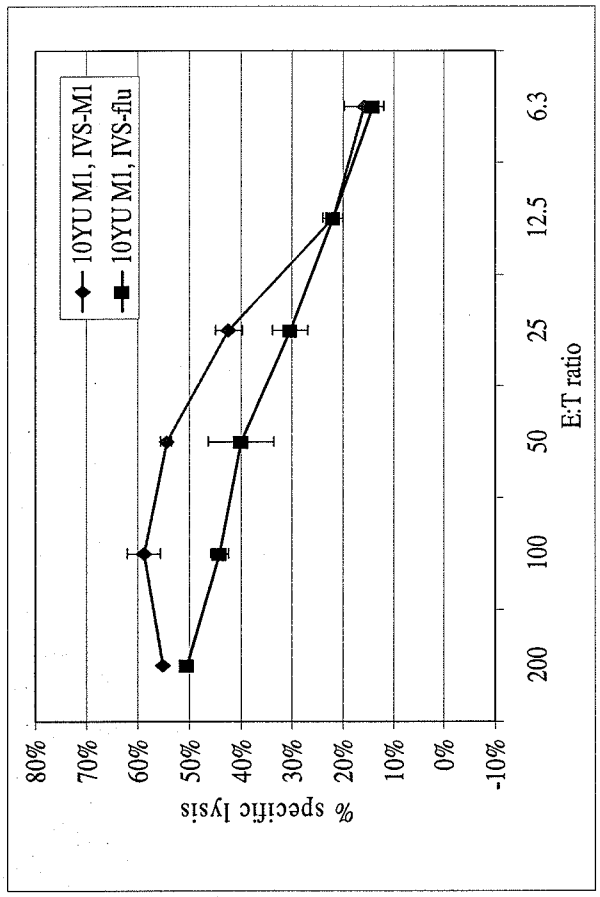
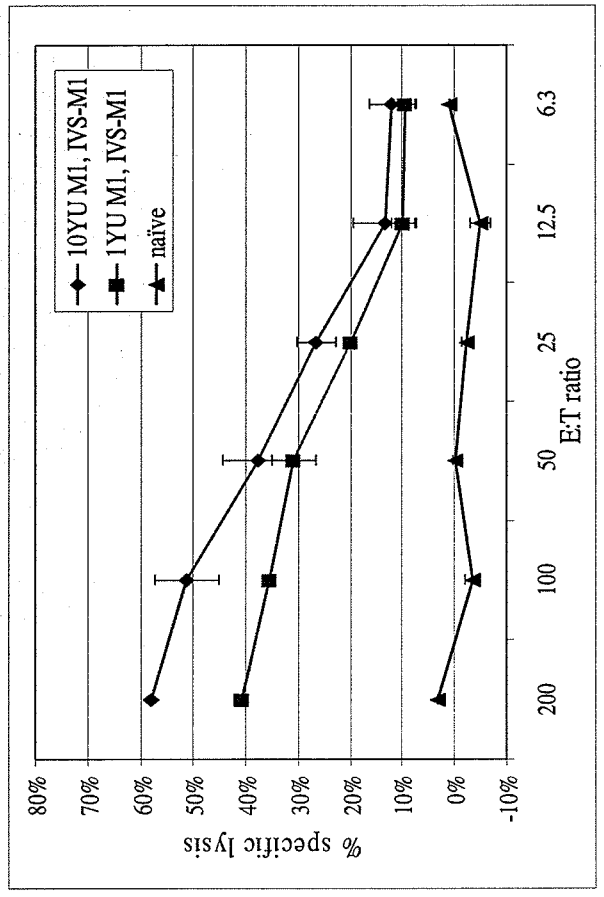

10YU M1, IVS-M1
10YU M1, IVS-flu
% specific lysis
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
200
100
50
25
12.5
6.3
E:T ratio

FIG. 3A


10YU M1, IVS-M1
1YU M1, IVS-M1
naïve
% specific lysis
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
200
100
50
25
12.5
6.3
E:T ratio

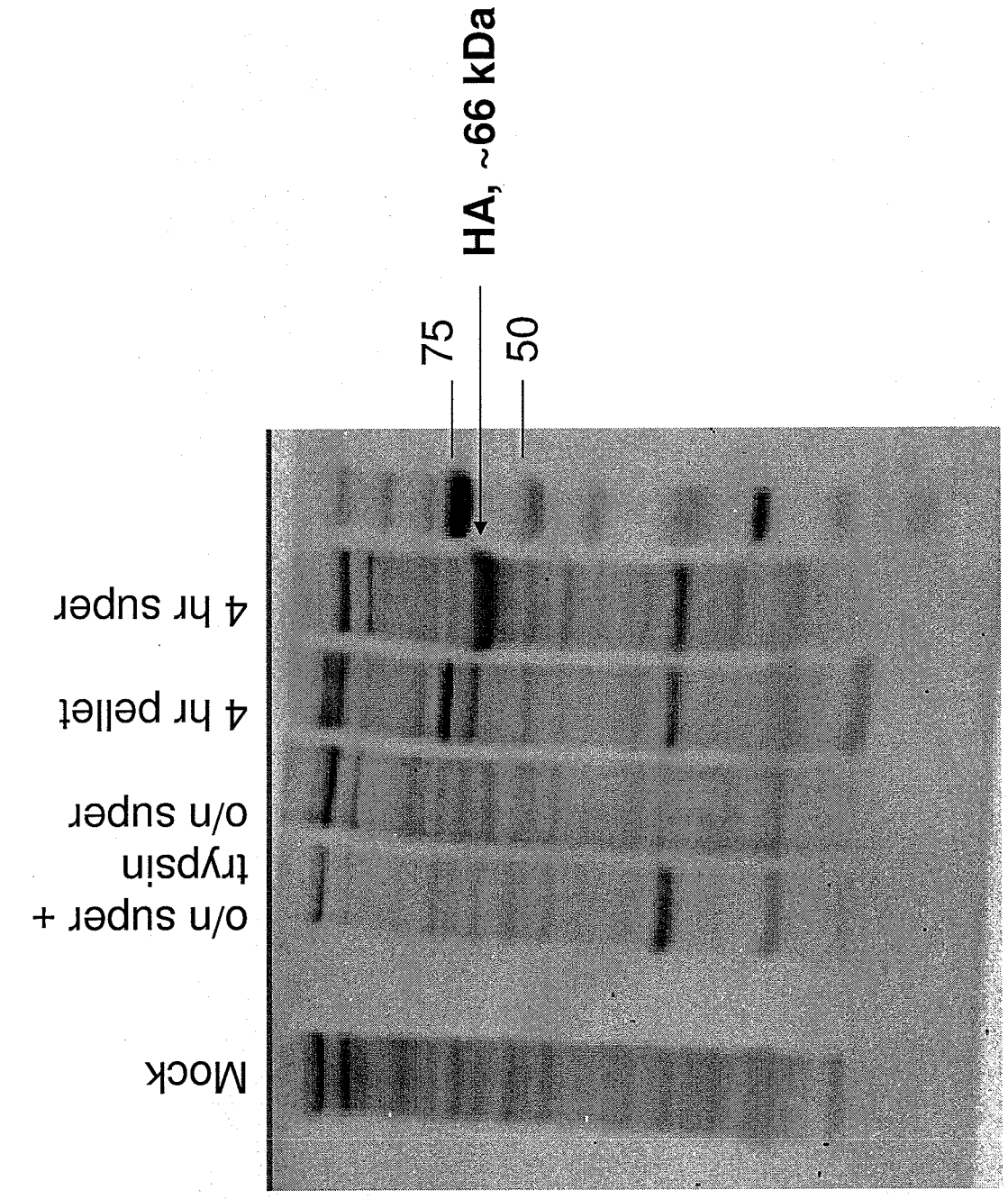
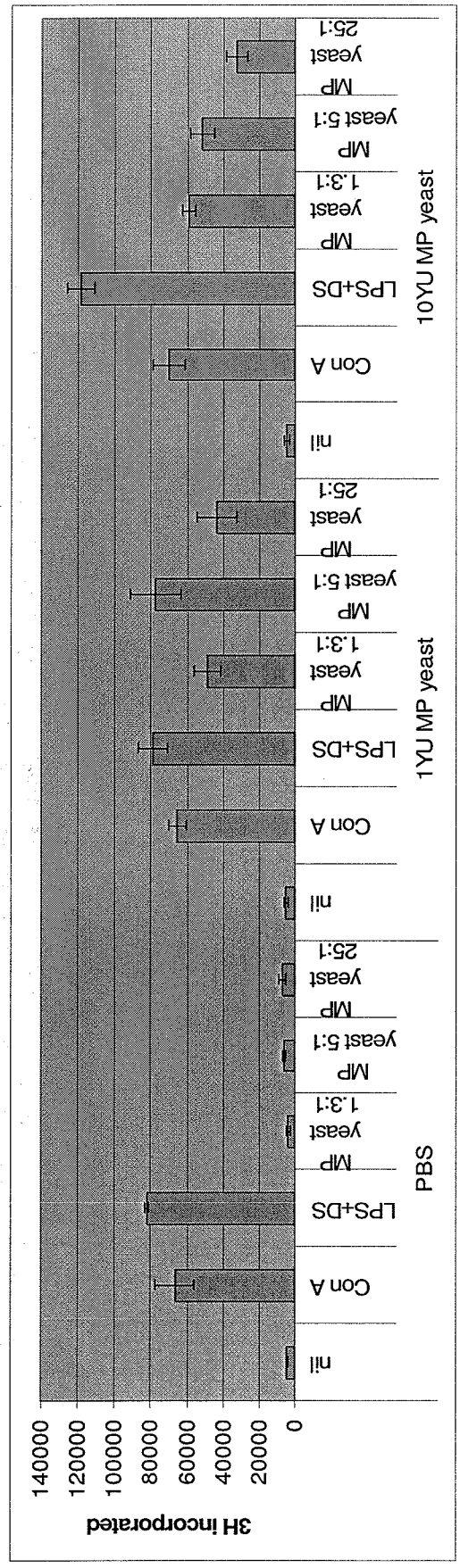

HA, ~66 kDa
75
50
Mock
o/n super +
trypsin
o/n super
4 hr pellet
4 hr super

FIG. 4

FIG. 5


3H incorporated
140000
120000
100000
80000
60000
40000
20000
0
nil
Con A
LPS+DS
MP yeast 1.3:1
MP yeast 5:1
MP yeast 25:1
PBS
1YU MP yeast
10YU MP yeast

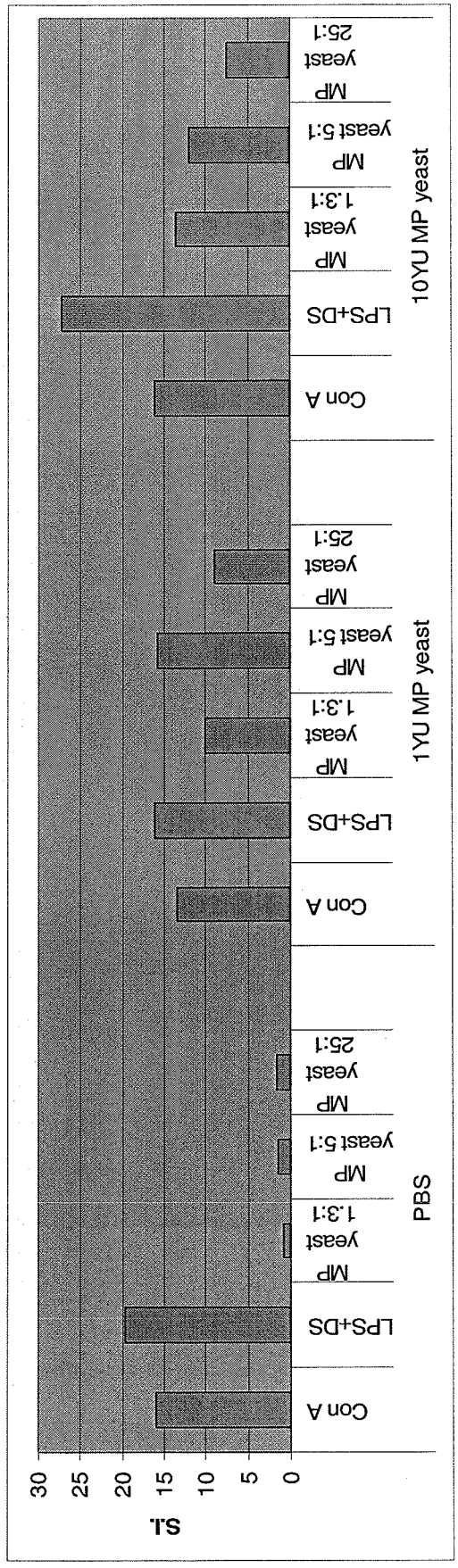

SI.
30
25
20
15
10
5
0
Con A
LPS+DS
MP yeast 1.3:1
MP yeast 5:1
MP yeast 25:1
PBS
1YU MP yeast
10YU MP yeast

FIG. 6

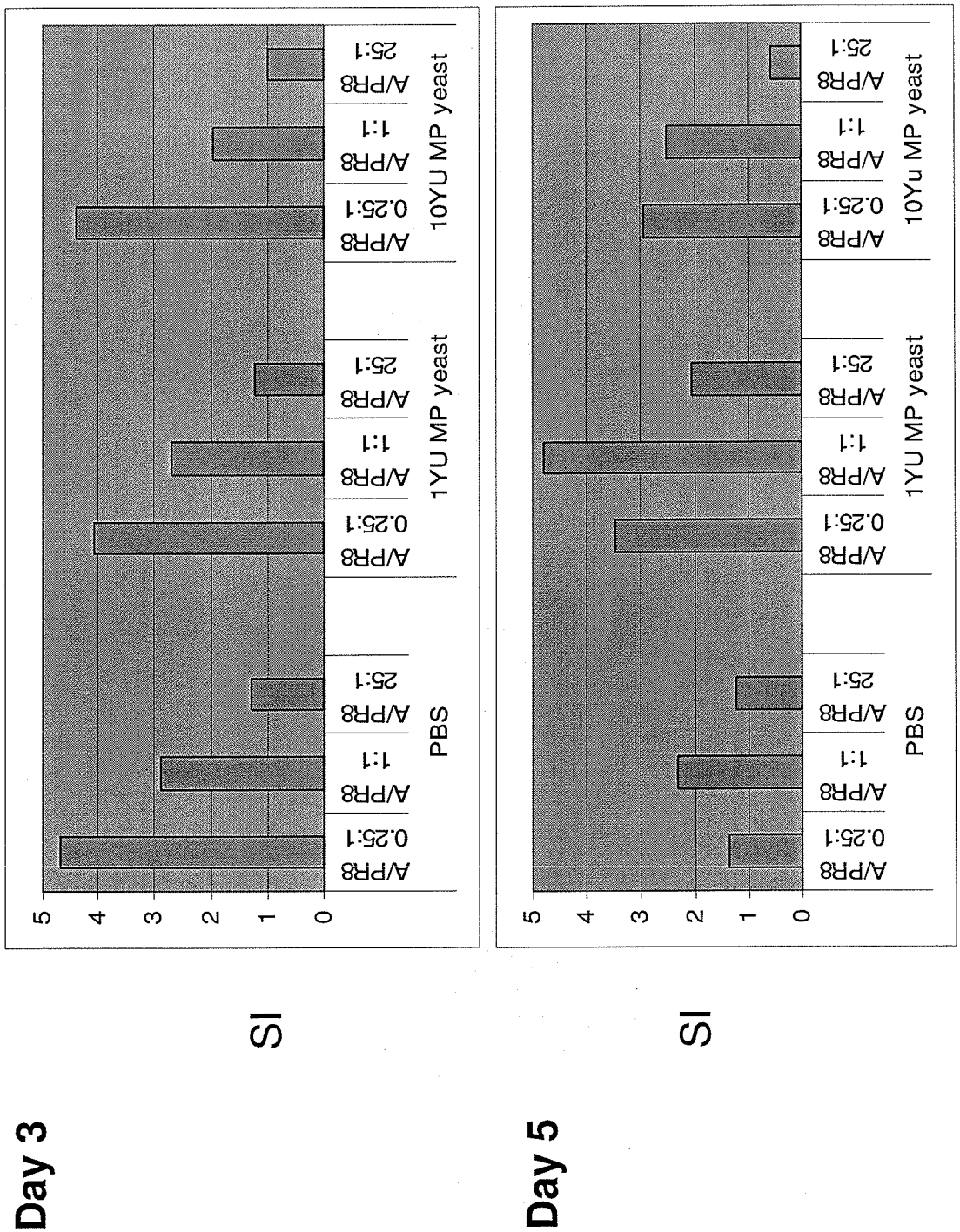

Day 3
SI
5
4
3
2
1
0
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
PBS
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
1YU MP yeast
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
10YU MP yeast
Day 5
SI
5
4
3
2
1
0
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
PBS
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
1YU MP yeast
A/PR8 0.25:1
A/PR8 1:1
A/PR8 25:1
10Yu MP yeast

FIG. 7

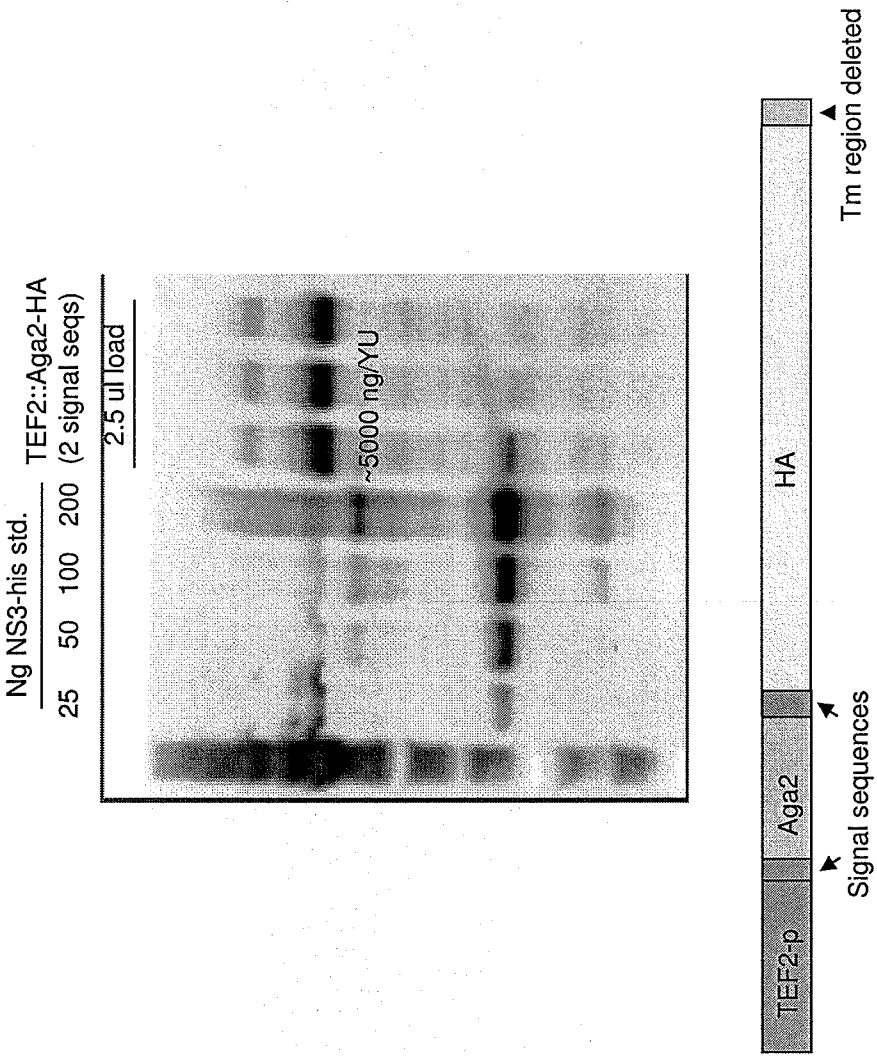
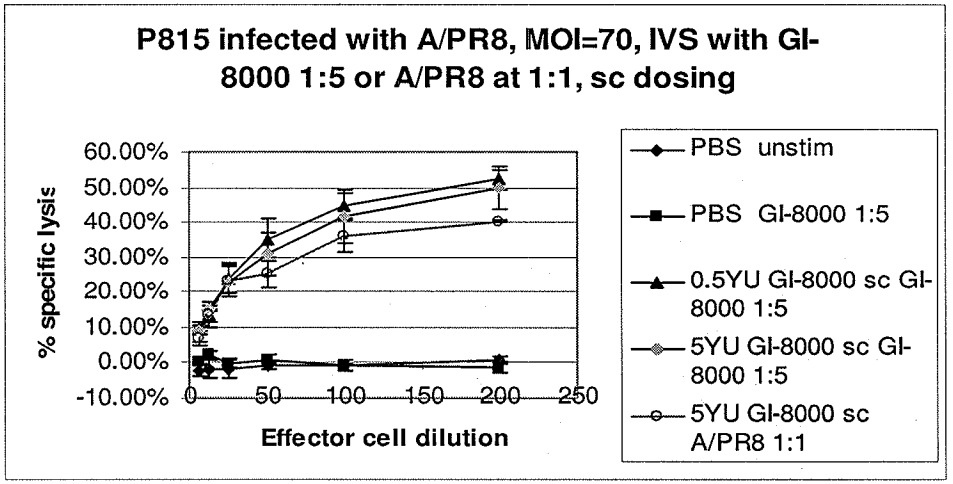
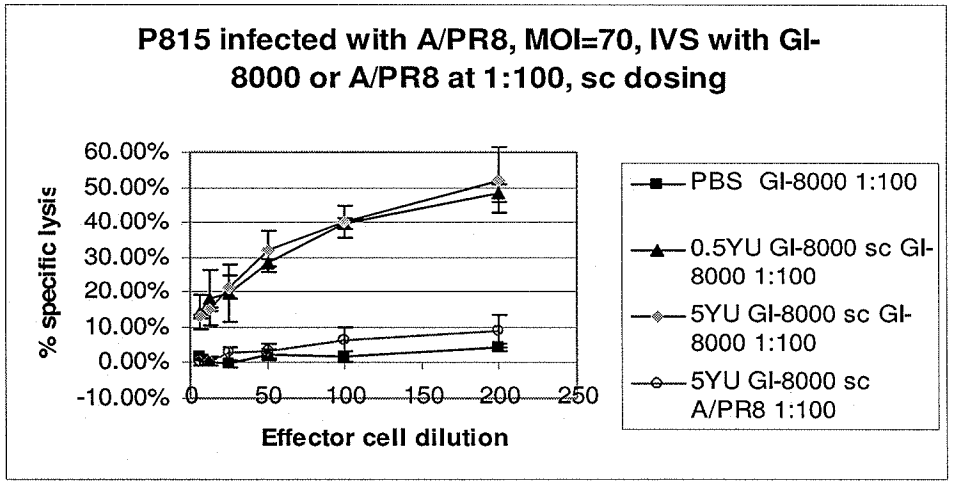
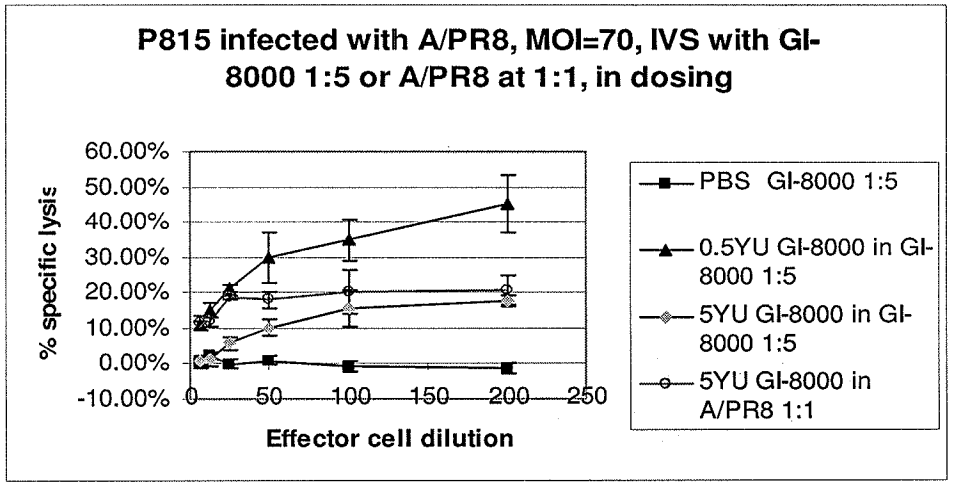

Ng NS3-his std.
25 50 100 200
TEF2::Aga2-HA
(2 signal seqs)
2.5 ul load
~5000 ng/YU
TEF2-p
Aga2
HA
Signal sequences
Tm region deleted

FIG. 8


P815 infected with A/PR8, MOI=70, IVS with GI-8000 1:5 or A/PR8 at 1:1, sc dosing
% specific lysis
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
-10.00%
0
50
100
150
200
250
Effector cell dilution
PBS unstim
PBS GI-8000 1:5
0.5YU GI-8000 sc GI-8000 1:5
5YU GI-8000 sc GI-8000 1:5
5YU GI-8000 sc A/PR8 1:1


P815 infected with A/PR8, MOI=70, IVS with GI-8000 or A/PR8 at 1:100, sc dosing
% specific lysis
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
-10.00%
0
50
100
150
200
250
Effector cell dilution
PBS GI-8000 1:100
0.5YU GI-8000 sc GI-8000 1:100
5YU GI-8000 sc GI-8000 1:100
5YU GI-8000 sc A/PR8 1:100


P815 infected with A/PR8, MOI=70, IVS with GI-8000 1:5 or A/PR8 at 1:1, in dosing
% specific lysis
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
-10.00%
0
50
100
150
200
250
Effector cell dilution
PBS GI-8000 1:5
0.5YU GI-8000 in GI-8000 1:5
5YU GI-8000 in GI-8000 1:5
5YU GI-8000 in A/PR8 1:1

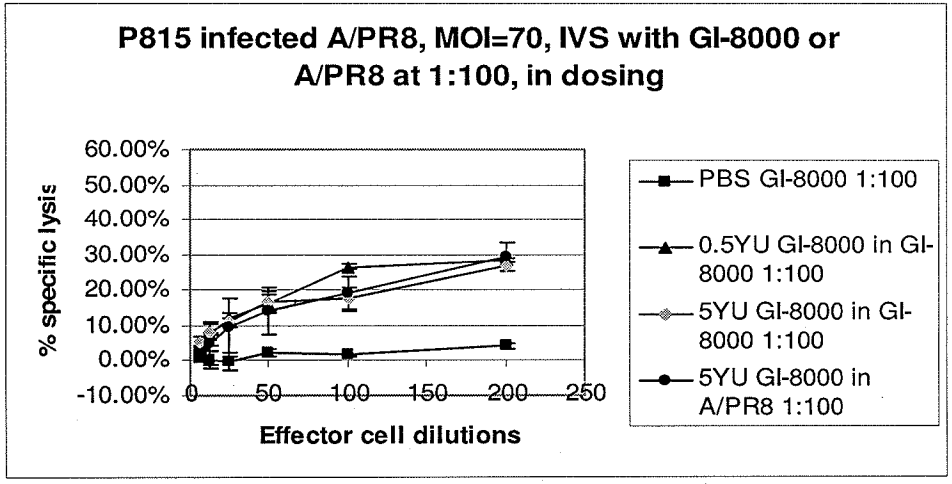

P815 infected A/PR8, MOI=70, IVS with GI-8000 or A/PR8 at 1:100, in dosing
% specific lysis
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
-10.00%
0
50
100
150
200
250
Effector cell dilutions
PBS GI-8000 1:100
0.5YU GI-8000 in GI-8000 1:100
5YU GI-8000 in GI-8000 1:100
5YU GI-8000 in A/PR8 1:100

FIG. 9

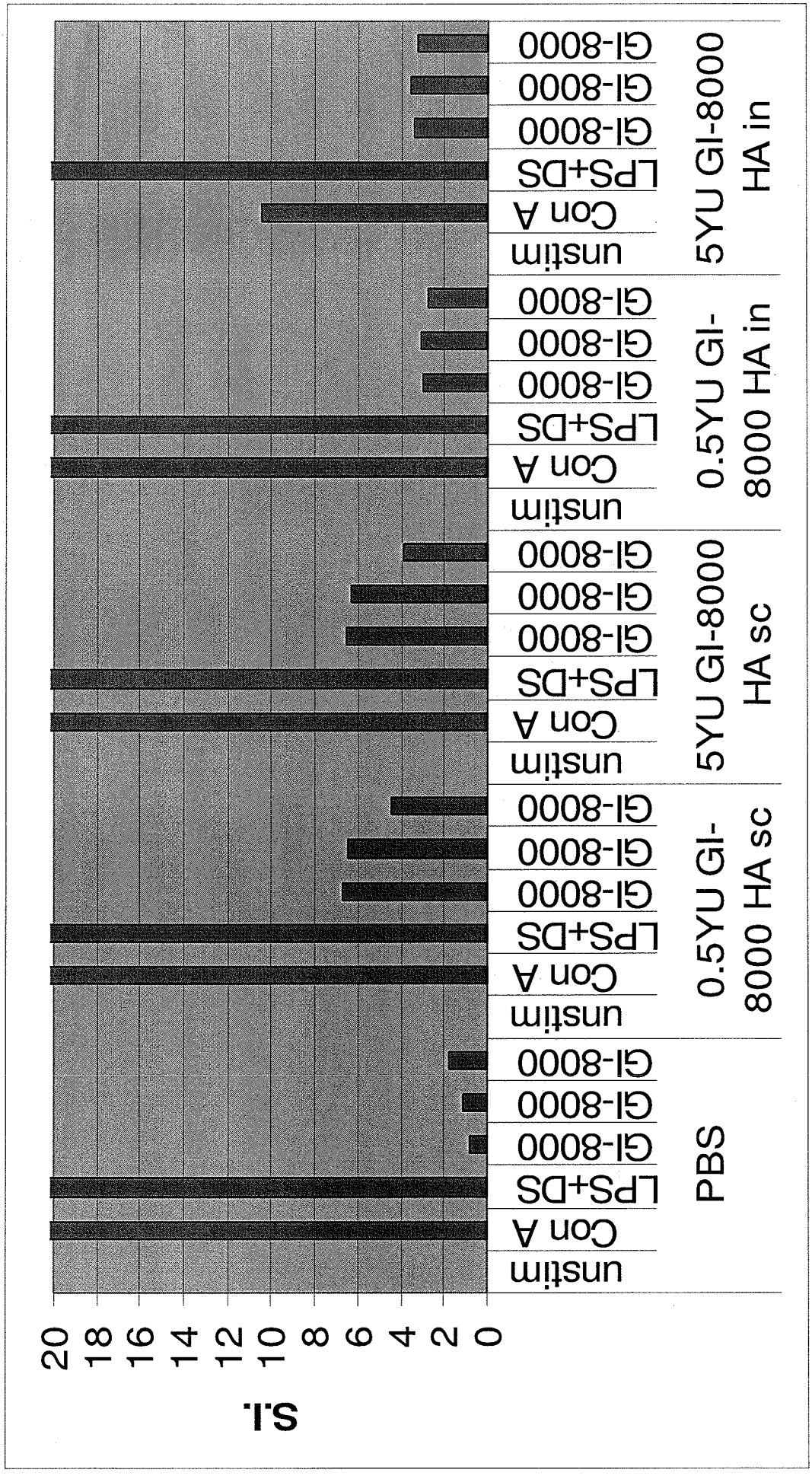
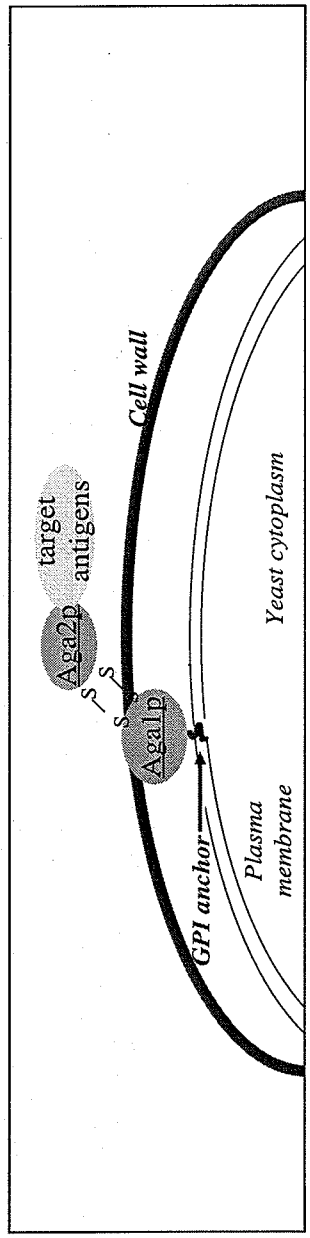
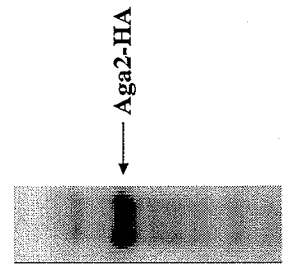
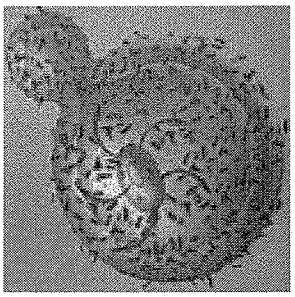

S.I.
0
2
4
6
8
10
12
14
16
18
20
unstim
Con A
LPS+DS
GI-8000
GI-8000
GI-8000
PBS
unstim
Con A
LPS+DS
GI-8000
GI-8000
GI-8000
0.5YU GI-8000 HA sc
unstim
Con A
LPS+DS
GI-8000
GI-8000
GI-8000
5YU GI-8000 HA sc
unstim
Con A
LPS+DS
GI-8000
GI-8000
GI-8000
0.5YU GI-8000 HA in
unstim
Con A
LPS+DS
GI-8000
GI-8000
GI-8000
5YU GI-8000 HA in

FIG. 10A







GI-8003 (prototype): A/PR/8/34 H1-HA expressed in yeast as Aga2-HA fusion protein

FIG. 10B

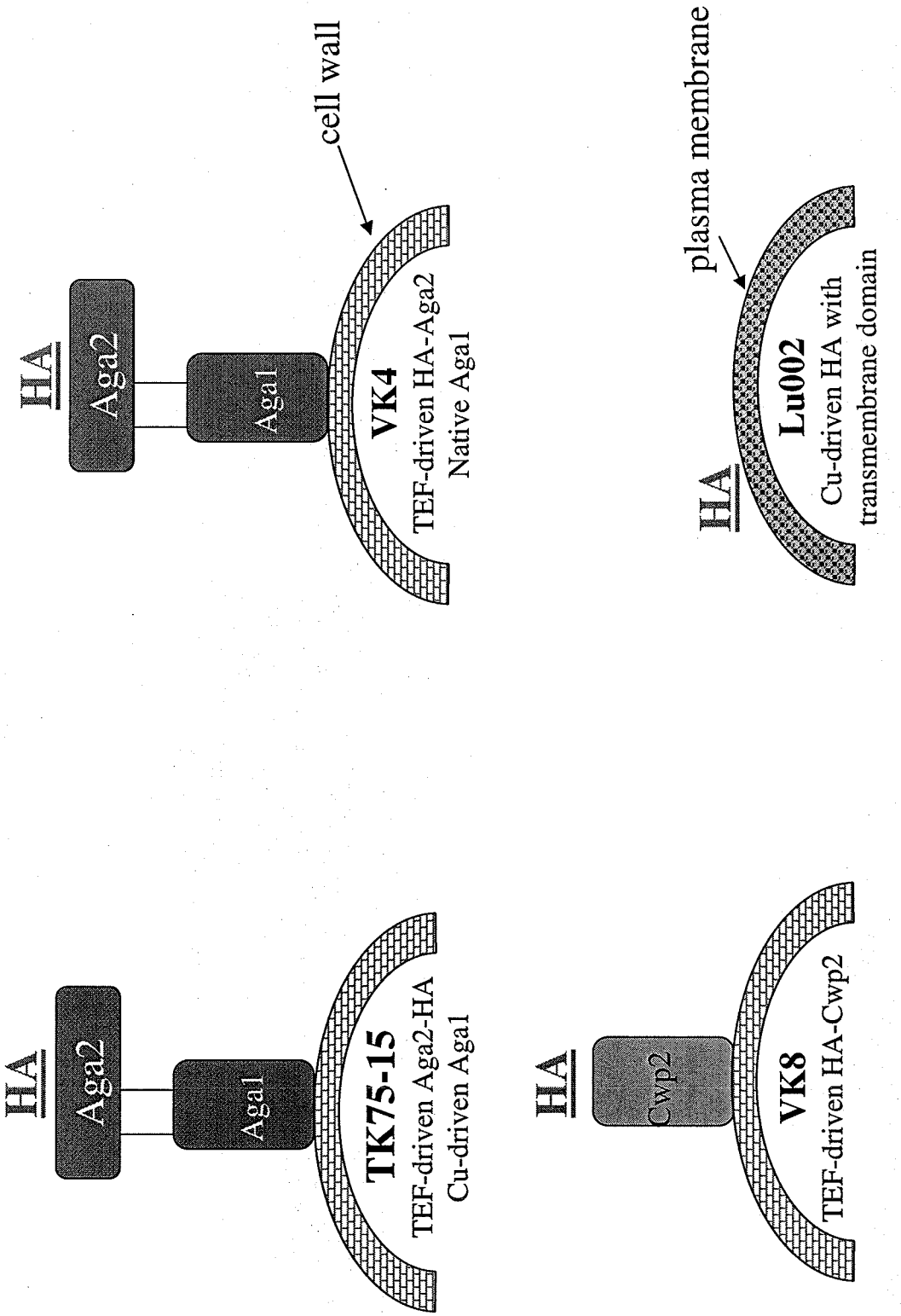
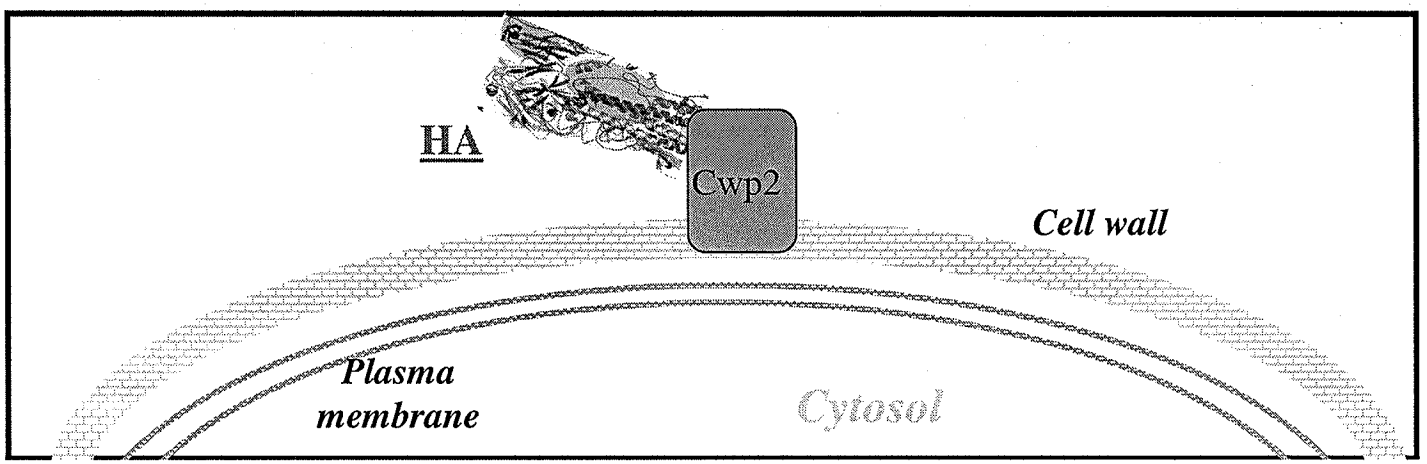
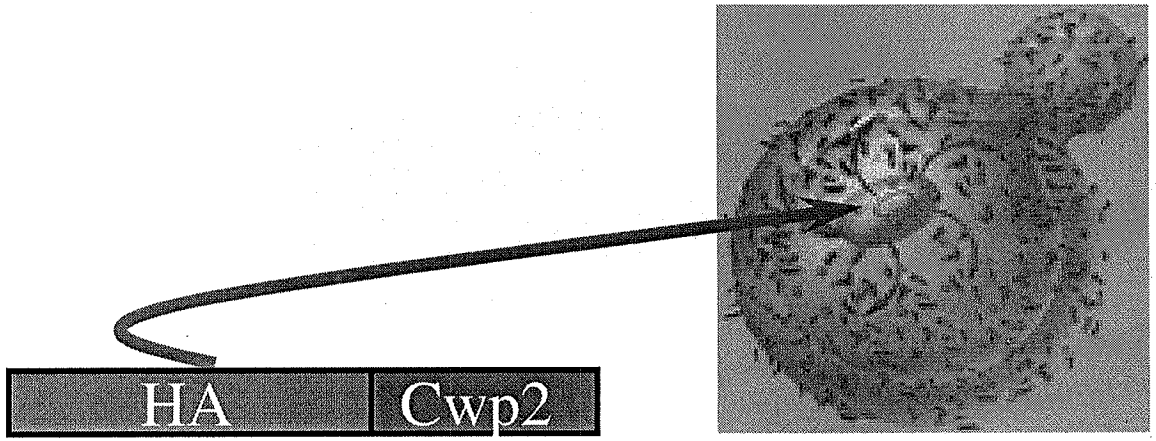
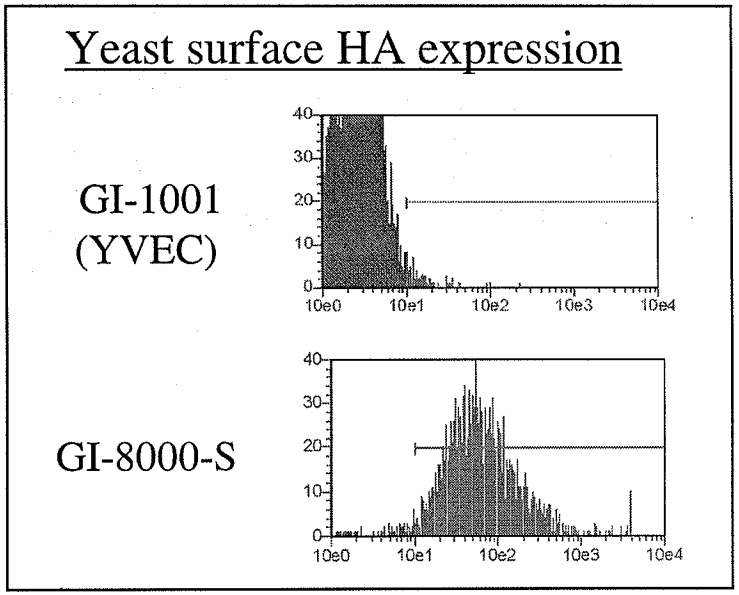

HA
Aga2
Aga1
TK75-15
TEF-driven Aga2-HA
Cu-driven Aga1
HA
Aga2
Aga1
cell wall
VK4
TEF-driven HA-Aga2
Native Aga1
HA
Cwp2
VK8
TEF-driven HA-Cwp2
plasma membrane
HA
Lu002
Cu-driven HA with
transmembrane domain

FIG. 11


HA
Cwp2
Cell wall
Plasma membrane
Cytosol


HA
Cwp2


Yeast surface HA expression
GI-1001
(YVEC)
GI-8000-S
40
30
20
10
0
10e0
10e1
10e2
10e3
10e4

Aga2 + HA fusion approach

YEX

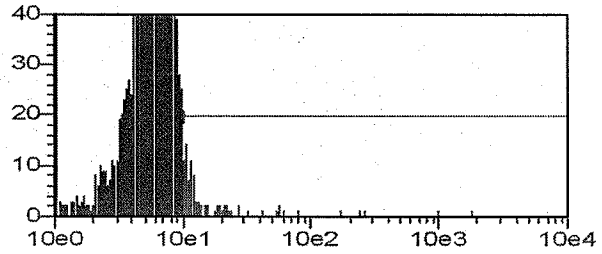


FIG. 12A

VK4
(HA-Aga2)

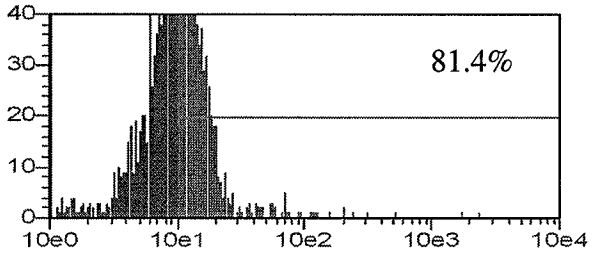


FIG. 12B

TK75-15
(Aga2-HA)

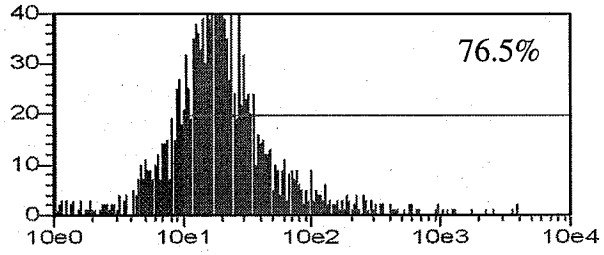


FIG. 12C

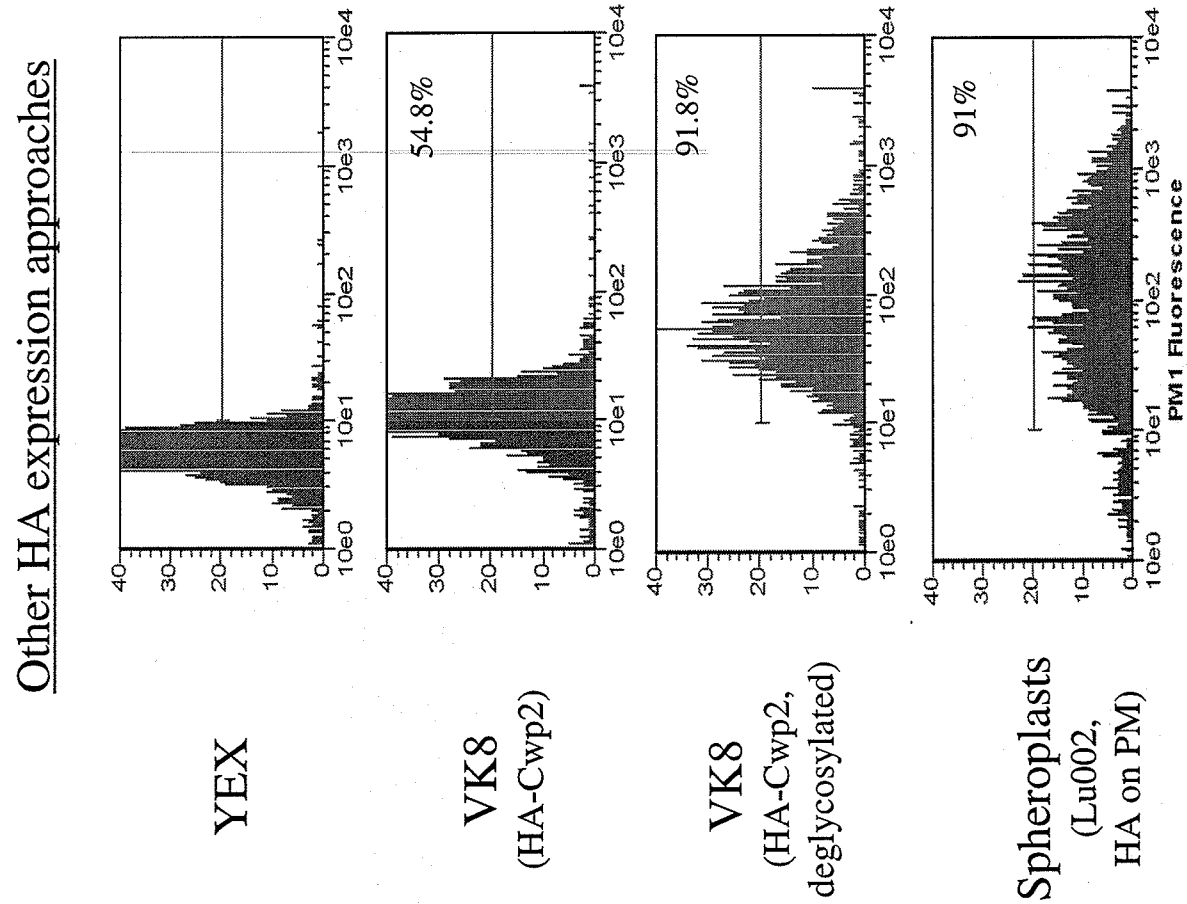


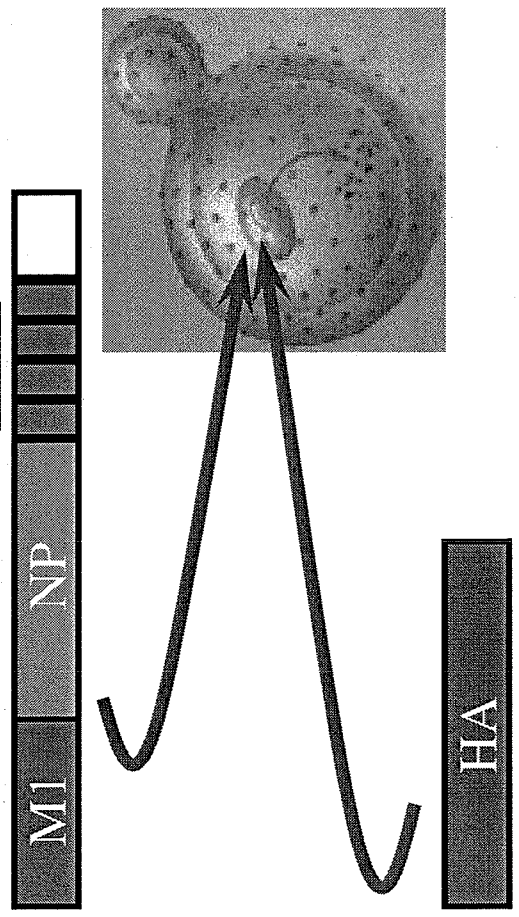
FIG. 12D

FIG. 12E

FIG. 12F

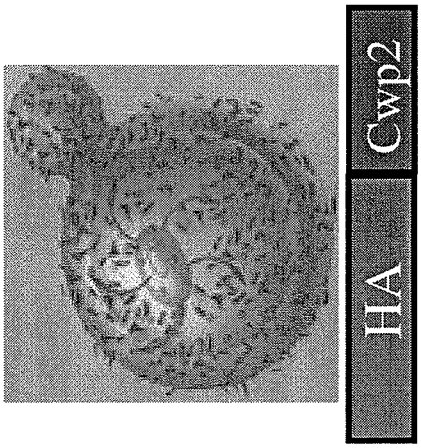
FIG. 12G

FIG. 13A



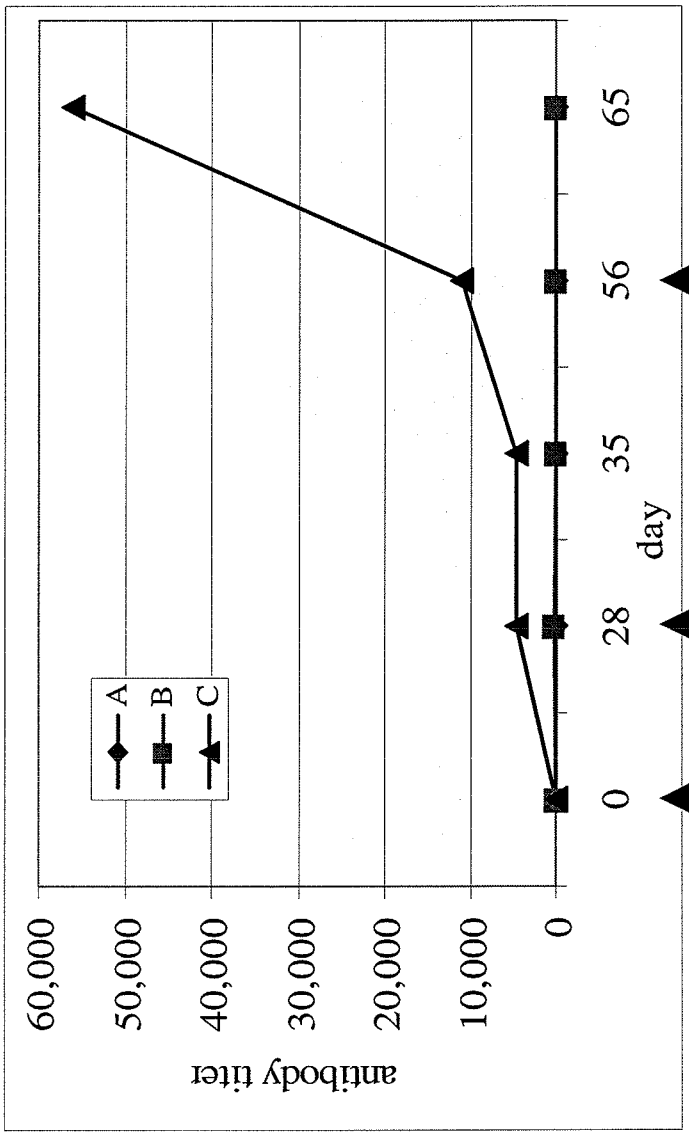
GI-8000-I

FIG. 13B



GI-8000-S

FIG. 14A



Regimen A: PBS PBS PBS

Regimen B: PBS PBS ova

Regimen C: OVAX OVAX ova

FIG. 14B

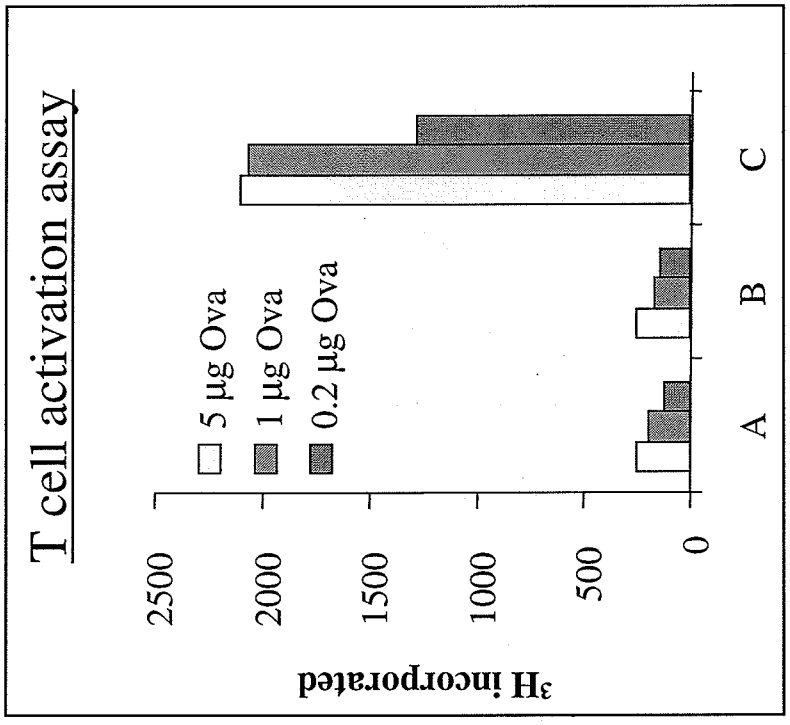


FIG. 15

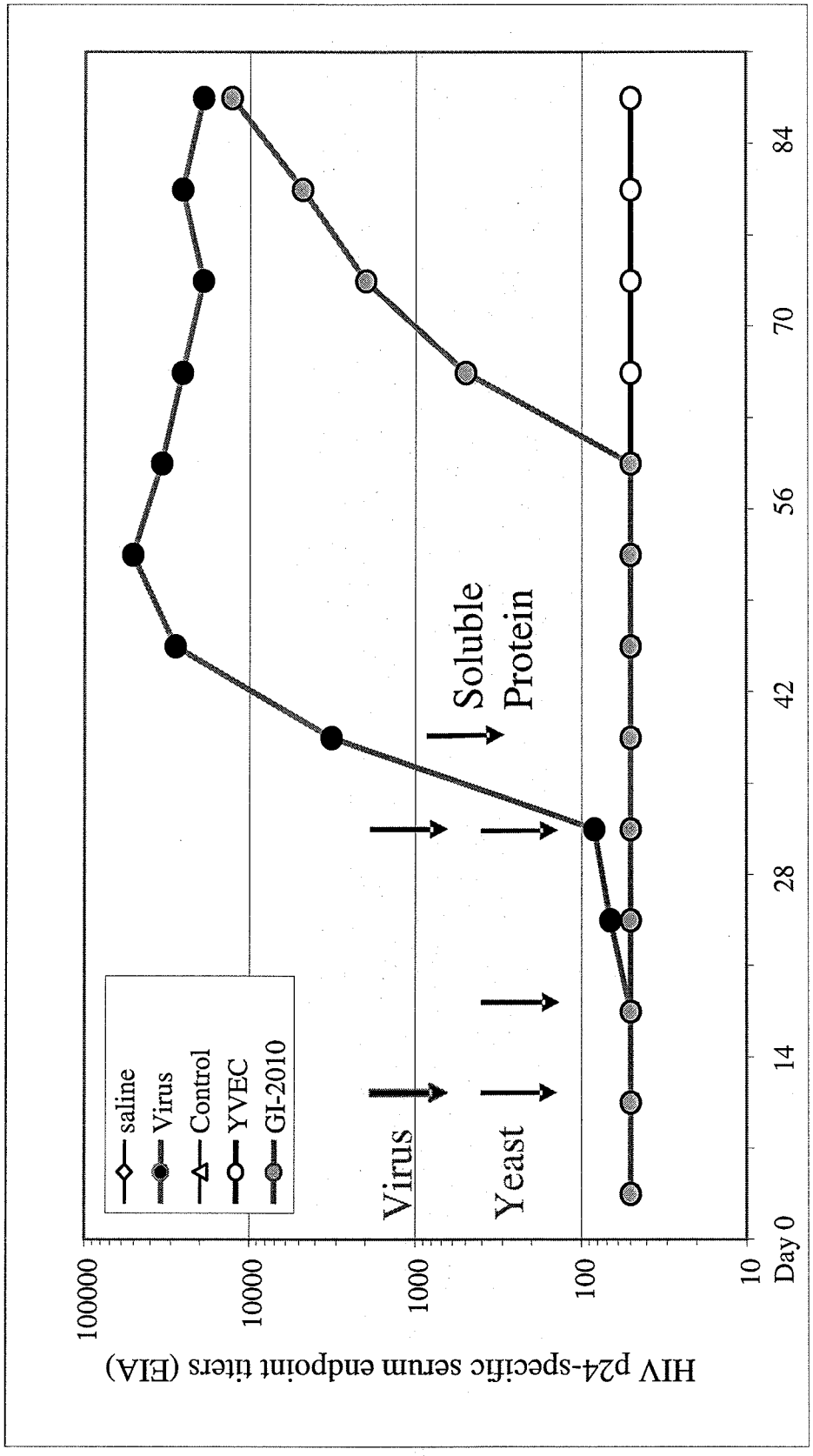
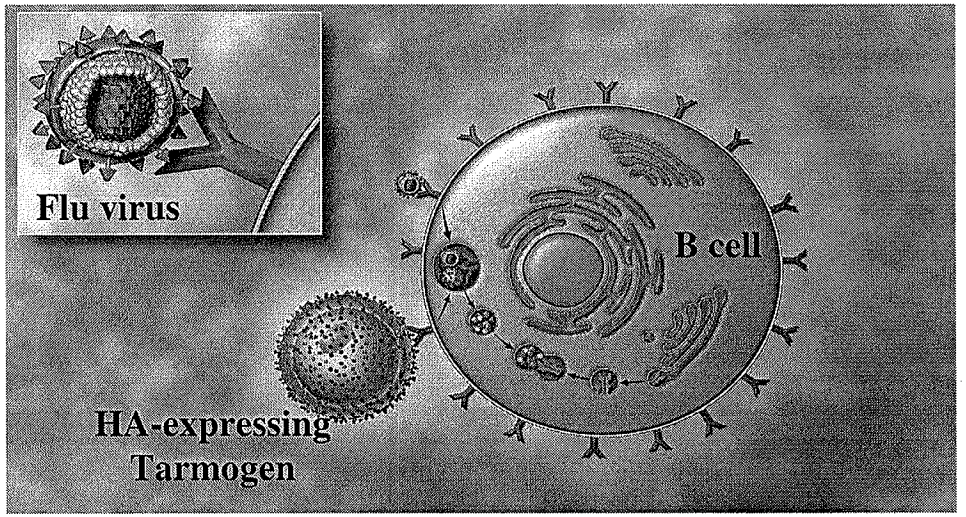
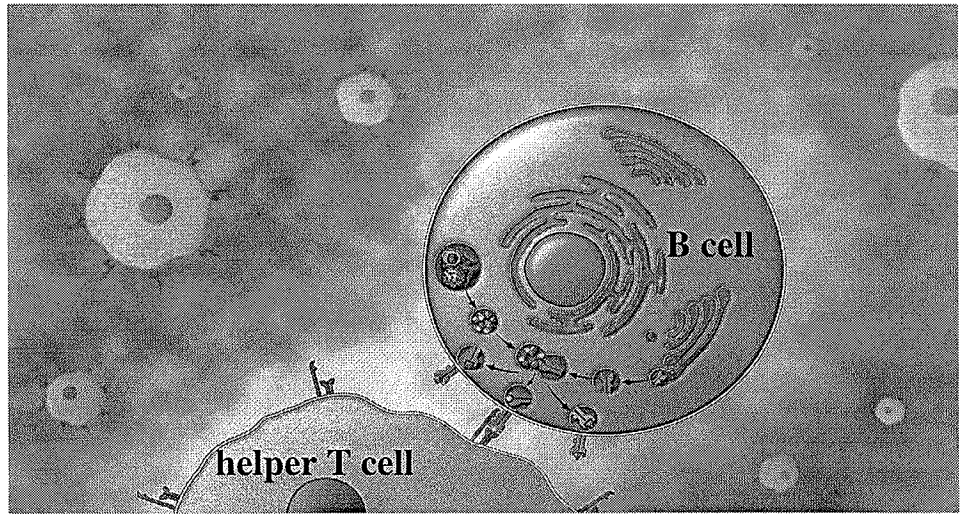

HIV p24-specific serum endpoint titers (EIA)
100000
10000
1000
100
10
Day 0
14
28
42
56
70
84
saline
Virus
Control
YVEC
GI-2010
Virus
Yeast
Soluble
Protein

FIG. 16A



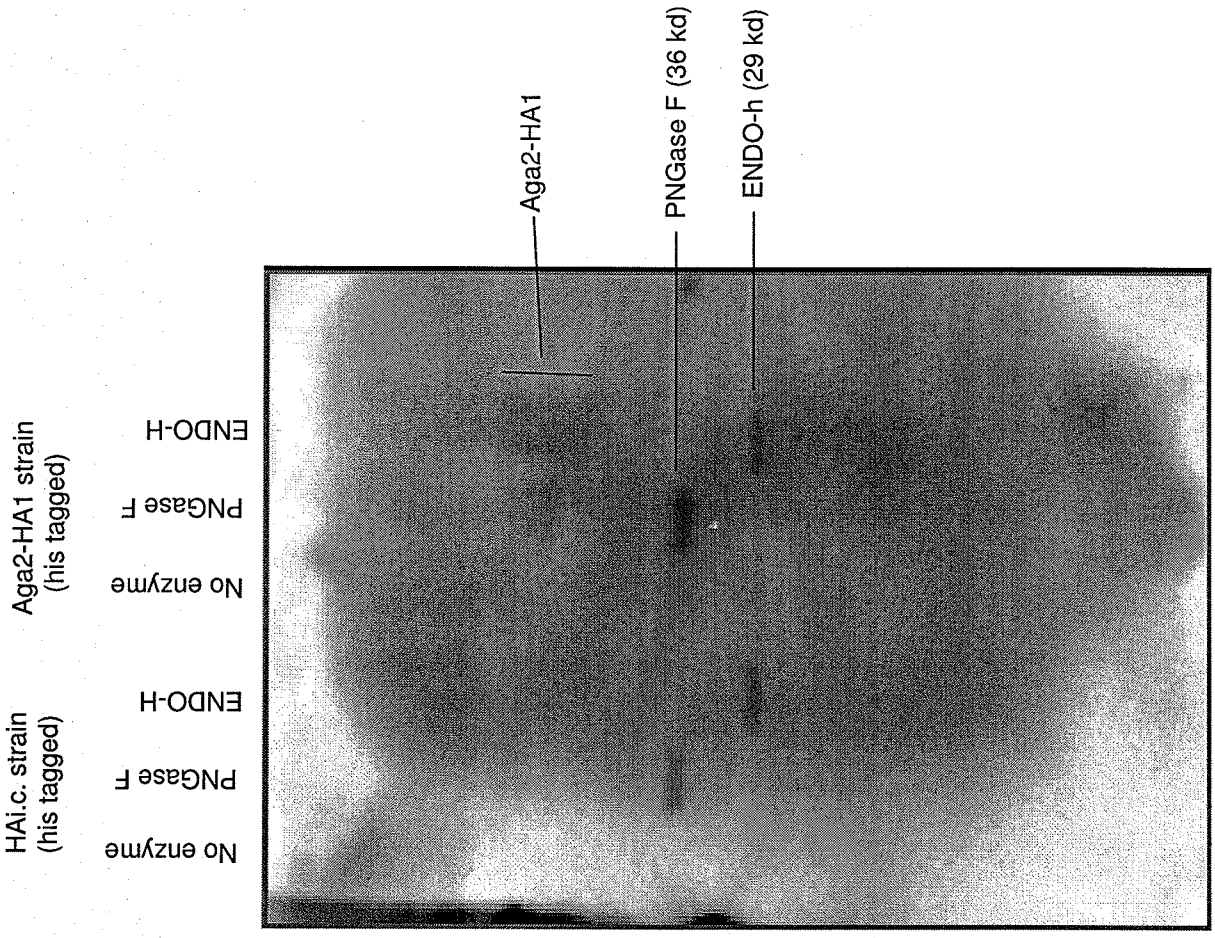
Signal 1 for Ab production

FIG. 16B



Signal 2 for Ab production

FIG. 17


Aga2-HA1
PNGase F (36 kd)
ENDO-h (29 kd)
Aga2-HA1 strain (his tagged)
No enzyme
PNGase F
ENDO-H
HAi.c. strain (his tagged)
No enzyme
PNGase F
ENDO-H