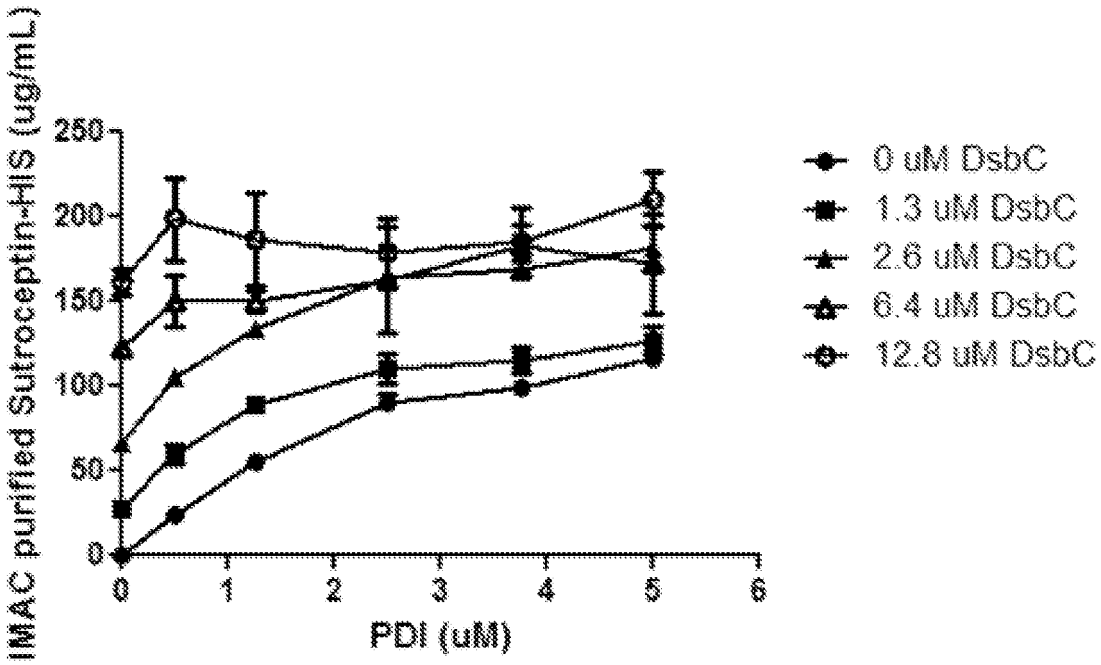

IMAC purified Sutroceptin-HIS (ug/mL)
250
200
150
100
50
0
0
1
2
3
4
5
6
PDI (uM)
0 uM DsbC
1.3 uM DsbC
2.6 uM DsbC
6.4 uM DsbC
12.8 uM DsbC

FIG. 1

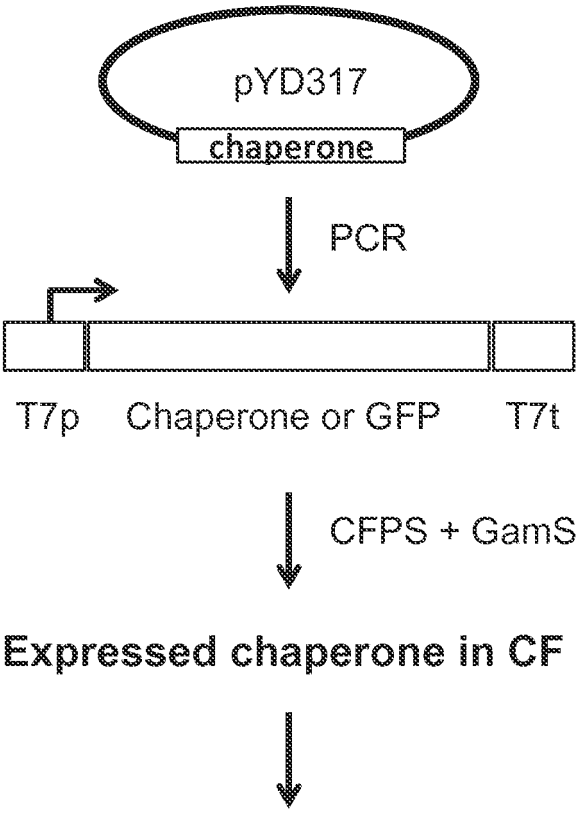

pYD317
chaperone
PCR
T7p
Chaperone or GFP
T7t
CFPS + GamS
Expressed chaperone in CF
IgG CFPS with ^{14}C incorporation
20% chaperone extract (v/v)

FIG. 2A

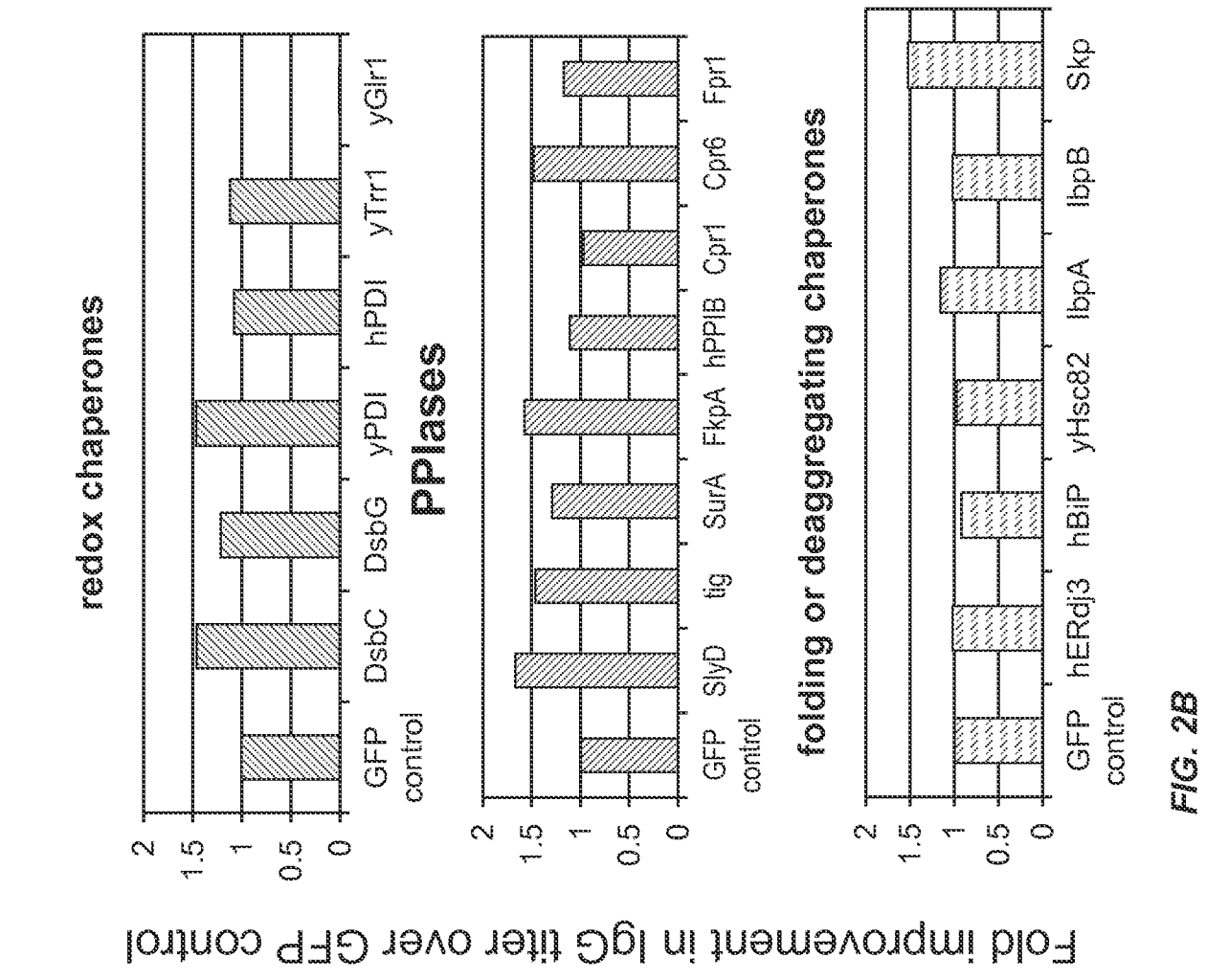


FIG. 2B

Concentration at which chaperones were expressed:

Redox proteins

	MW (kDa)	uM
DsbC	23.6	26
DsbG	29.8	11
yPDI	55.8	3
hPDI	55.3	3
yTrr1	34.2	18
yGlr1	53.4	3

Prolyl isomerases

	MW (kDa)	uM
SlyD	20.9	43
tig	48.2	15
SurA	45.1	8
FkpA	28.9	48
hPPIB	20.3	23
Cpr1	17.4	23
Cpr6	42.1	12
Fpr1	12.2	63

Deaggre-gases

	MW (kDa)	uM
hERdj3	38.2	12
hBiP	70.5	3
yHsc82	80.9	7
IbpA	15.8	52
IbpB	16.1	69
Skp	17.7	71

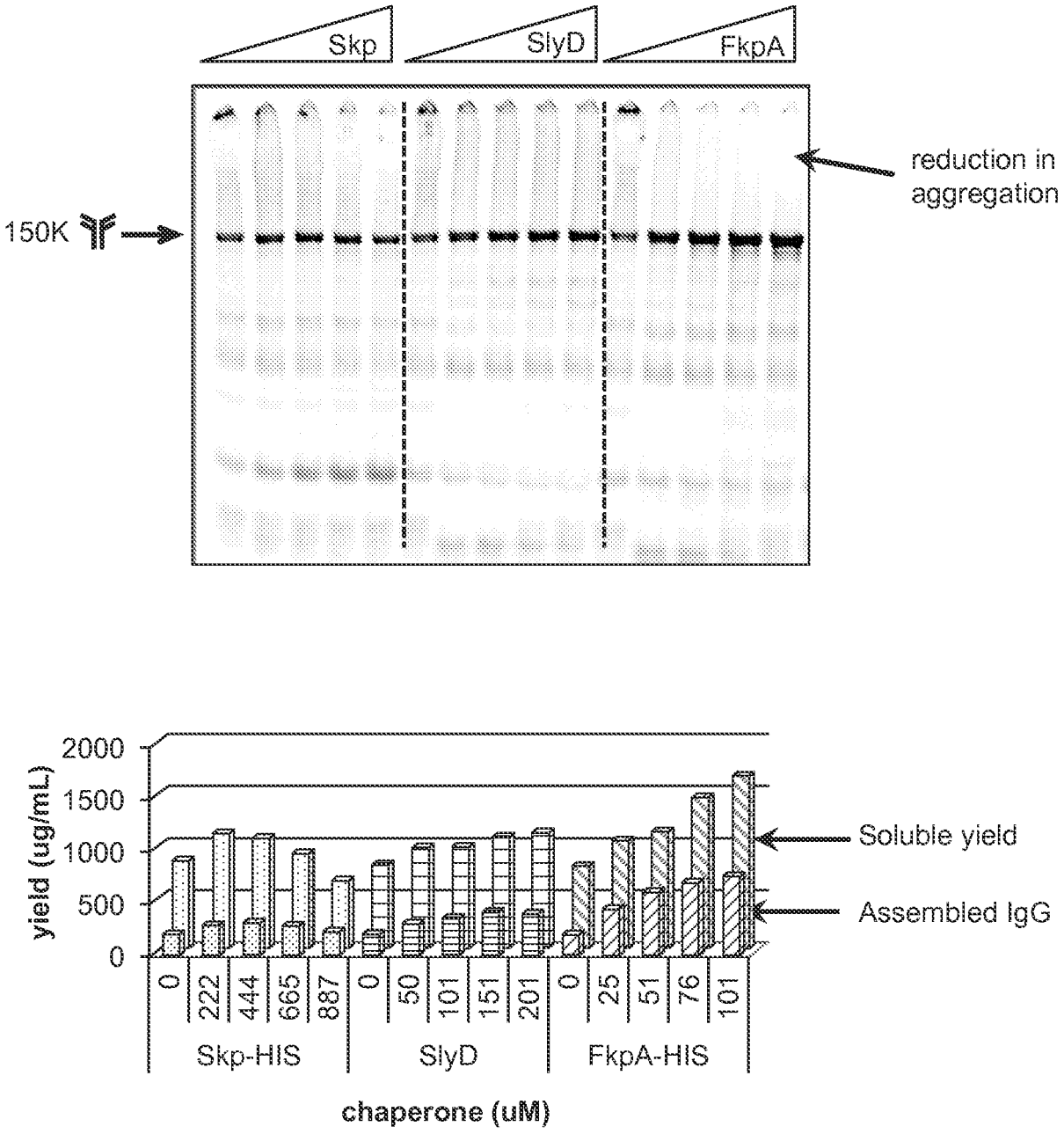
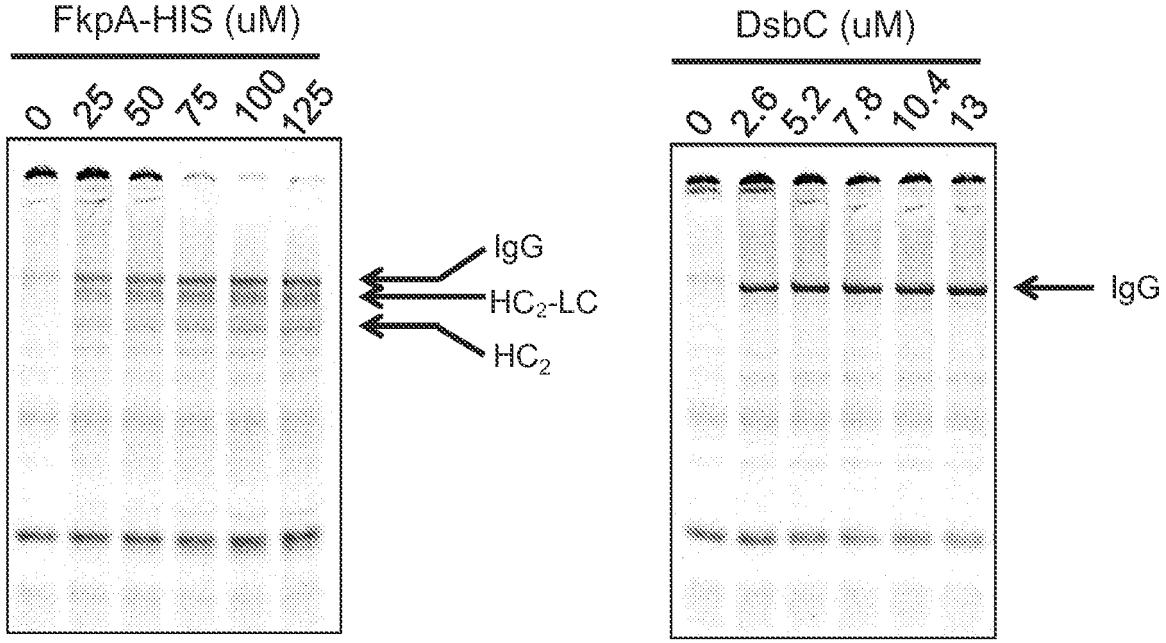
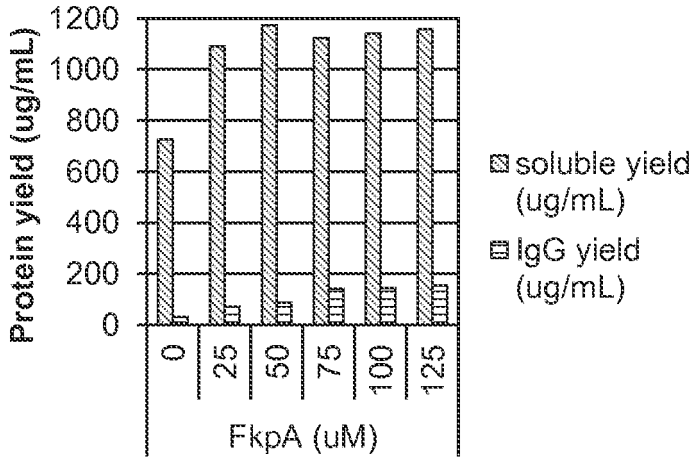

Skp
SlyD
FkpA
150K
reduction in aggregation
2000
1500
1000
500
0
yield (ug/mL)
0
222
444
665
887
0
50
101
151
201
0
25
51
76
101
Skp-HIS
SlyD
FkpA-HIS
chaperone (uM)
Soluble yield
Assembled IgG

FIG. 3


FkpA-HIS (uM)
0
25
50
75
100
125
IgG
HC2-LC
HC2
DsbC (uM)
0
2.6
5.2
7.8
10.4
13
IgG


Protein yield (ug/mL)
1200
1000
800
600
400
200
0
0
25
50
75
100
125
FkpA (uM)
soluble yield (ug/mL)
IgG yield (ug/mL)

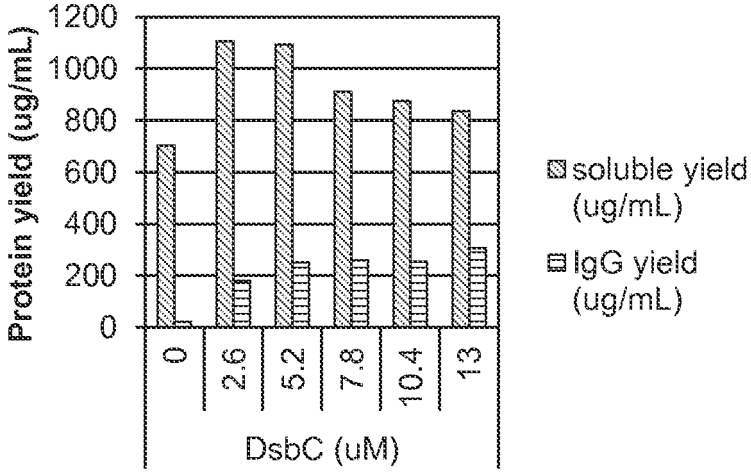
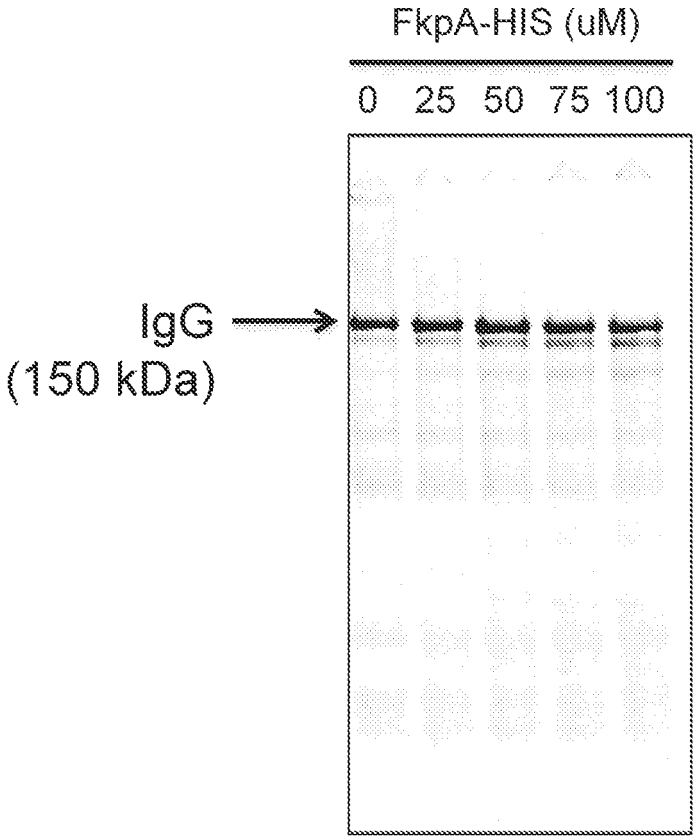

Protein yield (ug/mL)
1200
1000
800
600
400
200
0
0
2.6
5.2
7.8
10.4
13
DsbC (uM)
soluble yield (ug/mL)
IgG yield (ug/mL)

FIG. 4


FkpA-HIS (uM)
0 25 50 75 100
IgG
(150 kDa)

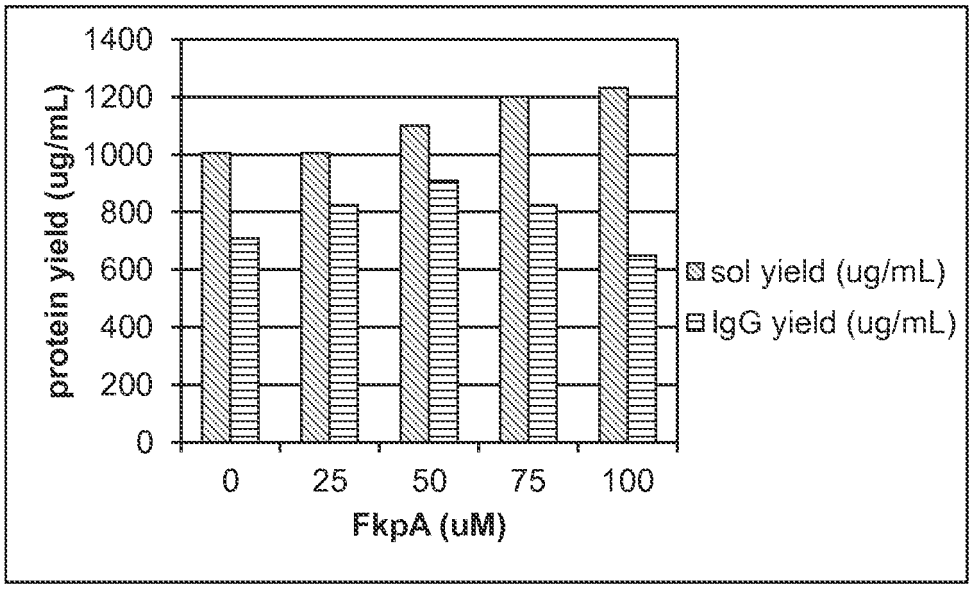

protein yield (ug/mL)
1400
1200
1000
800
600
400
200
0
0 25 50 75 100
FkpA (uM)
sol yield (ug/mL)
IgG yield (ug/mL)

FIG. 5

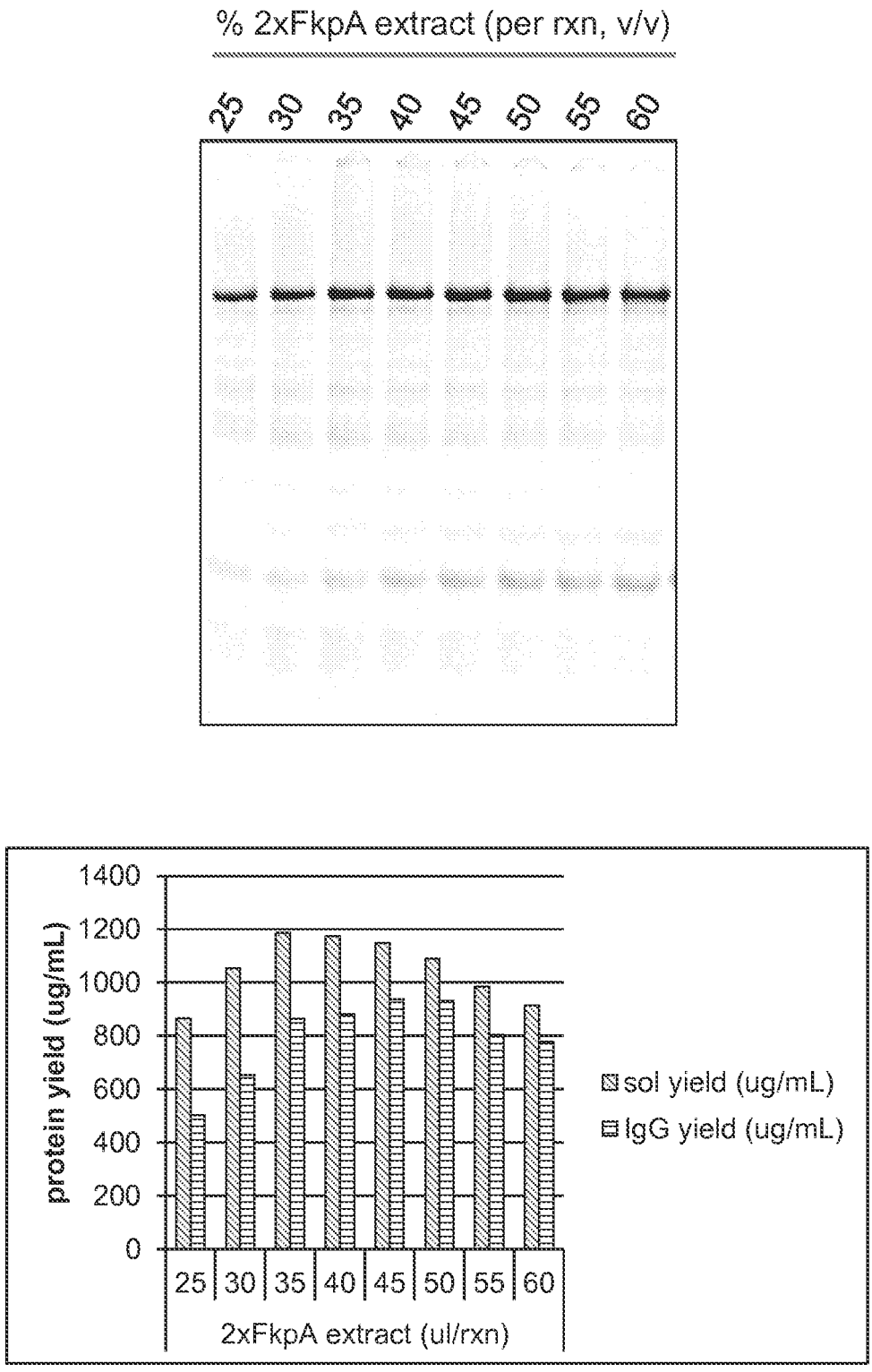

% 2xFkpA extract (per rxn, v/v)
25
30
35
40
45
50
55
60
protein yield (ug/mL)
1400
1200
1000
800
600
400
200
0
25
30
35
40
45
50
55
60
2xFkpA extract (ul/rxn)
sol yield (ug/mL)
IgG yield (ug/mL)

FIG. 6

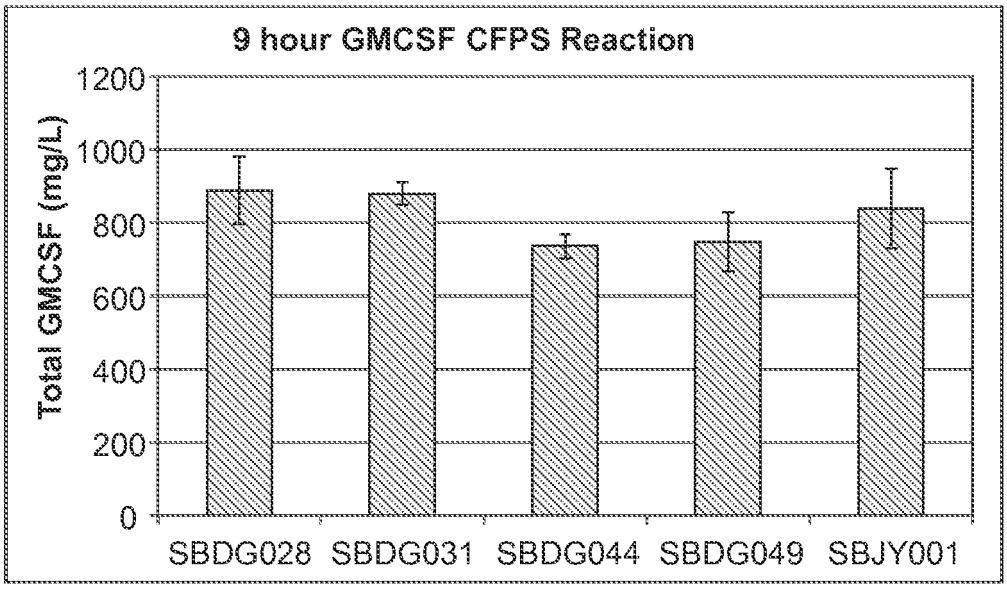
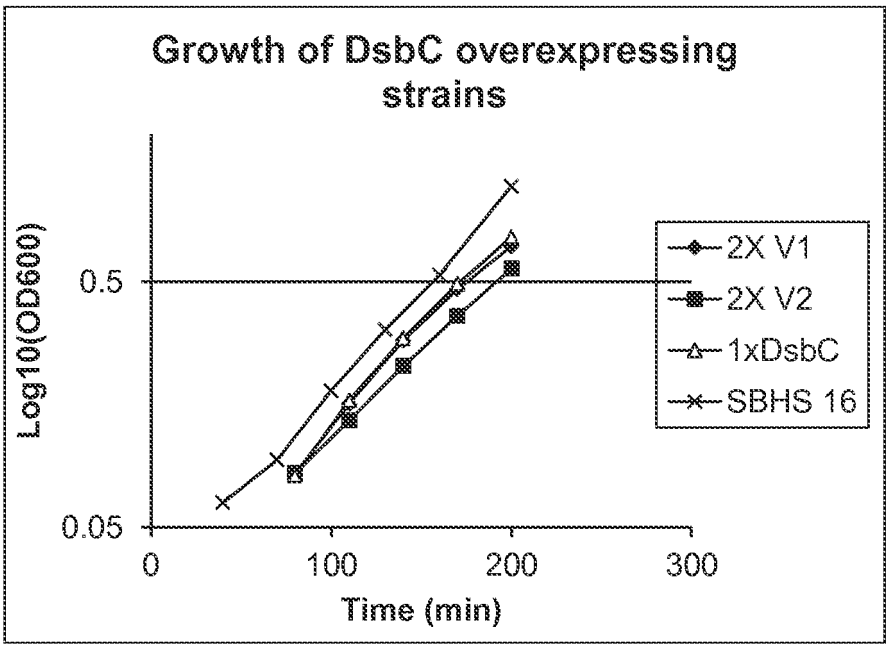

9 hour GMCSF CFPS Reaction
Total GMCSF (mg/L)
1200
1000
800
600
400
200
0
SBDG028
SBDG031
SBDG044
SBDG049
SBJY001

FIG. 7



Growth Rates

Strain	SBHS16	+1xDsbC	+2X ver 1	+2X ver 2
Doubling time (h)	.65	.64	.70	.73

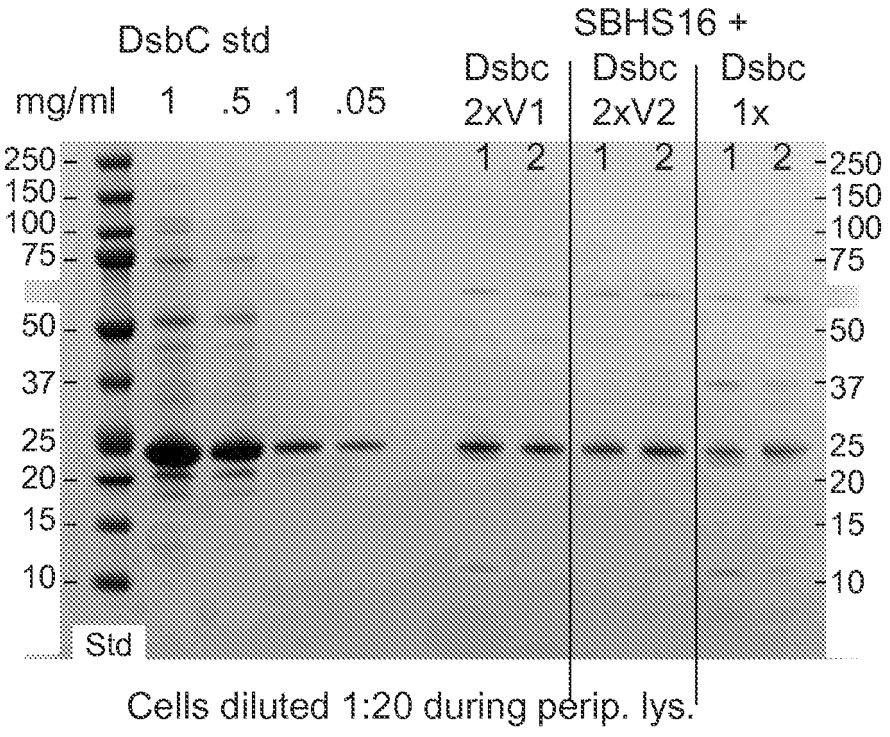
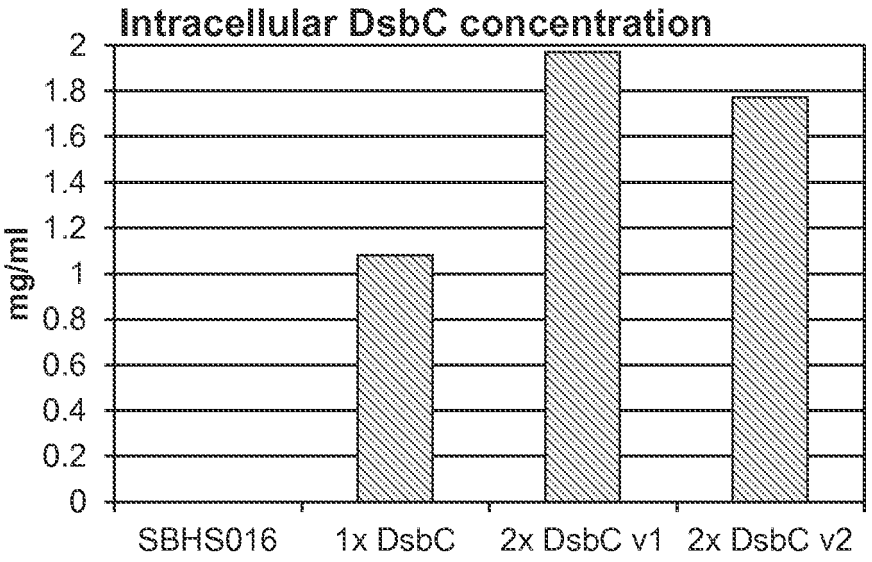


FIG. 8

Shake Flask expression
From densitometry



Extract Fermentation
From ELISA

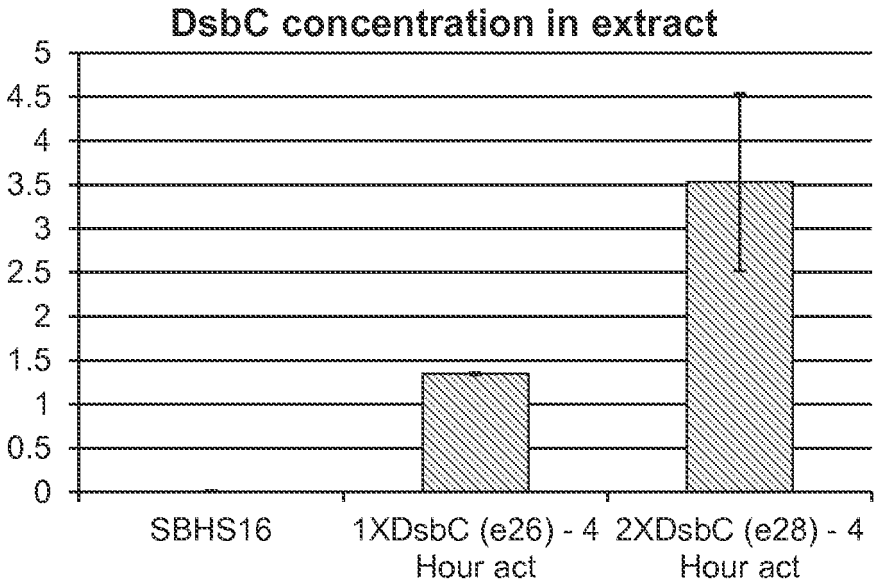
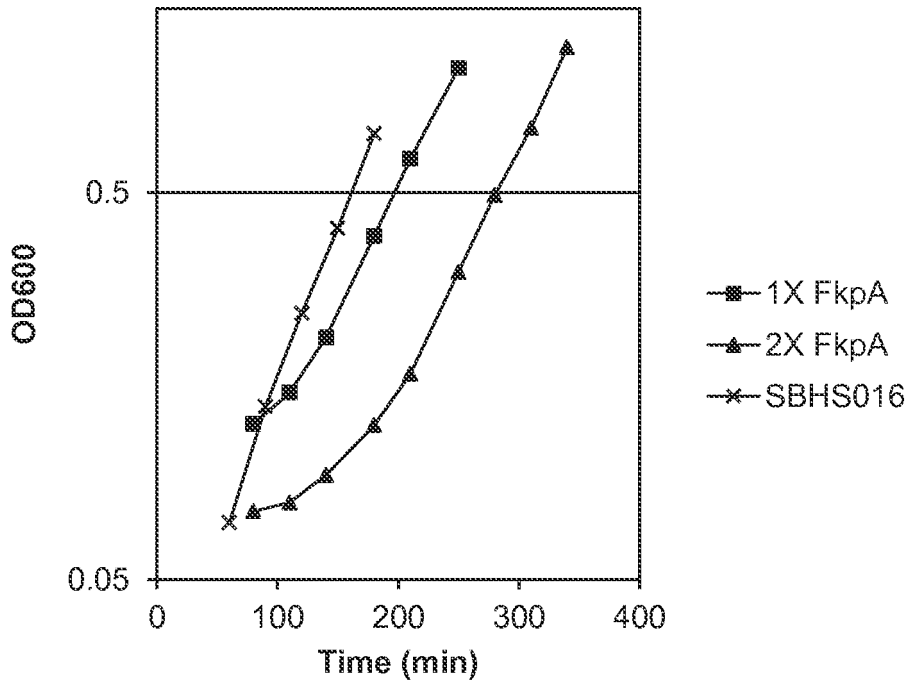
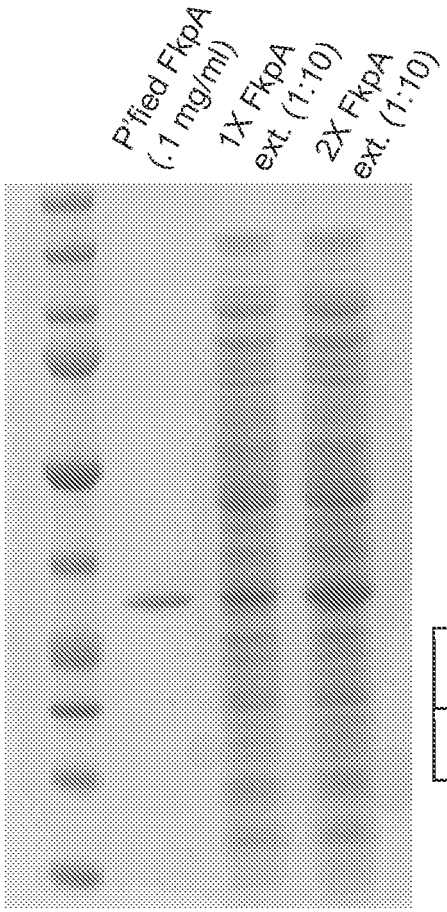


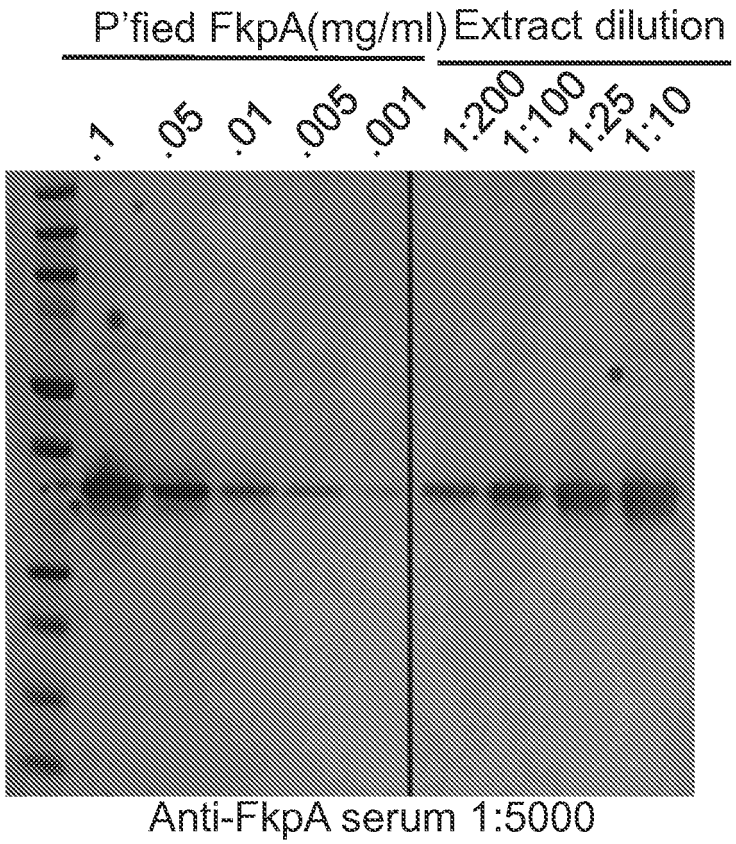
FIG. 9





Strain	SBHS16	+1xFkpA	+2xFkpA
Doubling time(h)	.65	.79	.79

FIG. 10


P'fied FkpA(mg/ml)
Extract dilution
.1
.05
.01
.005
.001
1:200
1:100
1:25
1:10
Anti-FkpA serum 1:5000

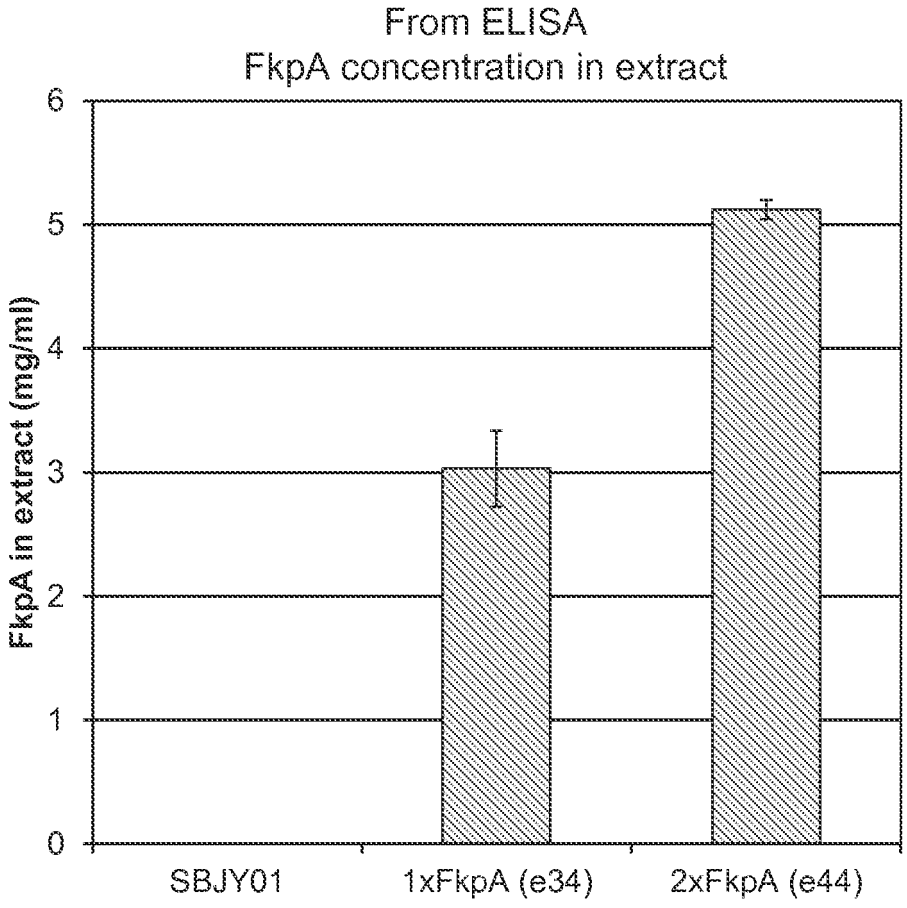

From ELISA
FkpA concentration in extract
FkpA in extract (mg/ml)
0
1
2
3
4
5
6
SBJY01
1xFkpA (e34)
2xFkpA (e44)

FIG. 11

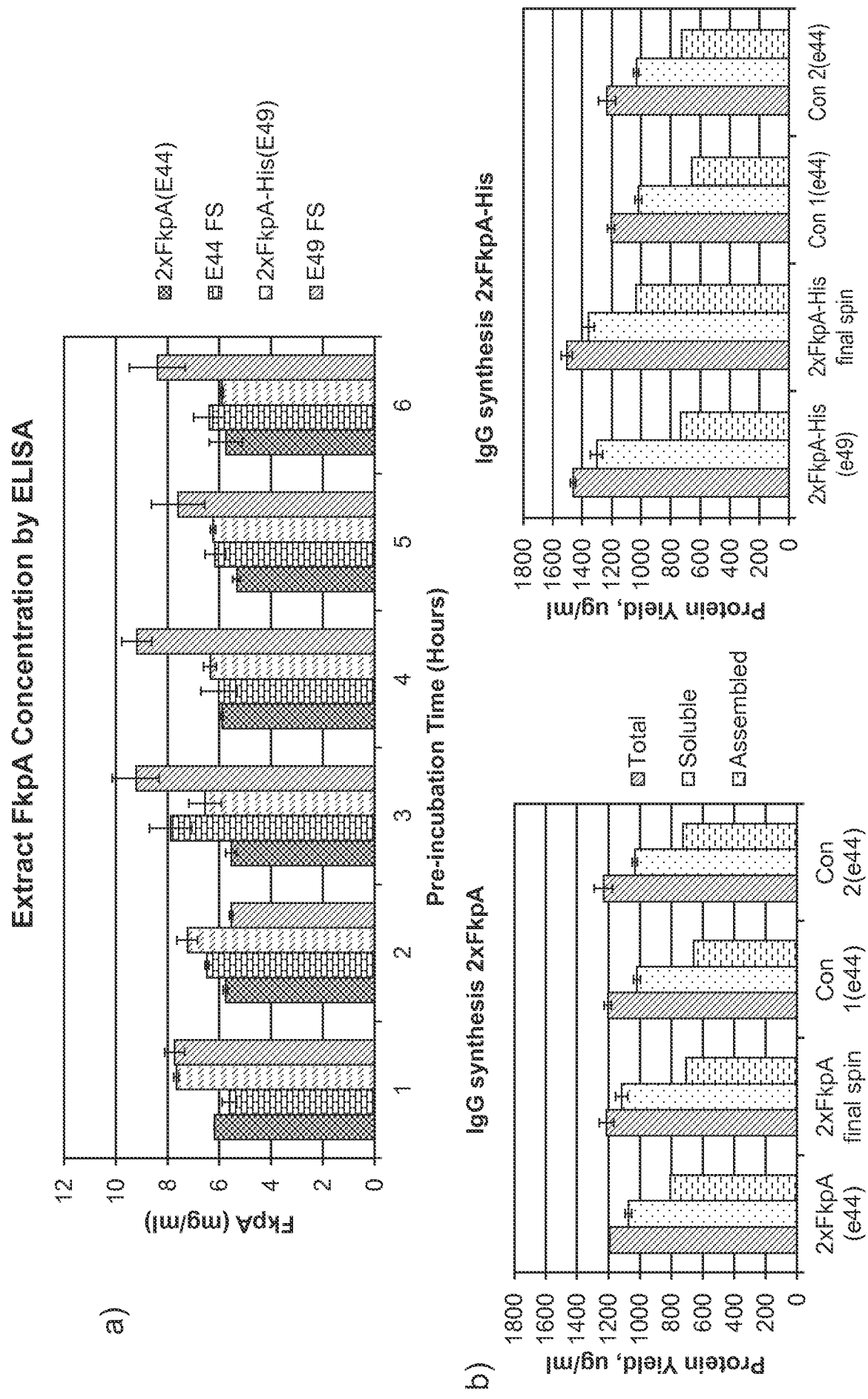
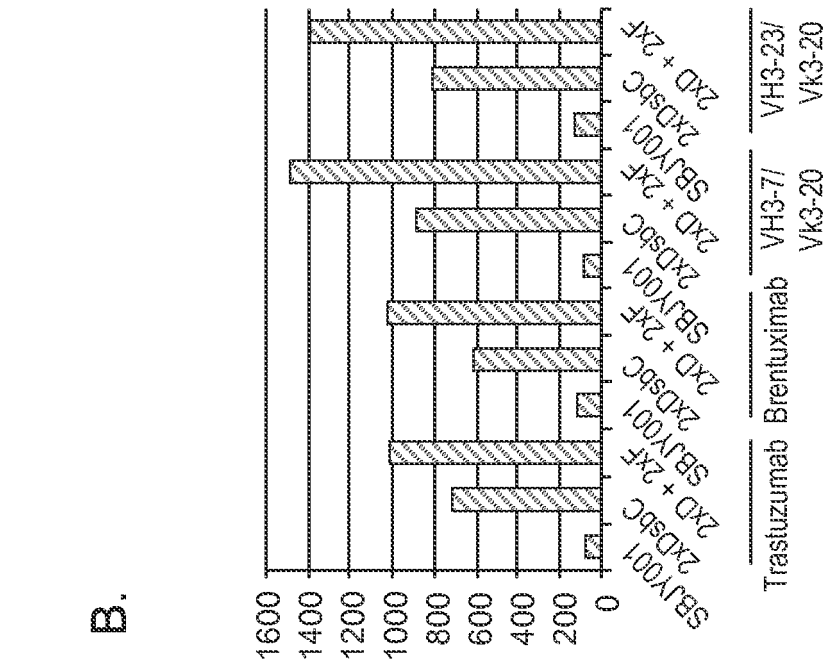

a)
Extract FkpA Concentration by ELISA
2xFkpA(E44)
E44 FS
2xFkpA-His(E49)
E49 FS
FkpA (mg/ml)
Pre-incubation Time (Hours)
IgG synthesis 2xFkpA-His
Protein Yield, ug/ml
2xFkpA-His (e49)
2xFkpA-His final spin
Con 1(e44)
Con 2(e44)
b)
IgG synthesis 2xFkpA
Total
Soluble
Assembled
Protein Yield, ug/ml
2xFkpA (e44)
2xFkpA final spin
Con 1(e44)
Con 2(e44)

FIG. 12


B.
1600
1400
1200
1000
800
600
400
200
0
SBJY001
2xDsbC
2xD + 2xF
Trastuzumab
Brentuximab
VH3-7/ Vk3-20
VH3-23/ Vk3-20

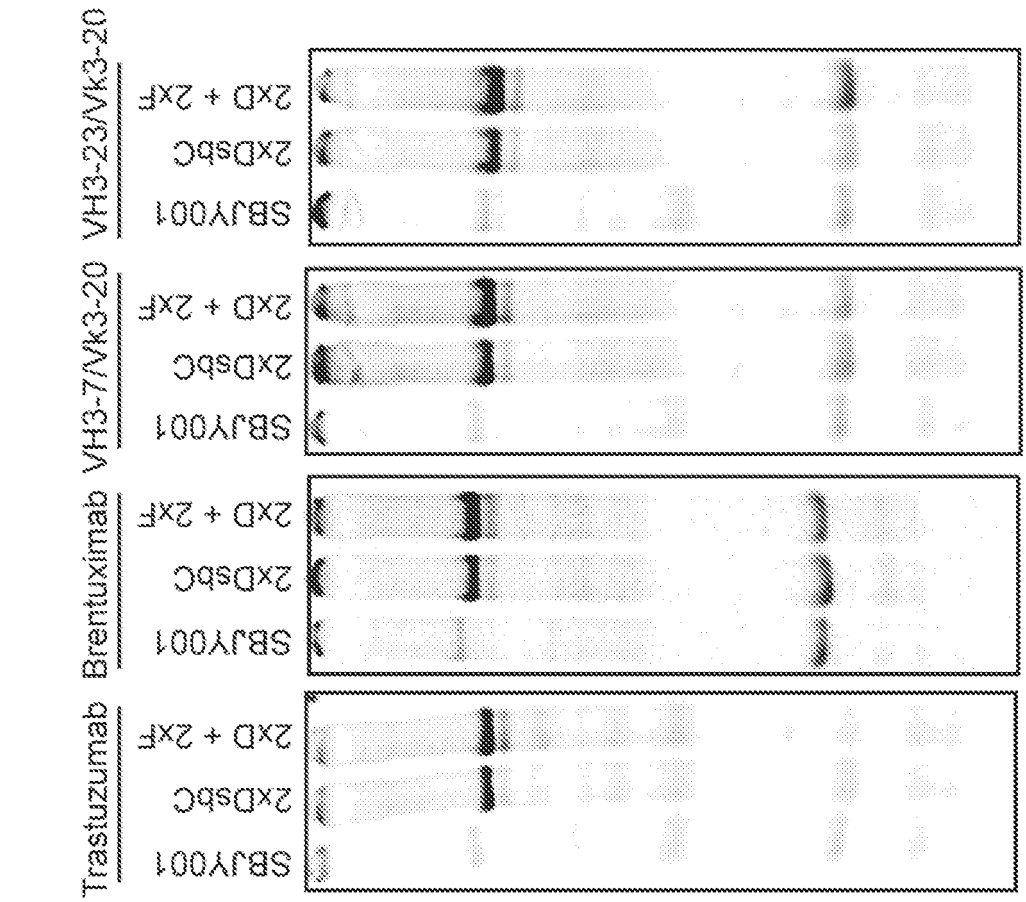

A.
Trastuzumab
Brentuximab
VH3-7/Vk3-20
VH3-23/Vk3-20
SBJY001
2xDsbC
2xD + 2xF

FIG. 13