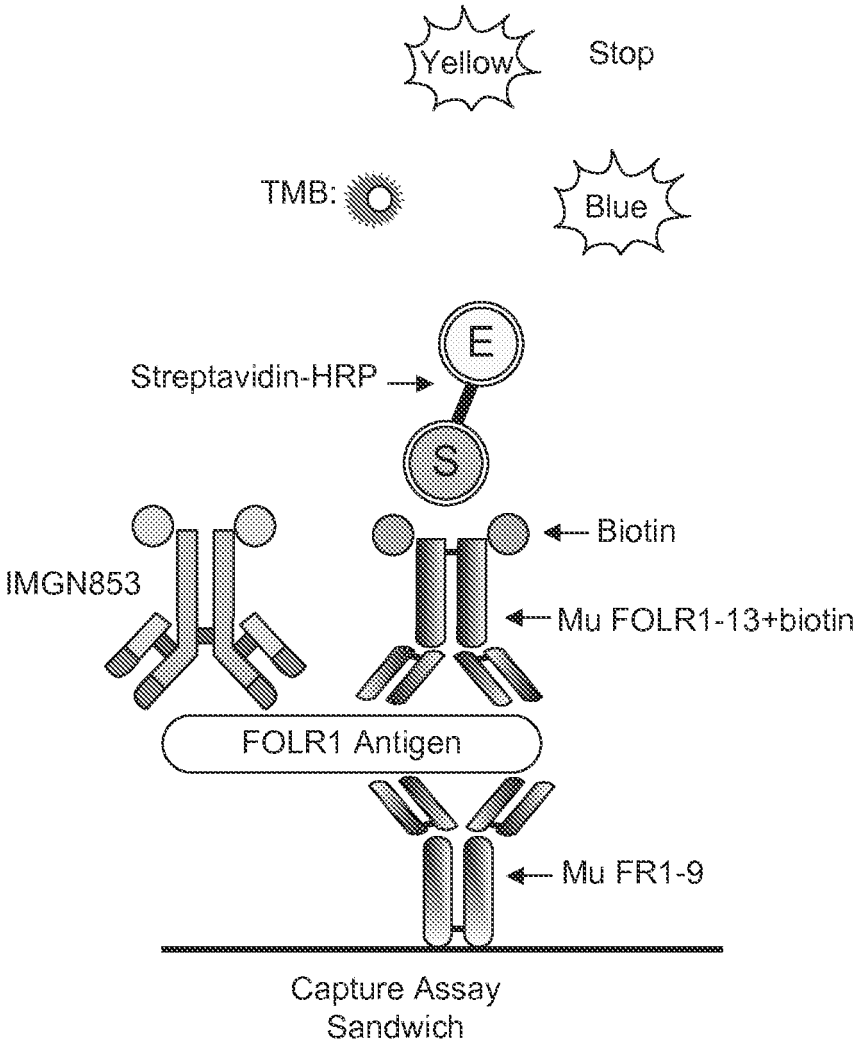

Yellow
Stop
TMB:
Blue
E
Streptavidin-HRP
S
Biotin
IMGN853
Mu FOLR1-13+biotin
FOLR1 Antigen
Mu FR1-9
Capture Assay
Sandwich

FIG. 1

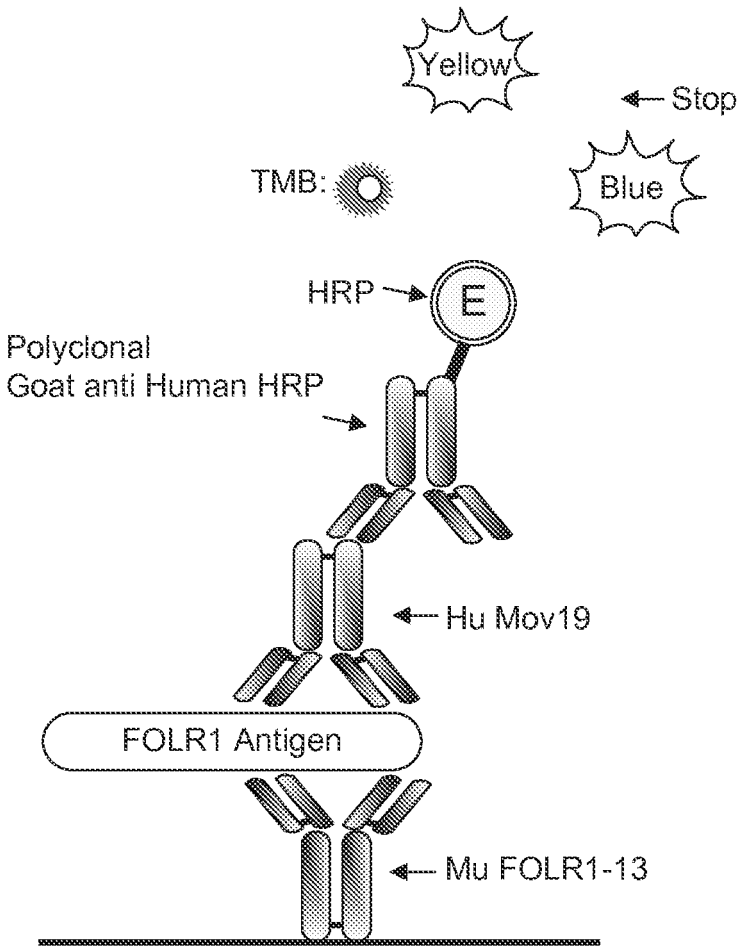

Yellow
Stop
TMB:
Blue
HRP
E
Polyclonal
Goat anti Human HRP
Hu Mov19
FOLR1 Antigen
Mu FOLR1-13

FIG. 2A

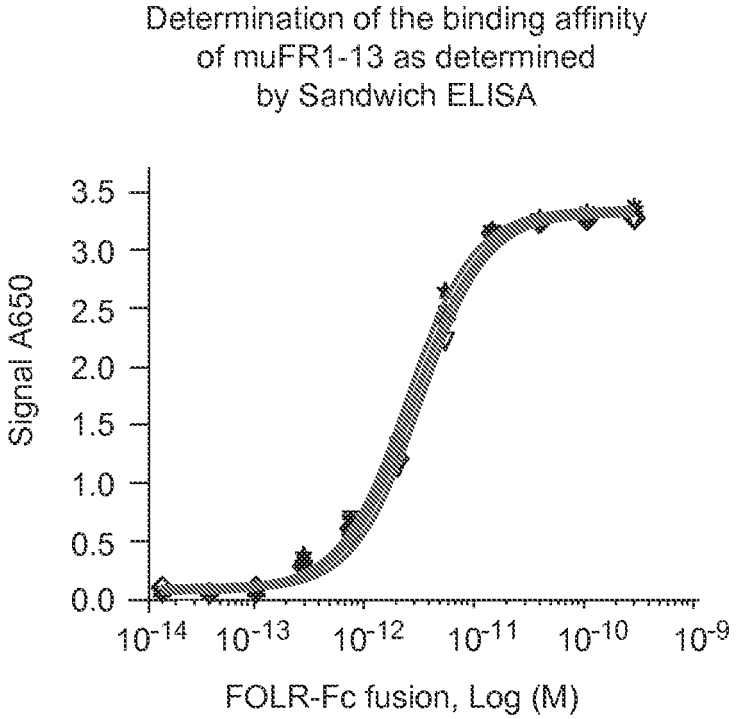

Determination of the binding affinity
of muFR1-13 as determined
by Sandwich ELISA
Signal A650
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10-14
10-13
10-12
10-11
10-10
10-9
FOLR-Fc fusion, Log (M)

FIG. 2B

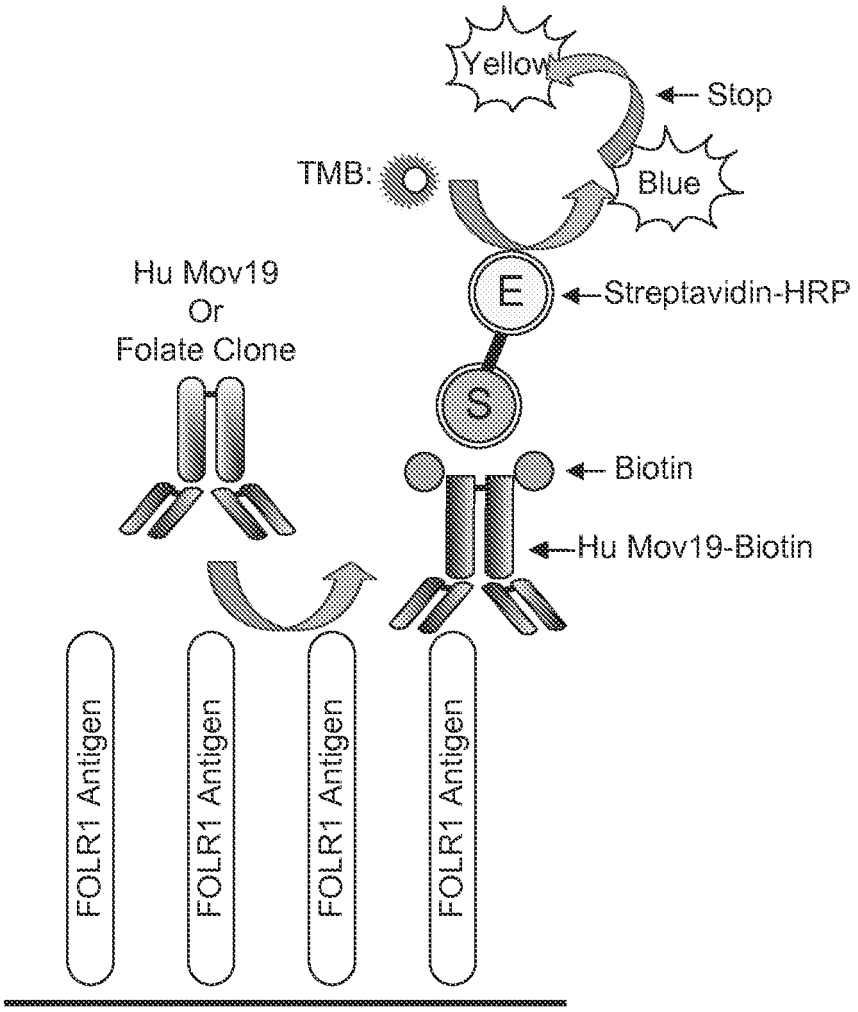

Yellow
Stop
TMB:
Blue
Hu Mov19
Or
Folate Clone
E
Streptavidin-HRP
S
Biotin
Hu Mov19-Biotin
FOLR1 Antigen
FOLR1 Antigen
FOLR1 Antigen
FOLR1 Antigen

FIG. 3A

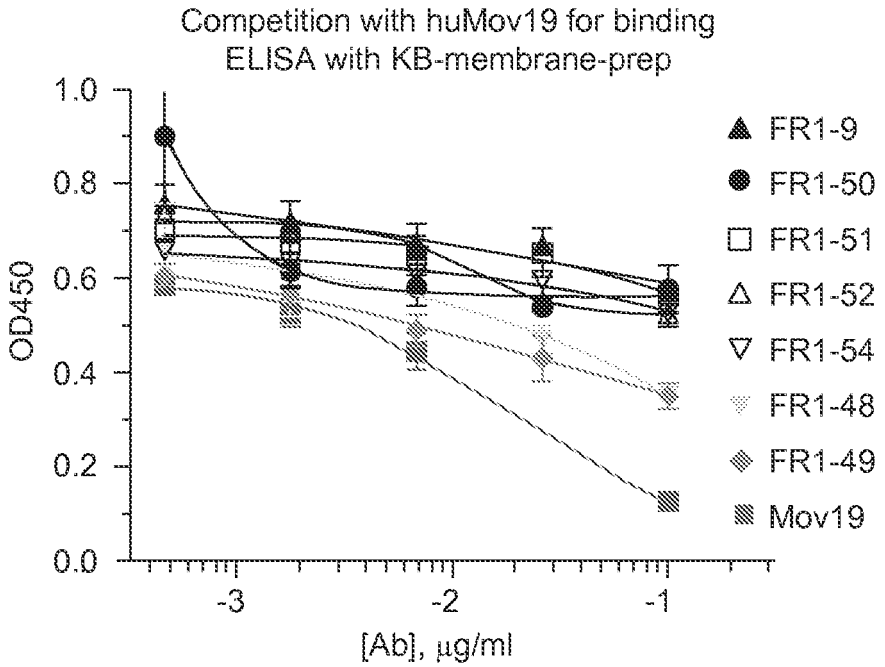

Competition with huMov19 for binding
ELISA with KB-membrane-prep
OD450
1.0
0.8
0.6
0.4
0.2
0.0
-3
-2
-1
[Ab], µg/ml
FR1-9
FR1-50
FR1-51
FR1-52
FR1-54
FR1-48
FR1-49
Mov19

FIG. 3B

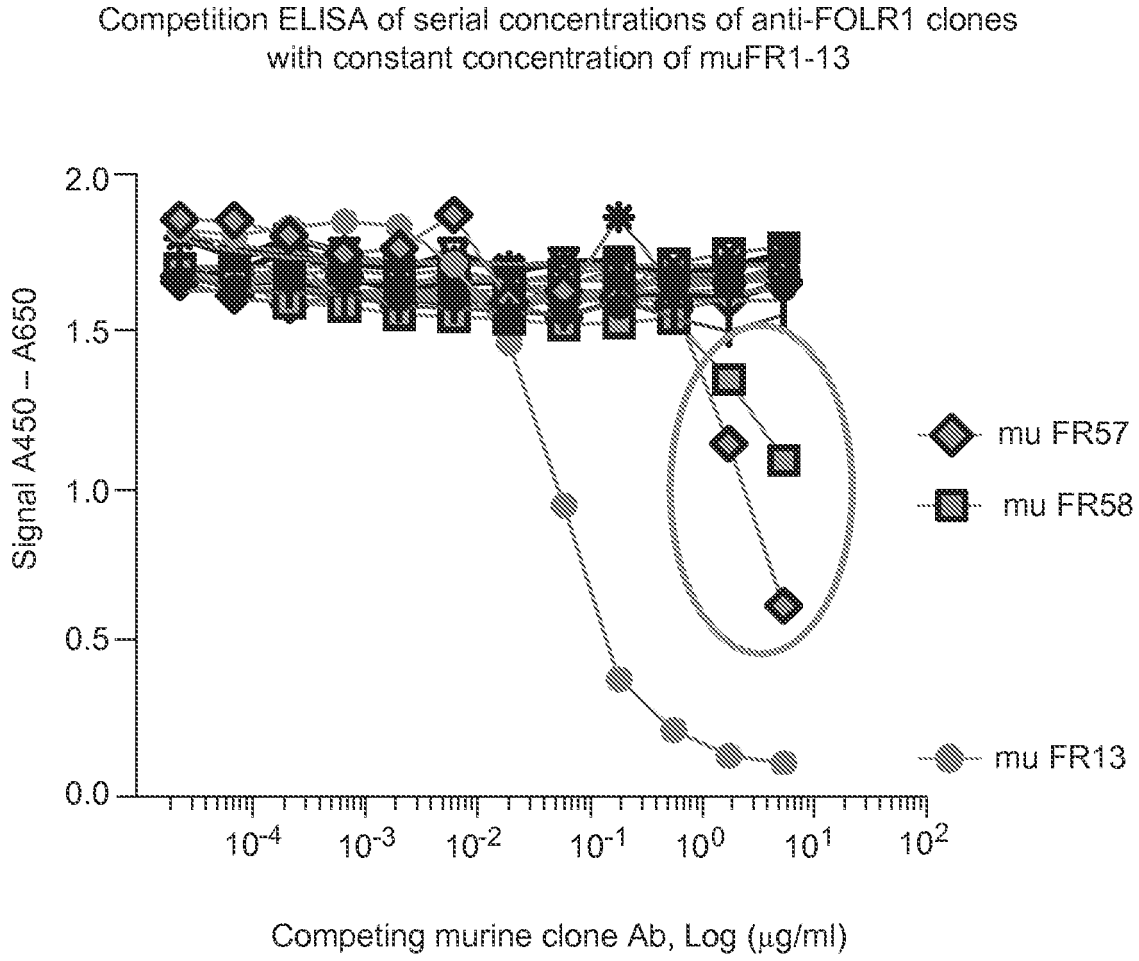

Competition ELISA of serial concentrations of anti-FOLR1 clones
with constant concentration of muFR1-13
Signal A450 – A650
2.0
1.5
1.0
0.5
0.0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Competing murine clone Ab, Log (µg/ml)
mu FR57
mu FR58
mu FR13

FIG. 4

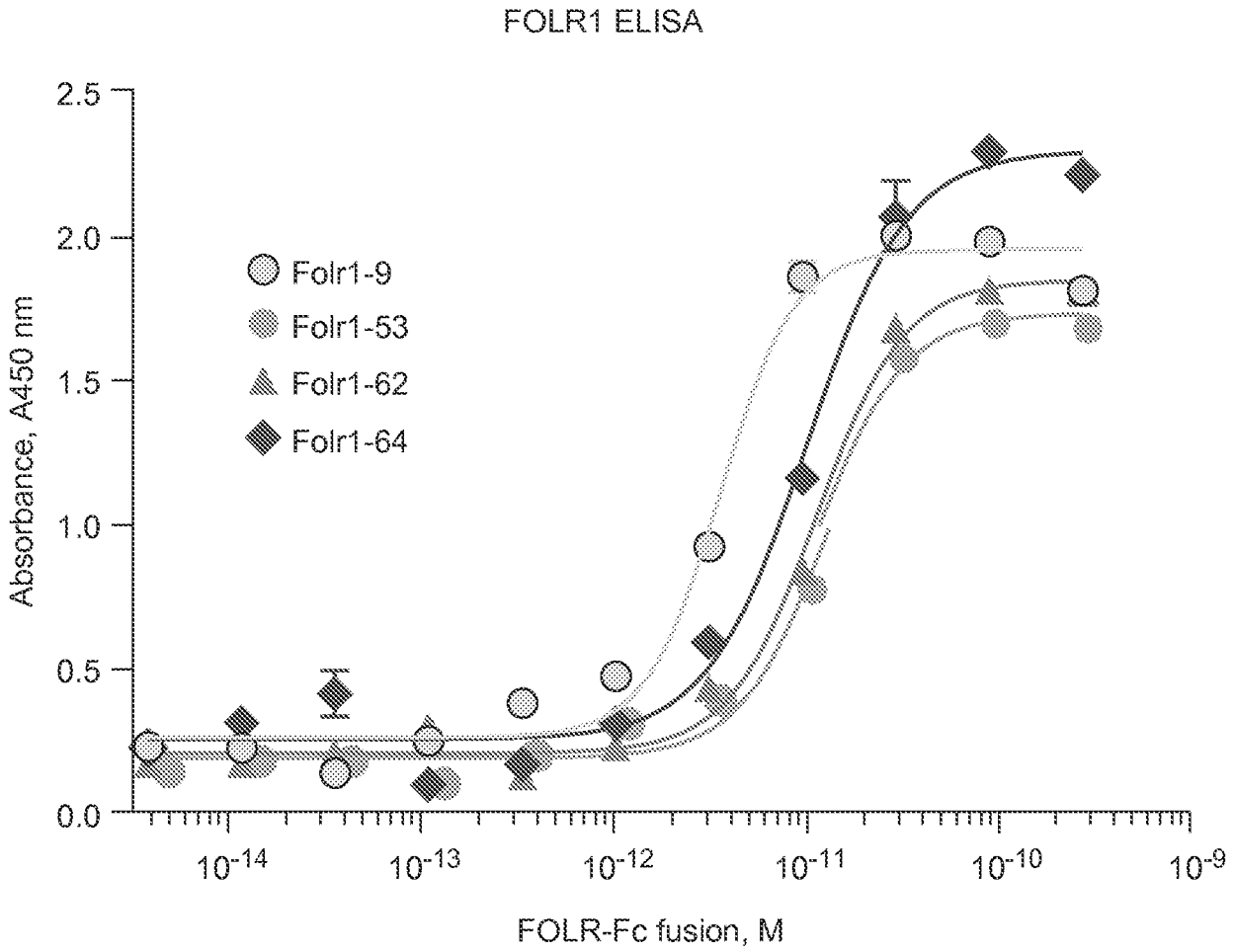

FOLR1 ELISA
Absorbance, A450 nm
2.5
2.0
1.5
1.0
0.5
0.0
Folr1-9
Folr1-53
Folr1-62
Folr1-64
10-14
10-13
10-12
10-11
10-10
10-9
FOLR-Fc fusion, M

FIG. 5

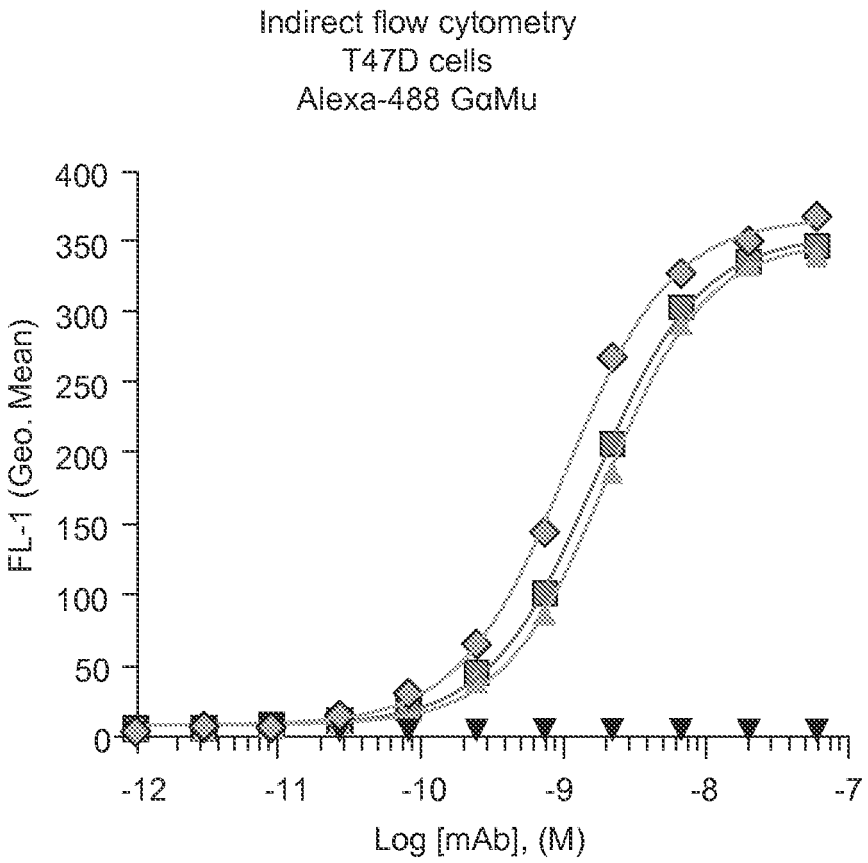

Indirect flow cytometry
T47D cells
Alexa-488 GαMu
FL-1 (Geo. Mean)
400
350
300
250
200
150
100
50
0
-12
-11
-10
-9
-8
-7
Log [mAb], (M)

FIG. 6A

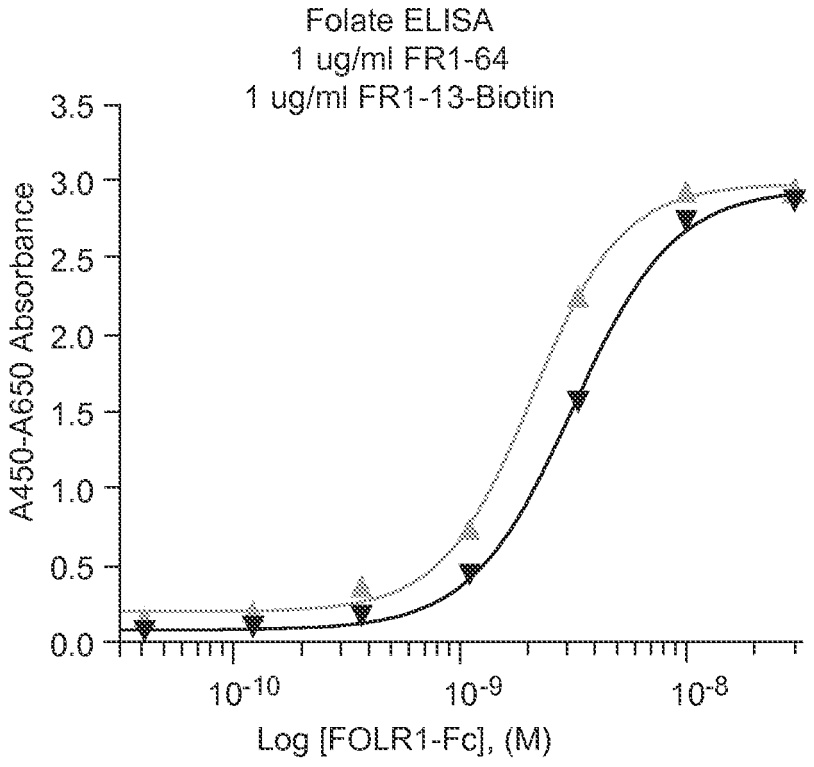

Folate ELISA
1 ug/ml FR1-64
1 ug/ml FR1-13-Biotin
A450-A650 Absorbance
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10-10
10-9
10-8
Log [FOLR1-Fc], (M)

FIG. 6B

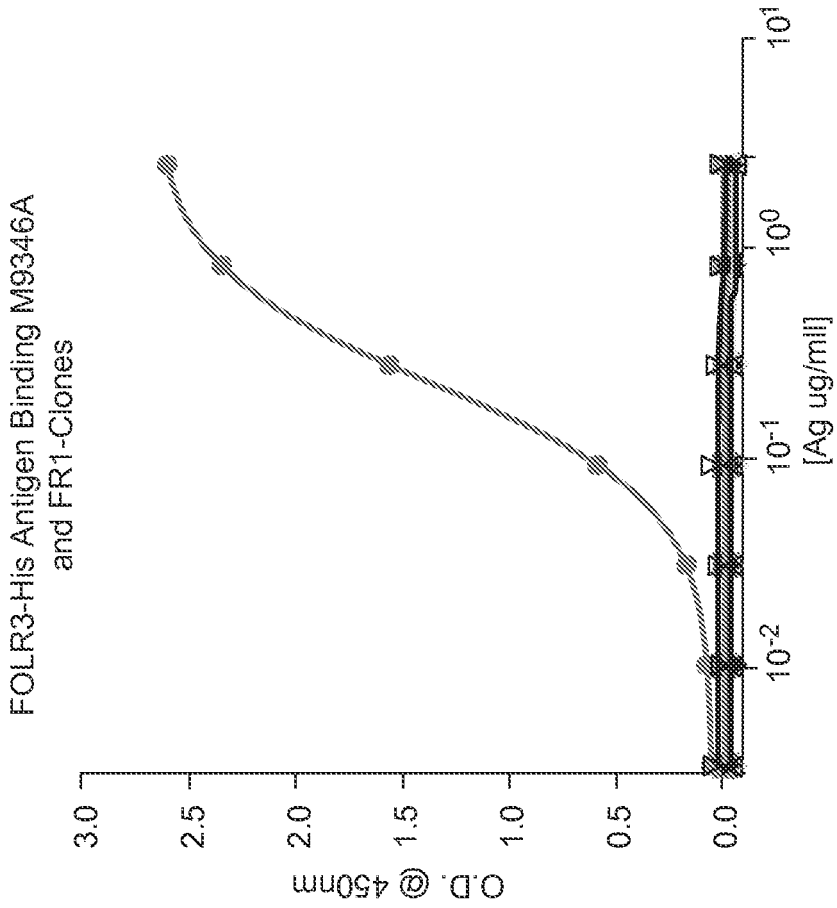


FIG. 7B

Note: FR3 Curve= 3 min development
all other curves = 20 min development

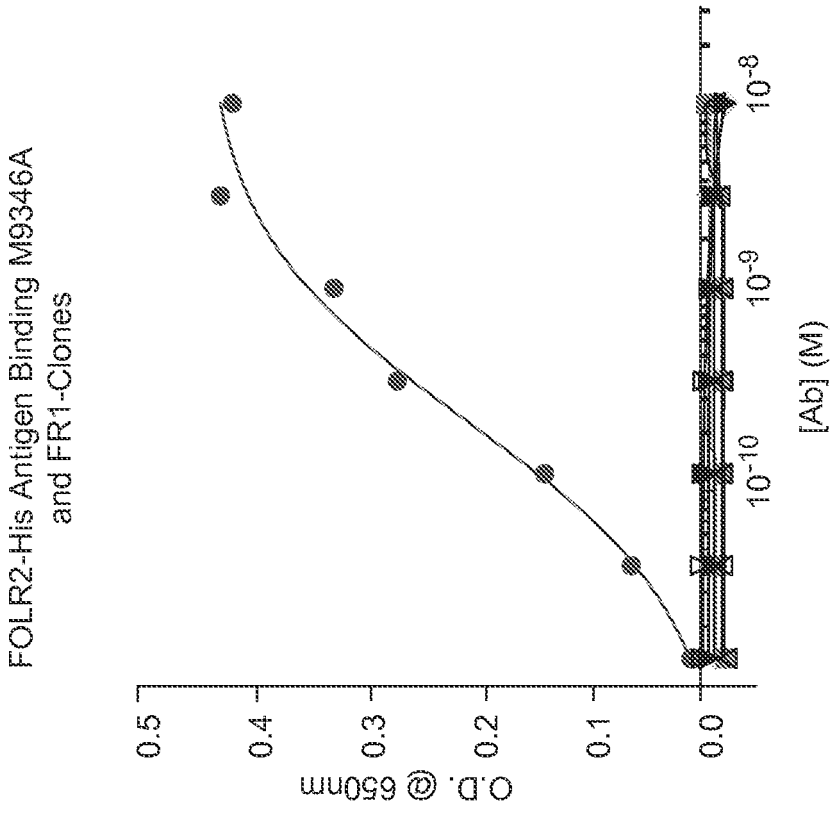
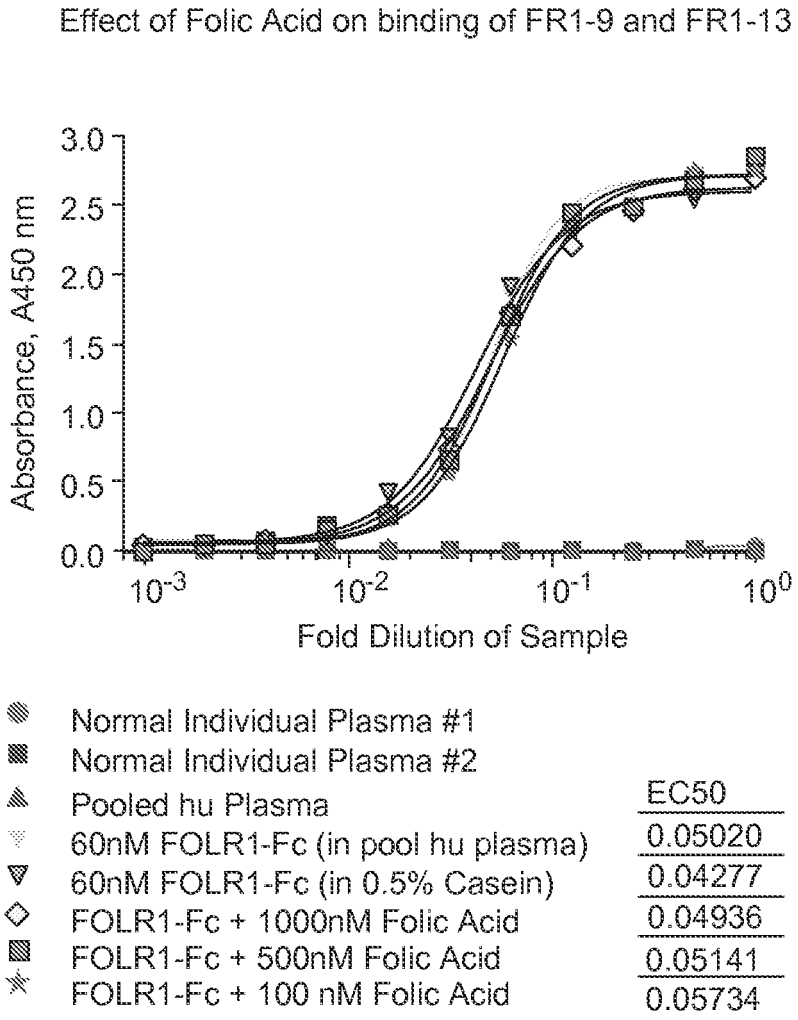


FIG. 7A



	EC50
60nM FOLR1-Fc (in pool hu plasma)	0.05020
60nM FOLR1-Fc (in 0.5% Casein)	0.04277
FOLR1-Fc + 1000nM Folic Acid	0.04936
FOLR1-Fc + 500nM Folic Acid	0.05141
FOLR1-Fc + 100 nM Folic Acid	0.05734

FIG. 8

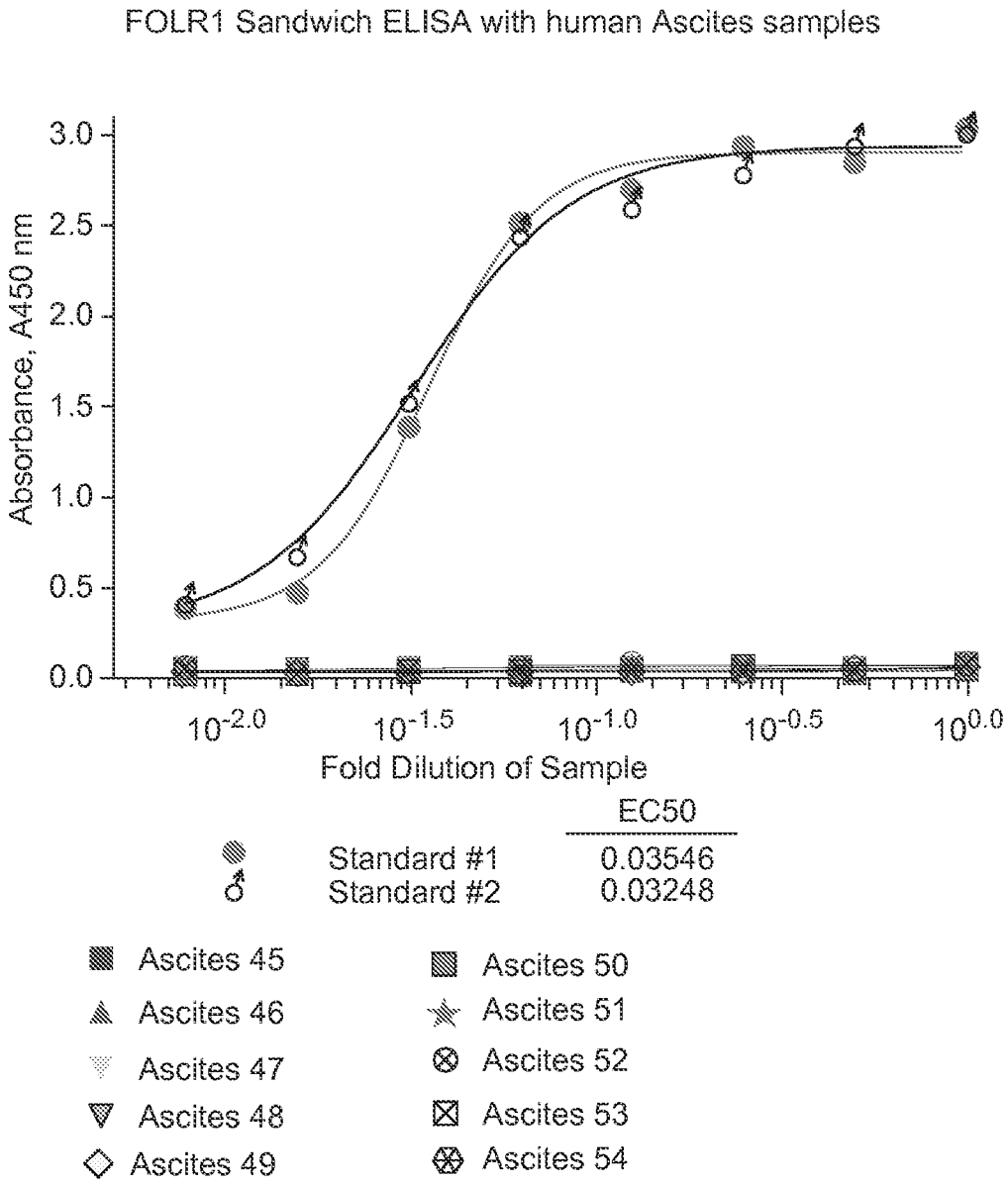

FOLR1 Sandwich ELISA with human Ascites samples
Absorbance, A450 nm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$10^{-2.0}$
$10^{-1.5}$
$10^{-1.0}$
$10^{-0.5}$
$10^{0.0}$
Fold Dilution of Sample
EC50
Standard #1 0.03546
Standard #2 0.03248
Ascites 45
Ascites 46
Ascites 47
Ascites 48
Ascites 49
Ascites 50
Ascites 51
Ascites 52
Ascites 53
Ascites 54

FIG. 9

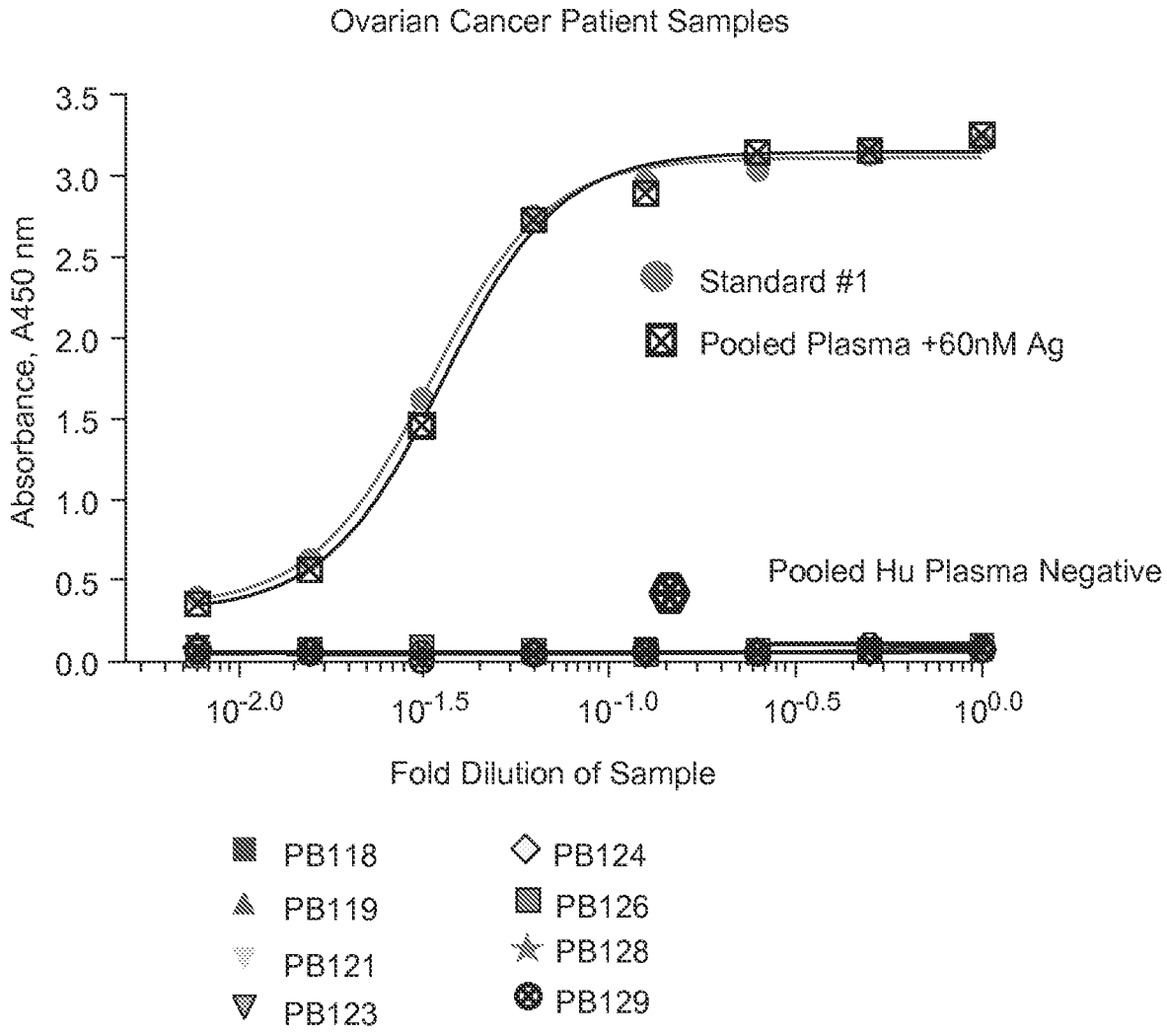
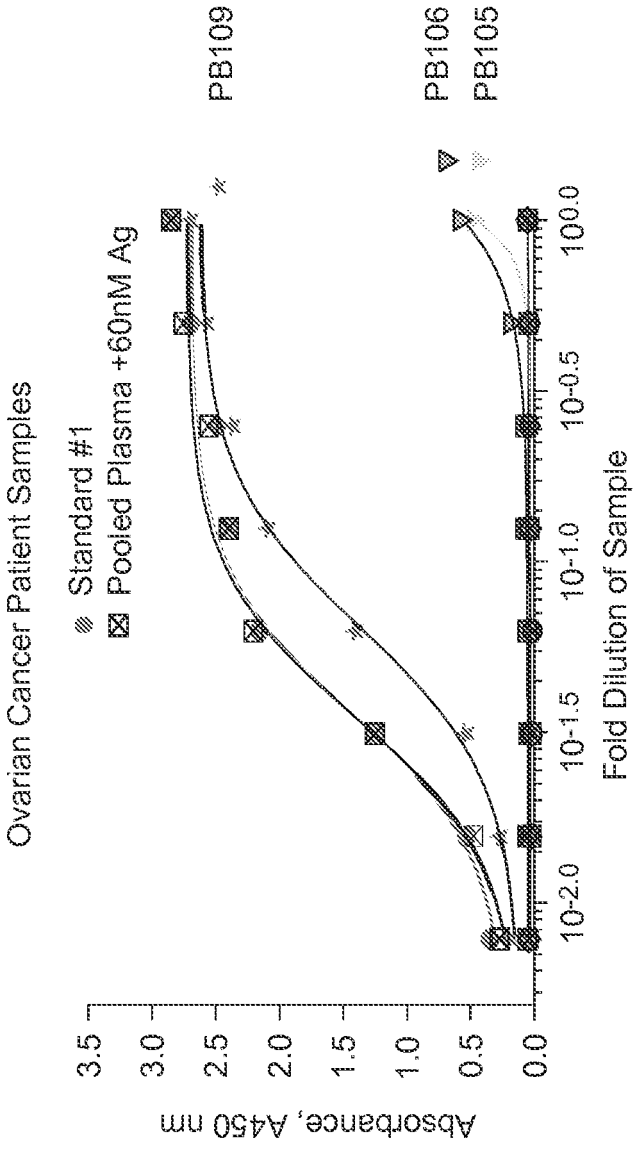

Ovarian Cancer Patient Samples
Absorbance, A450 nm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Standard #1
Pooled Plasma +60nM Ag
Pooled Hu Plasma Negative
$10^{-2.0}$
$10^{-1.5}$
$10^{-1.0}$
$10^{-0.5}$
$10^{0.0}$
Fold Dilution of Sample
PB118
PB119
PB121
PB123
PB124
PB126
PB128
PB129

FIG. 10



	Standard						Recovery	%CV
Sample	Y-values	Log(x)	x	Conc	Dil Factor	Interpolated Folr1-Fc		
PB105	0.4536	-1.90938	0.01232	0.74 nM	1	0.74 nM	0.74 nM	N/a
PB106	0.5628	-1.82047	0.015119	0.91 nM	1	0.91 nM	0.91 nM	N/a

FIG. 11

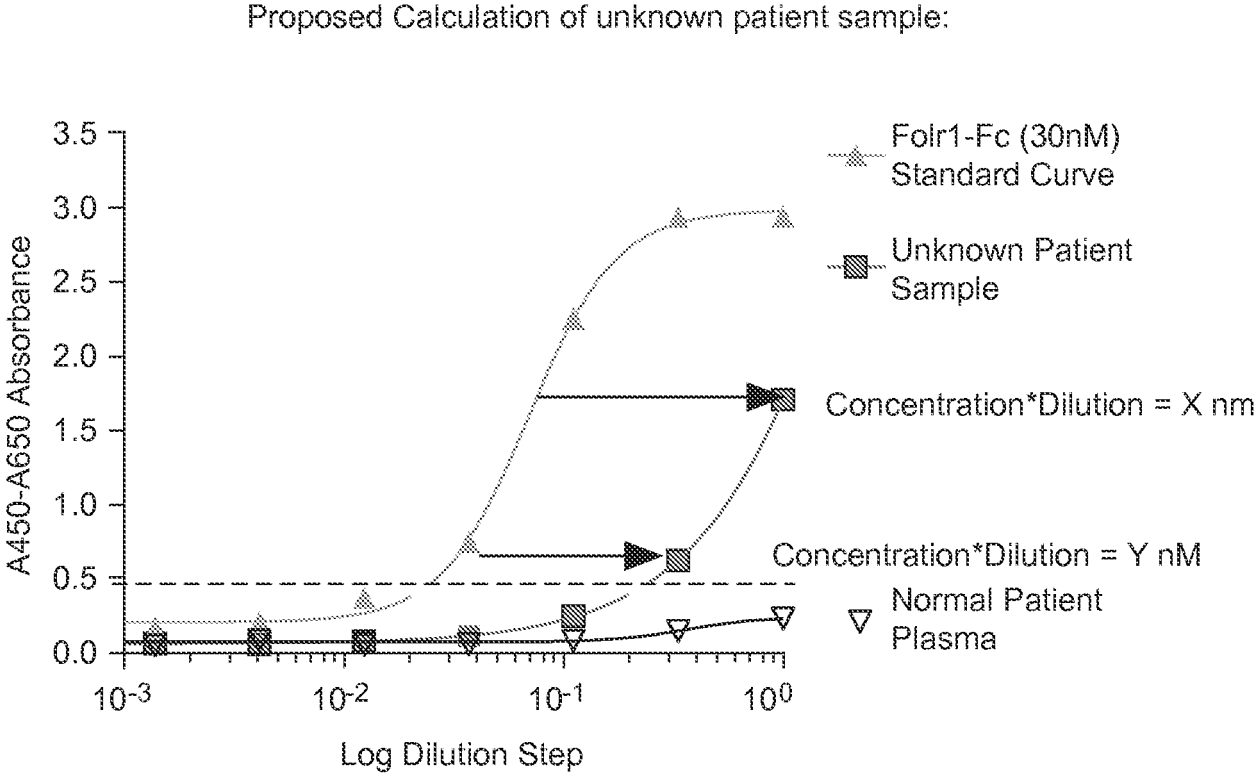
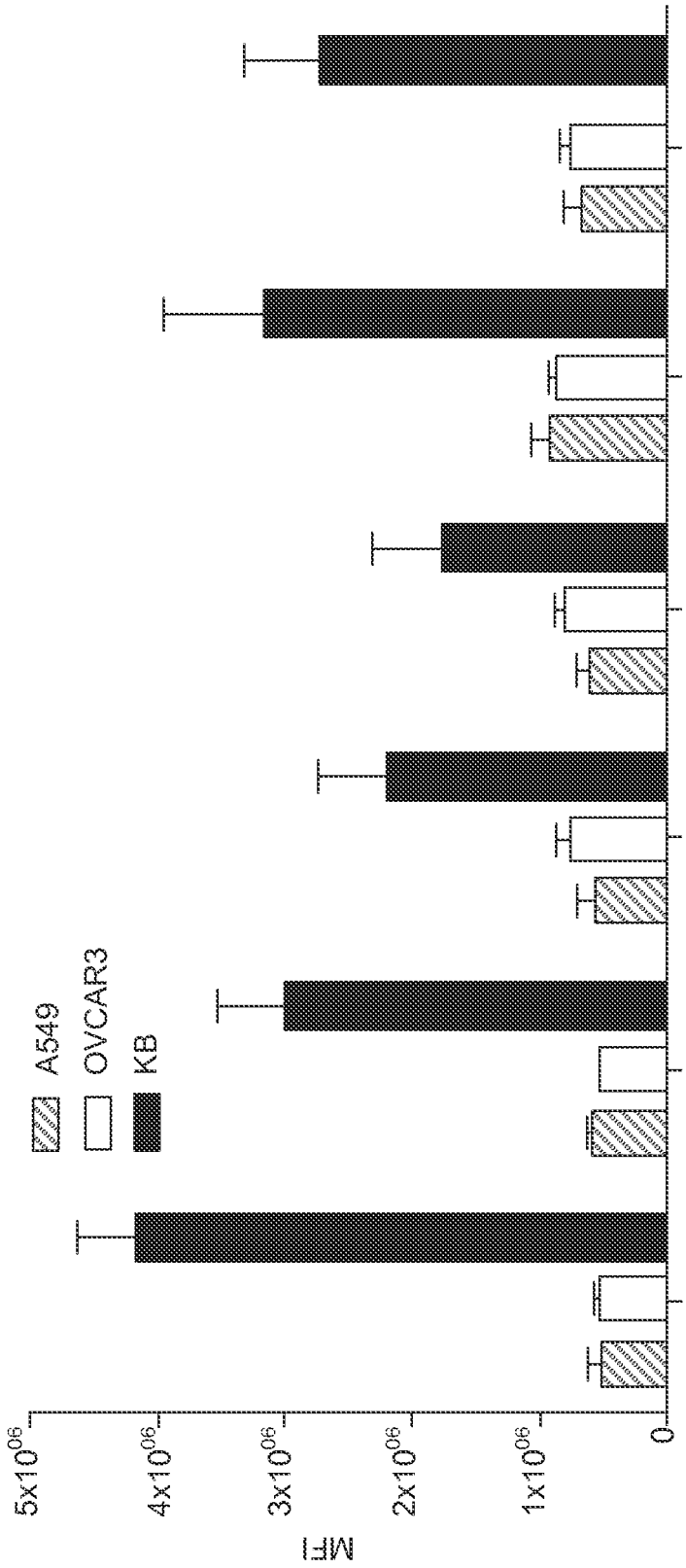

Proposed Calculation of unknown patient sample:
A450-A650 Absorbance
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10-3
10-2
10-1
100
Log Dilution Step
Folr1-Fc (30nM) Standard Curve
Unknown Patient Sample
Concentration*Dilution = X nm
Concentration*Dilution = Y nM
Normal Patient Plasma

FIG. 12



Antibody		A549 (Negative)	OVCAR3 (Low)	KB (High)
Commercial FRA	1:5	522300 ± 63250	524733 ± 16815	4176667 ± 462343
	1:10	539733 ± 71517	500200 ± 7932	3015667 ± 471776
MuFR1-9	1:100	542333 ± 153328	744667 ± 105706	2206667 ± 523461
	1:200	606000 ± 86309	802000 ± 61441	1783333 ± 541501
MuFR1-13	1:100	914000 ± 127582	886333 ± 25621	3170000 ± 757386
	1:200	653000 ± 144420	744667 ± 89631	2733333 ± 542965

FIG. 13

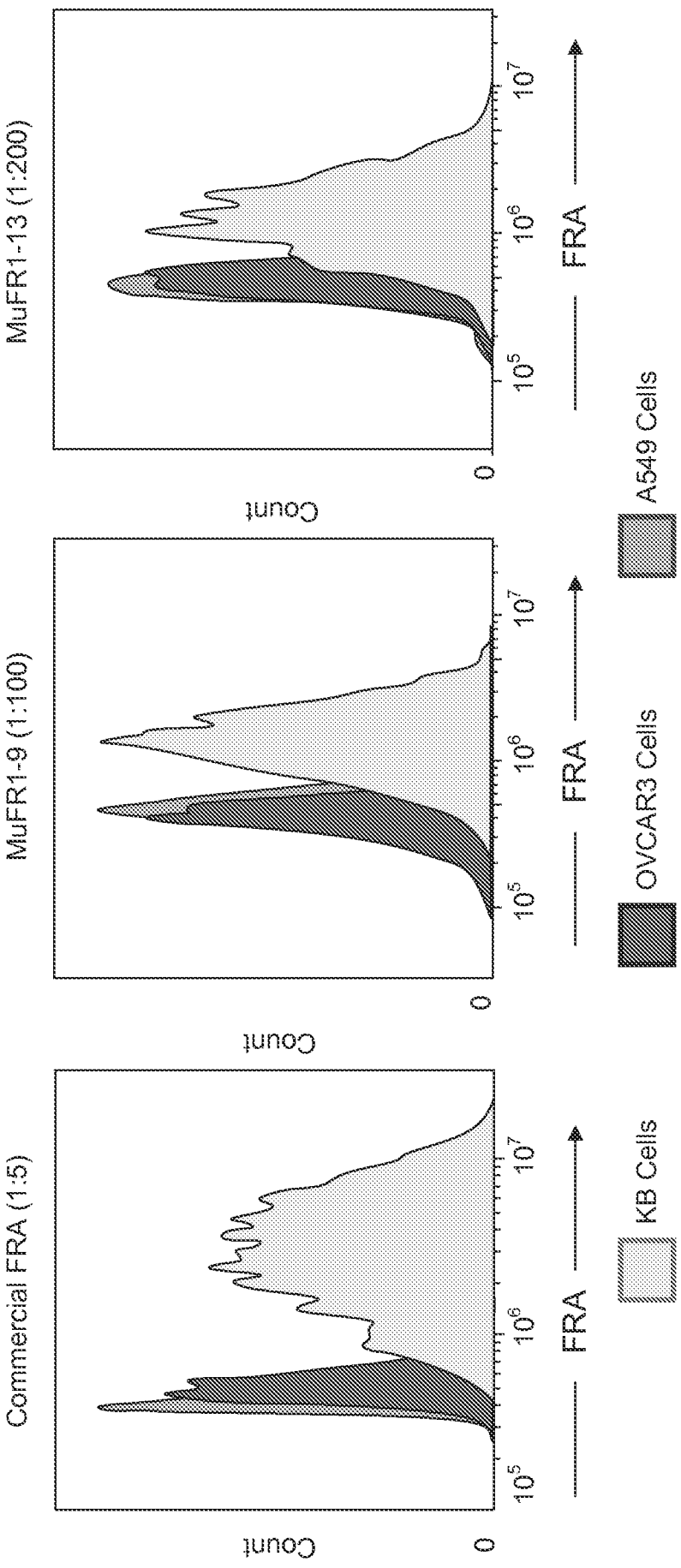
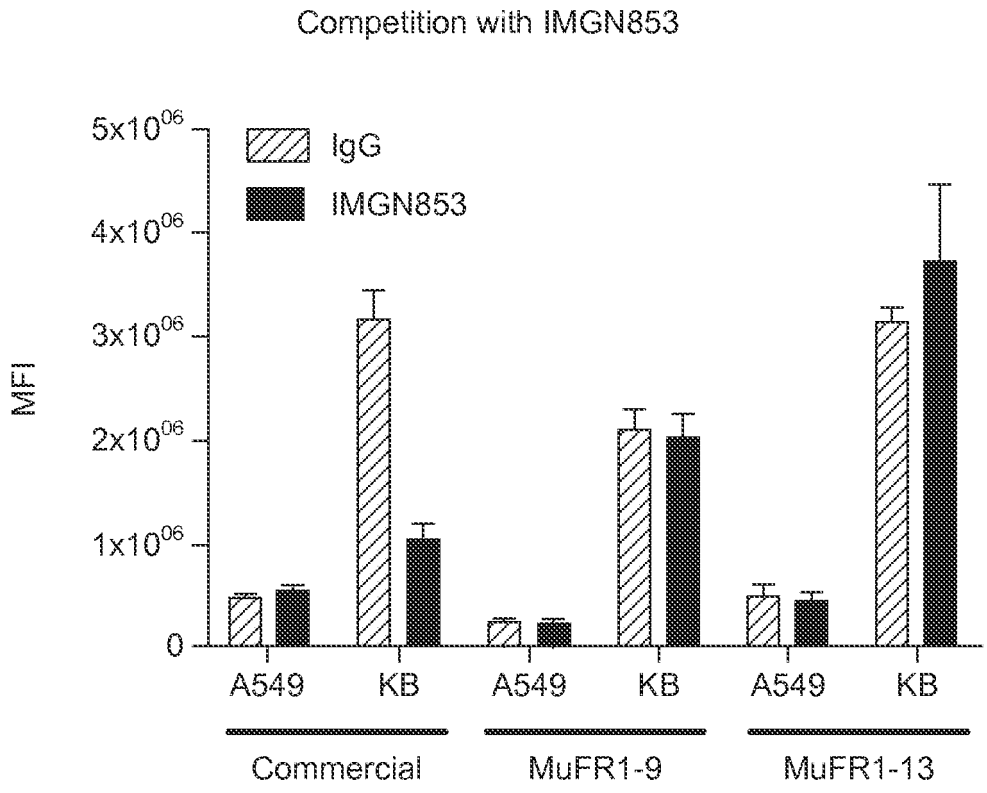

Commercial FRA (1:5)
MuFR1-9 (1:100)
MuFR1-13 (1:200)
Count
0
10^5
10^6
10^7
FRA
KB Cells
OVCAR3 Cells
A549 Cells

FIG. 14



Antibody	Cells	Treatment	
		IgG (MFI)	IMGN853 (MFI)
Commercial FRA	A549	458333 ± 36085	524333 ± 62307
	KB	3173333 ± 298124	1006333 ± 195297
MuFR1-9	A549	224667 ± 27936	221667 ± 32380
	KB	2076667 ± 202759	2010000 ± 225167
MuFR1-13	A549	459000 ± 129230	404667 ± 117689
	KB	3113333 ± 190117	3726667 ± 768339

FIG. 15