P1A3 (lambda)

QAVVTQEPSL SVSPGGTVTL TCGLSSGSVS TGHYASWYQQ TPGQAPRTLF YNTNTRSSGV
PDRFSGSIVG NKAALTITGA QADDESDYYC VLYVGDGIWV FGGGTKLTVL GQPKAAPSVT
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK QSNNKYAASS
YLSLTPEQWK SHKSYSCQVT HEGSTVEKTV APTECS

Figure 1A

P1C5 (lambda)

QTVVTQEPSF SVSPGGTVTL TCGLSSGSVS TGYYPSWYQQ TPGQAPRTLI YSTNSRSSGV
PDRFSGSILG NKAALTITGA QADDESDYYC VLYMGSGISV FGGGTKLTVL GQPKAAPSVT
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK QSNNKYAASS
YLSLTPEQWK SHKSYSCQVT HEGSTVEKTV APTECS

Figure 1B

P1E4 (lambda)

QTVVTQEPSF SVSPGGTVTL TCGLSSGSVS TSYYPSWYQQ IPGQAPRTLI YTTNIRSSGV
PDRFGGSILG NKAALTITGA QAEDESDYYC MLYMGSGIWV FGGGTKLTVL GQPKTAPSVT
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK QSNNKYAASS
YLSLTPEQWK SHKSYSCQVT HEGSTVEKTV APAECS

Figure 1C

P1F1 (lambda)

QTVVTQEPSF SVSPGGTVTL TCGLSSGSVS TSYYPSWYQQ TPGQAPRTLI YSTNTRSSGV
PDRFSGSILG NKAALTITGA QADDESDYYC VLYMGSGIWV FGGGTKLTVL GQPKAAPSVT
LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK QSNNKYAASS
YLSLTPEQWK SHKSYSCQVT HEGSTVEKTV APTECS

Figure 1D

P1A3

EVQLVQSGTE VKKPGASVRV SCKSSGYTFT **SYYIH**WVRQA PGQGLEWMA**I INPGNGDTNY AQRFQG**RVTM TRDTSTSTVY MELRSLRSDD TAVYFCAR**EI ASYSGSYYDY** WGQGTLVTVS S

Figure 2A

P1C5

QVQLQESGPG LVKPSETLSL TCTVSGGSIS **SSSYYWG**WIR QPPGKGLEWI G**SIYYSGSTY YNPSLKS**RVT ISVDTSKNQF SLKLSSVTAA DTAVYYCAR**Y APDSSGYLVA FDI**WGQGTMV TVSS

Figure 2B

P1E4

QVQLQQWGAG LLKPSETLSL TCAVYGGSFS **GYYWS**WIRQP PGKGLEWIG**E INHSGSTNYN PSLKS**RVTIS VDTSKNQFSL KLSSVTTADT AVYYCAR**MGI NSGGYLYGMD VW**GQGTTVTV SS

Figure 2C

P1F1

QVQLVESGPG LVKPSGTLSL TCAVSGGSIS **SSNWWS**WVRQ PPGKGLEWIG **EIYHSGSTNY NPSLKS**RVTI SVDTSKNQFS LKLSSVTAAD TAVYYCAR**MG ANSGGYLYGM DV**WGQGTTVT VSS

Figure 2D

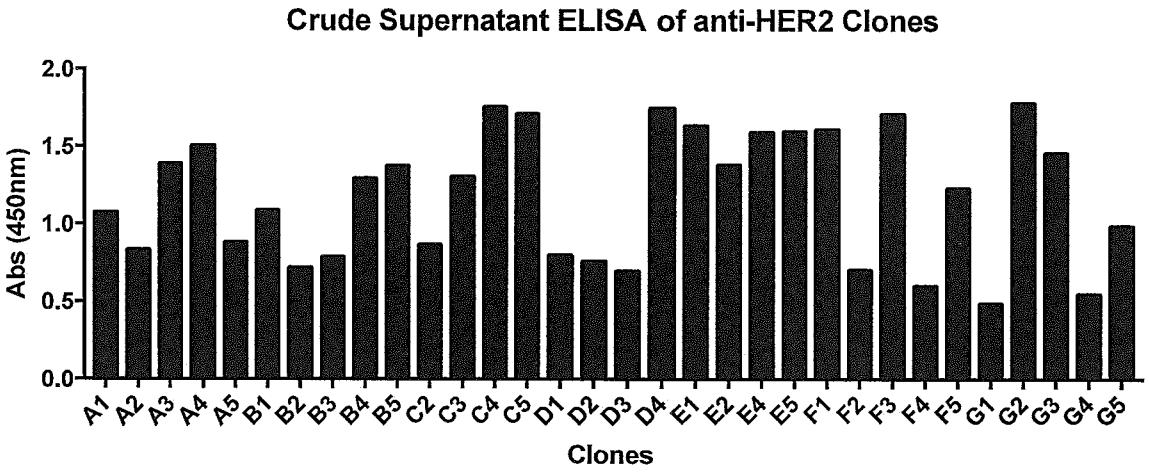

Crude Supernatant ELISA of anti-HER2 Clones
Abs (450nm)
2.0
1.5
1.0
0.5
0.0
A1 A2 A3 A4 A5 B1 B2 B3 B4 B5 C2 C3 C4 C5 D1 D2 D3 D4 E1 E2 E4 E5 F1 F2 F3 F4 F5 G1 G2 G3 G4 G5
Clones

Figure 3

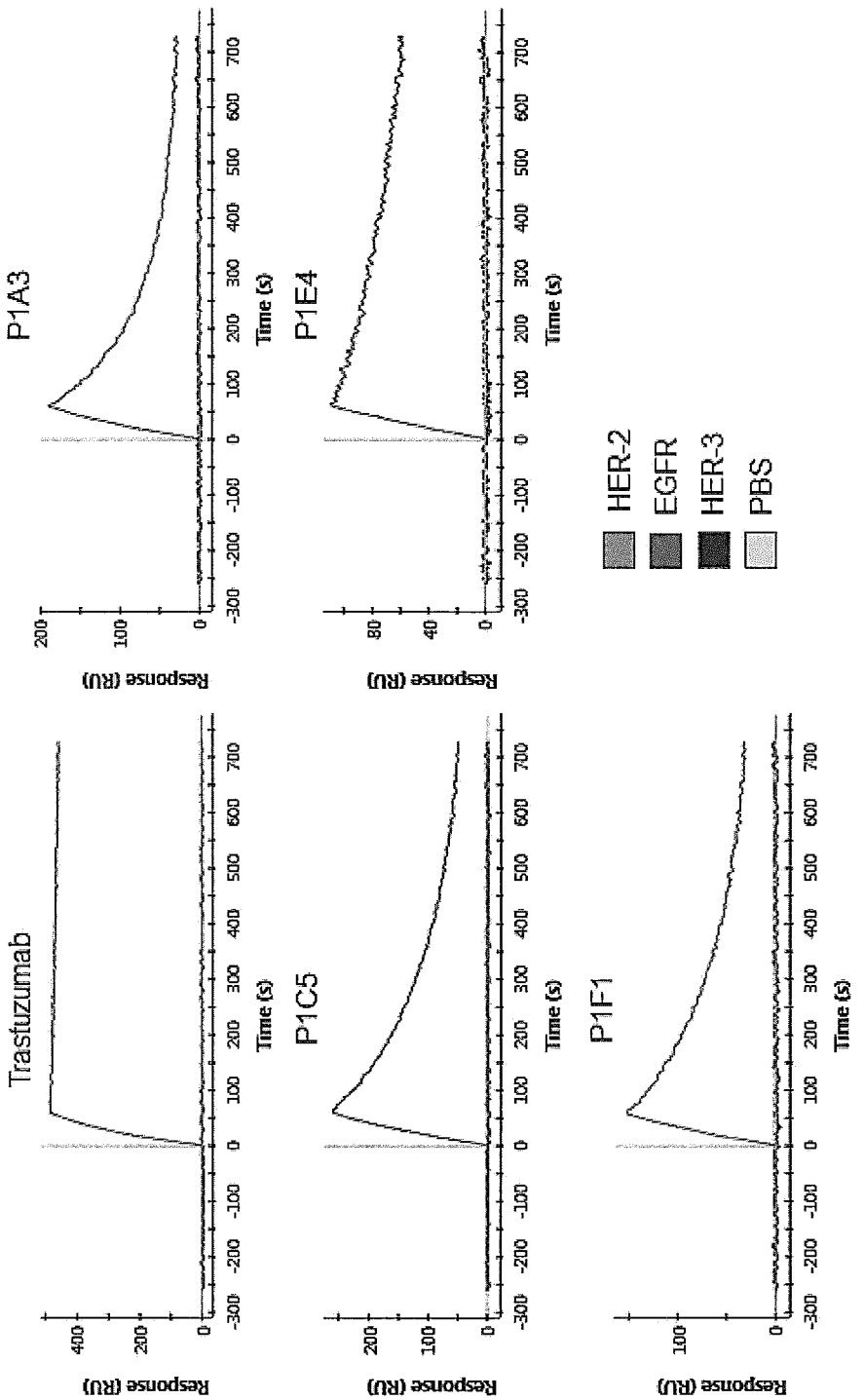

Trastuzumab
P1A3
P1E4
P1C5
P1F1
Response (RU)
Time (s)
HER-2
EGFR
HER-3
PBS

Figure 4

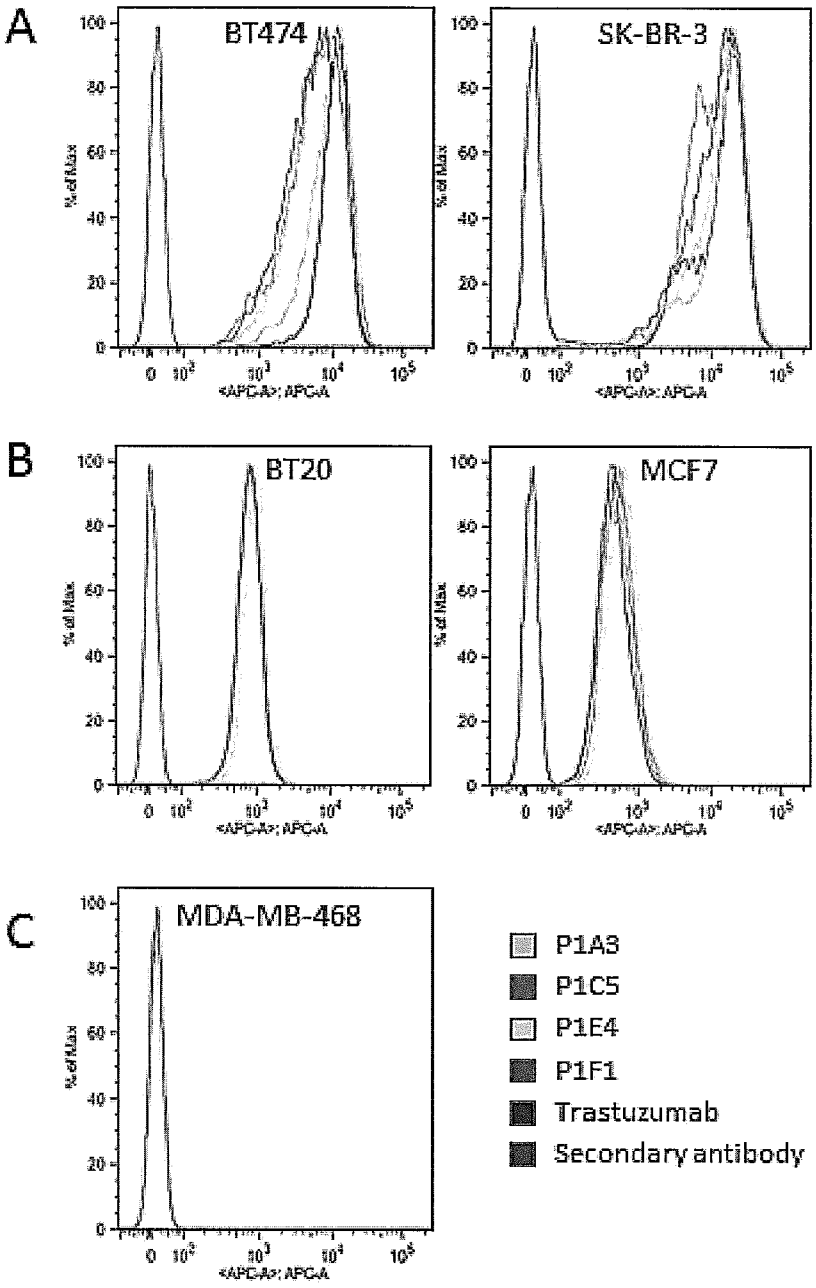

A
BT474
SK-BR-3
B
BT20
MCF7
C
MDA-MB-468
P1A3
P1C5
P1E4
P1F1
Trastuzumab
Secondary antibody
% of Max
<APC-A>: APC-A

Figure 5

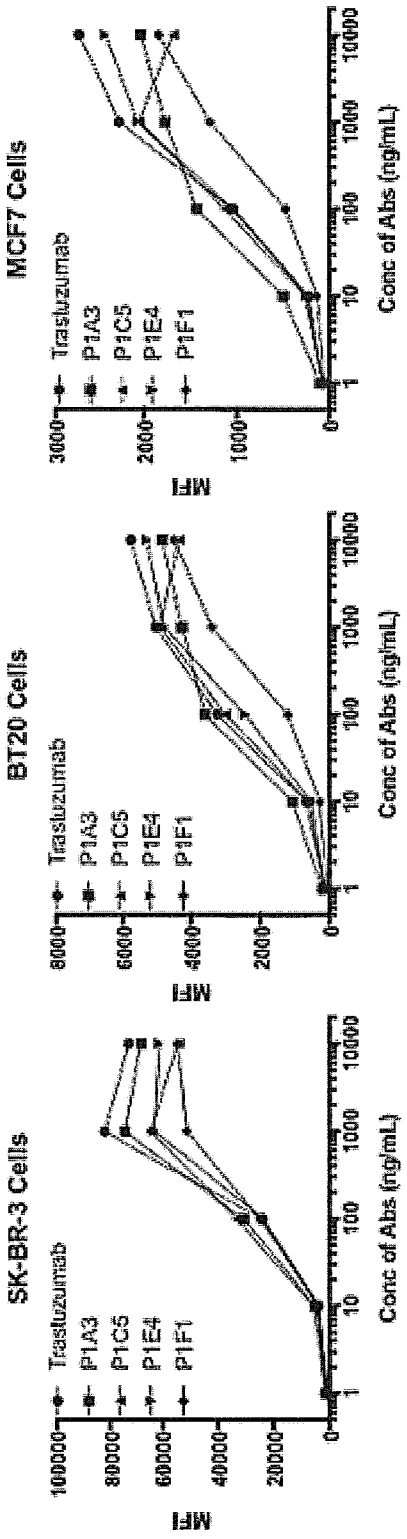

SK-BR-3 Cells
BT20 Cells
MCF7 Cells
Trastuzumab
P1A3
P1C5
P1E4
P1F1
MFI
Conc of Abs (ng/mL)

Figure 6

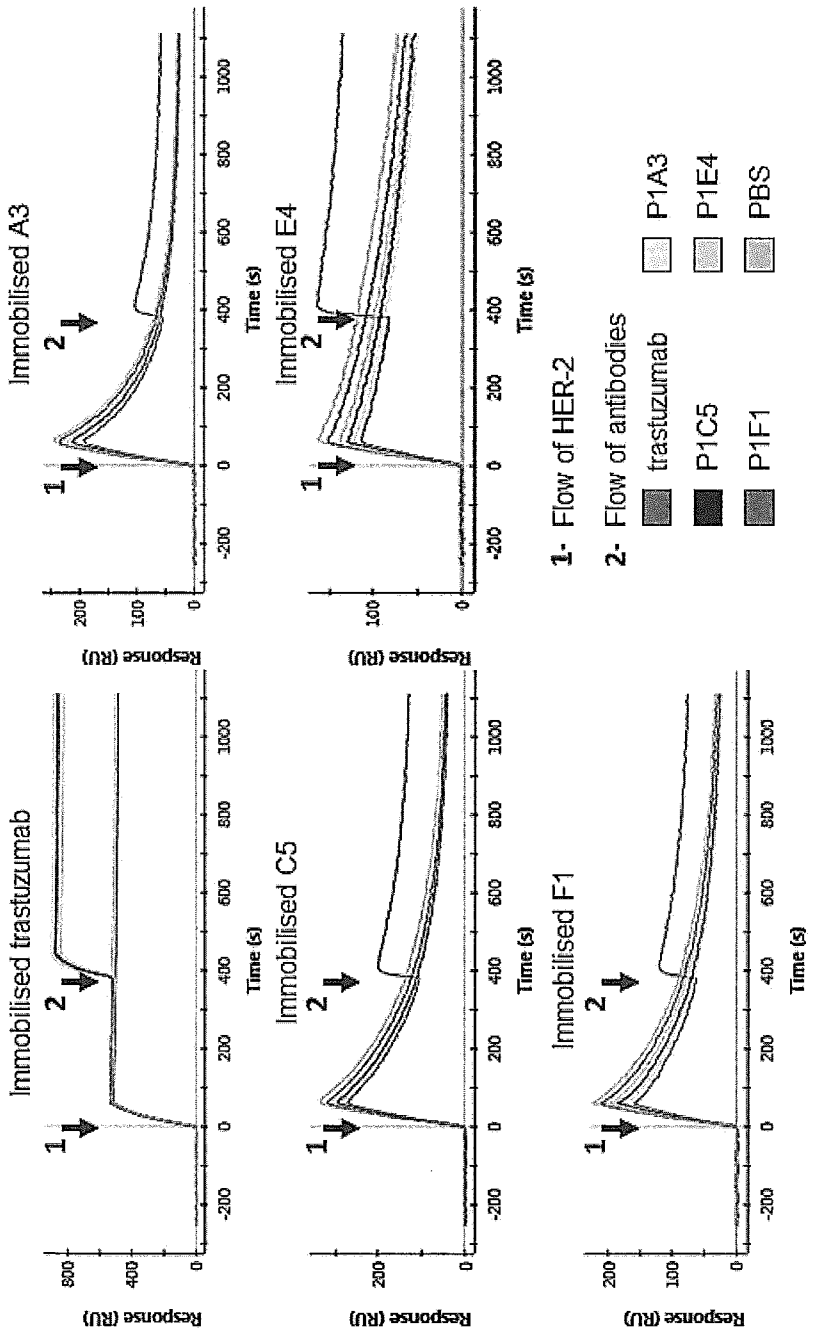

Immobilised A3
Immobilised E4
Immobilised trastuzumab
Immobilised C5
Immobilised F1
1
2
Response (RU)
Time (s)
1- Flow of HER-2
2- Flow of antibodies
trastuzumab
P1C5
P1F1
P1A3
P1E4
PBS

Figure 7

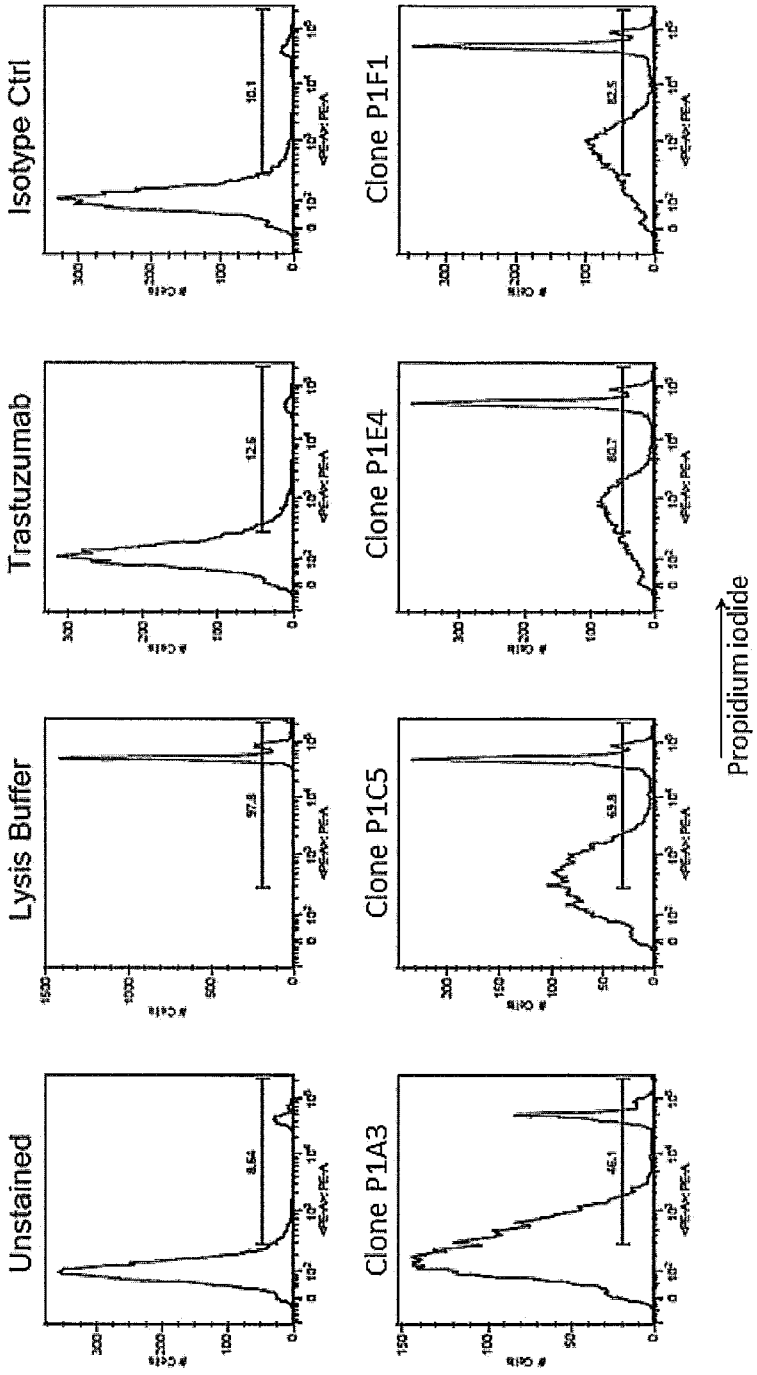

Unstained
Lysis Buffer
Trastuzumab
Isotype Ctrl
Clone P1A3
Clone P1C5
Clone P1E4
Clone P1F1
Propidium iodide

Figure 8

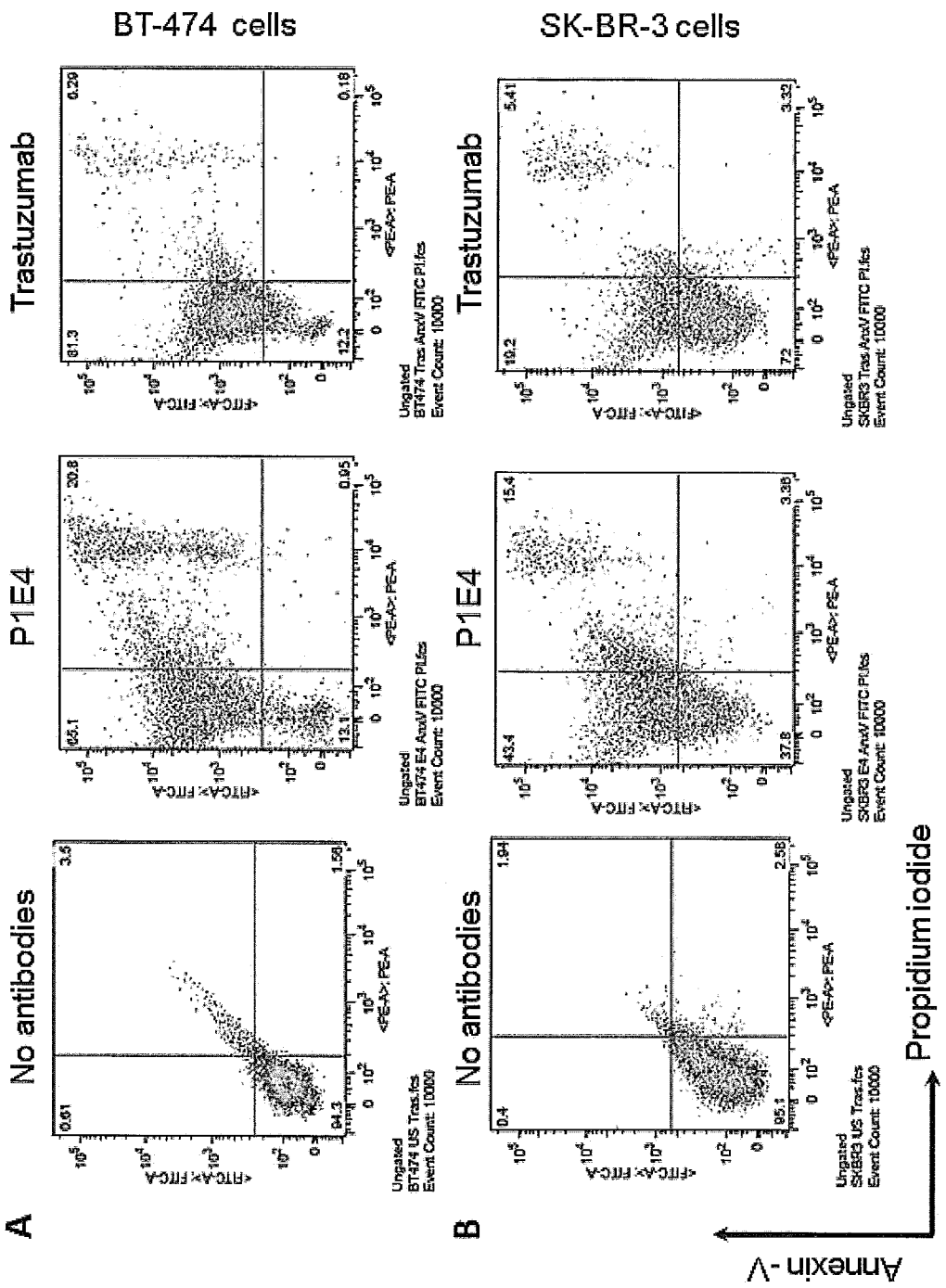

BT-474 cells
SK-BR-3 cells
Trastuzumab
P1E4
No antibodies
A
B
Annexin -V
Propidium iodide

Figure 9

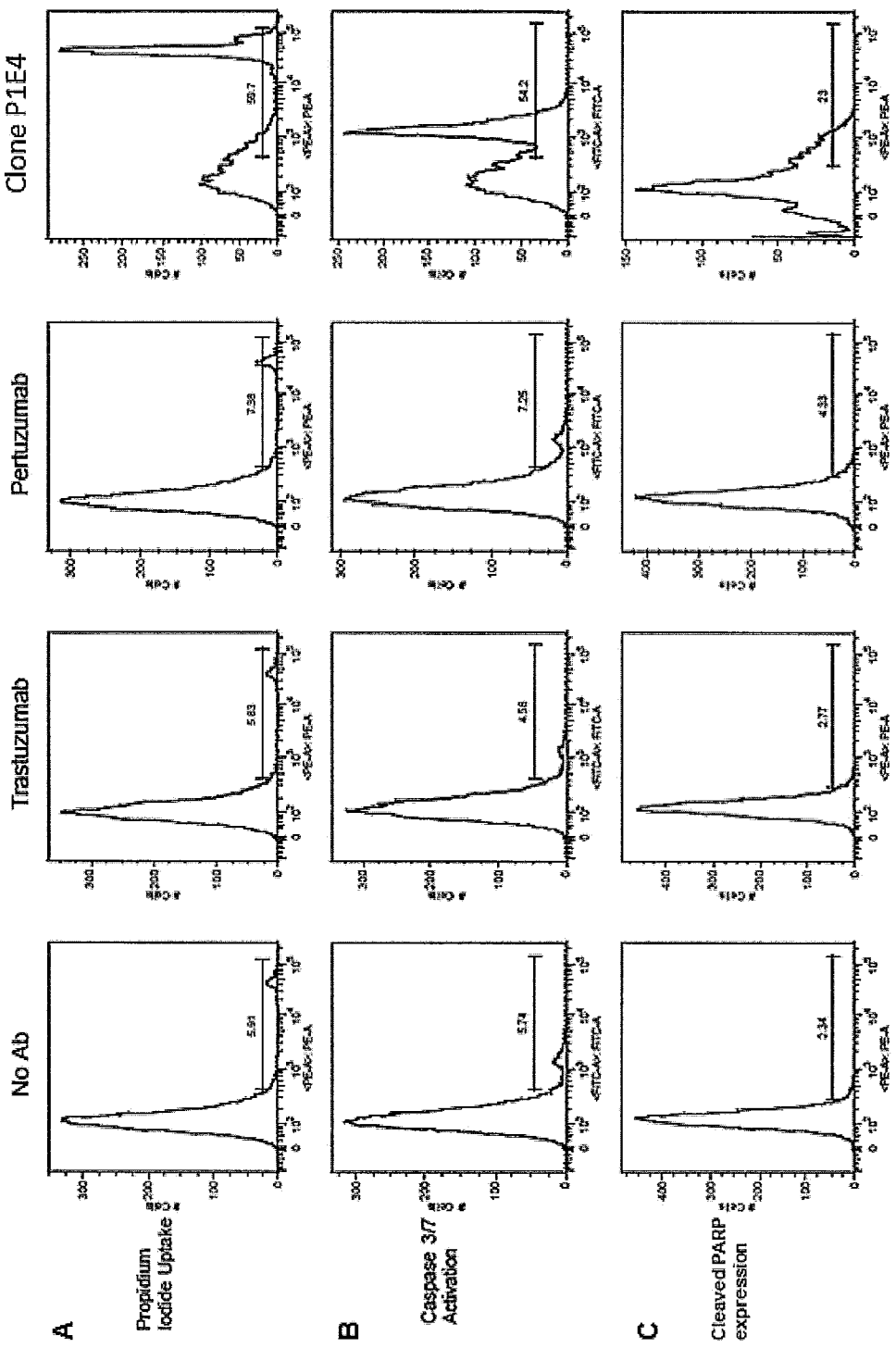

No Ab
Trastuzumab
Pertuzumab
Clone P1E4
A
Propidium Iodide Uptake
B
Caspase 3/7 Activation
C
Cleaved PARP expression
5.91
5.83
7.38
59.7
5.74
4.58
7.25
54.2
2.34
2.77
4.33
23
Cells
<PE-A>: PE-A
<FITC-A>: FITC-A

Figure 10

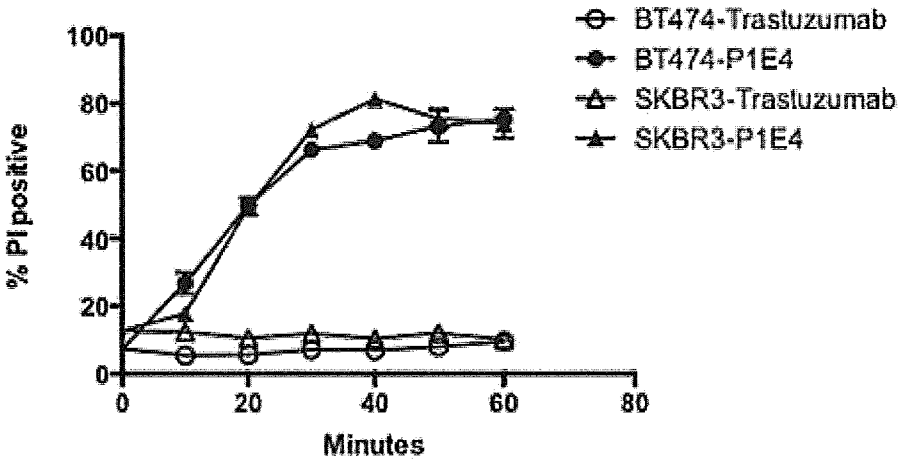

BT474-Trastuzumab
BT474-P1E4
SKBR3-Trastuzumab
SKBR3-P1E4
% PI positive
100
80
60
40
20
0
0
20
40
60
80
Minutes

Figure 11

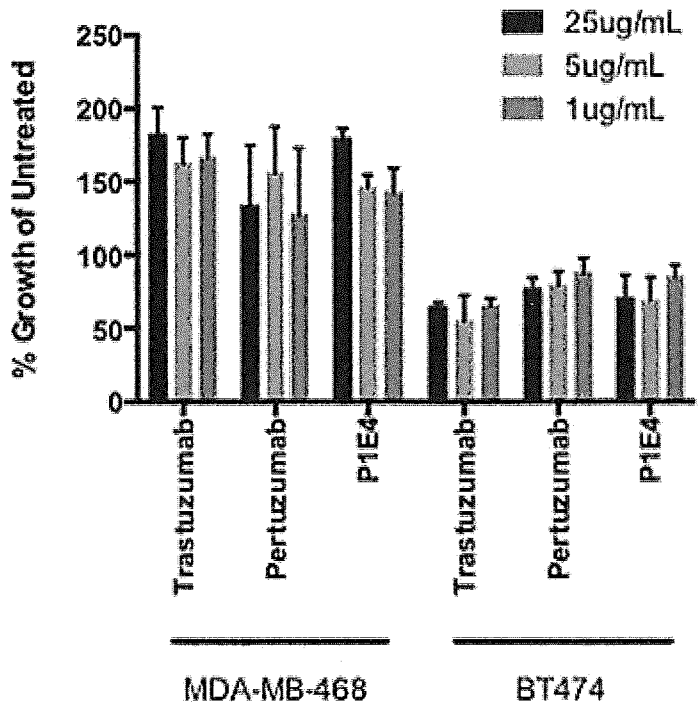

25ug/mL
5ug/mL
1ug/mL
% Growth of Untreated
250
200
150
100
50
0
Trastuzumab
Pertuzumab
P1E4
Trastuzumab
Pertuzumab
P1E4
MDA-MB-468
BT474

Figure 12

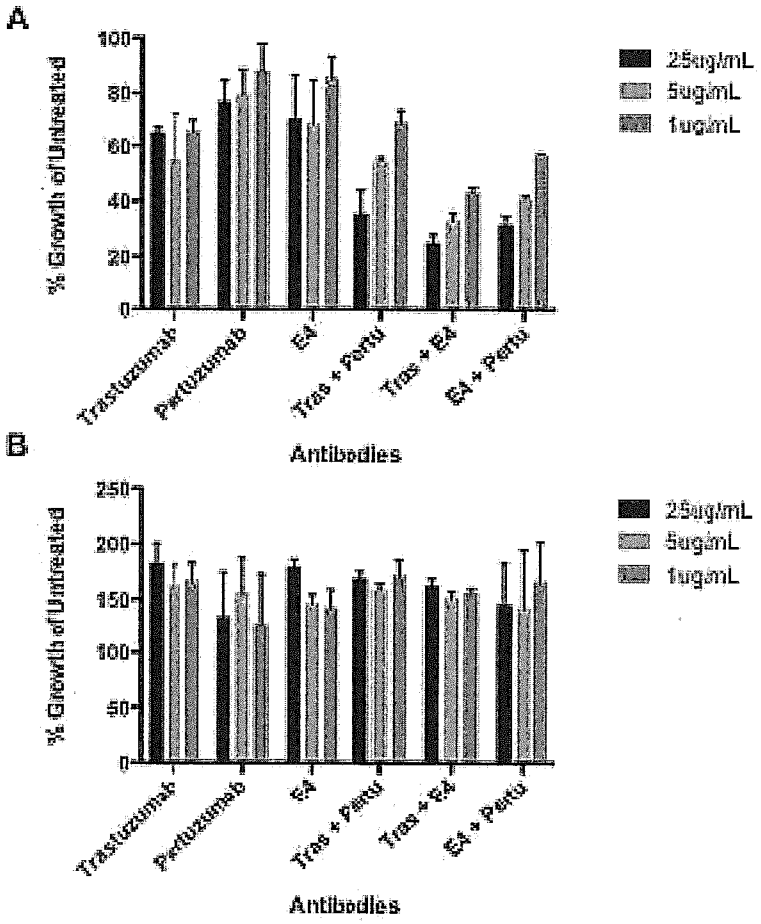

A
% Growth of Untreated
100
80
60
40
20
0
Trastuzumab
Pertuzumab
EA
Tras + Pertu
Tras + EA
EA + Pertu
Antibodies
25ug/mL
5ug/mL
1ug/mL
B
% Growth of Untreated
250
200
150
100
50
0
Trastuzumab
Pertuzumab
EA
Tras + Pertu
Tras + EA
EA + Pertu
Antibodies
25ug/mL
5ug/mL
1ug/mL

Figure 13

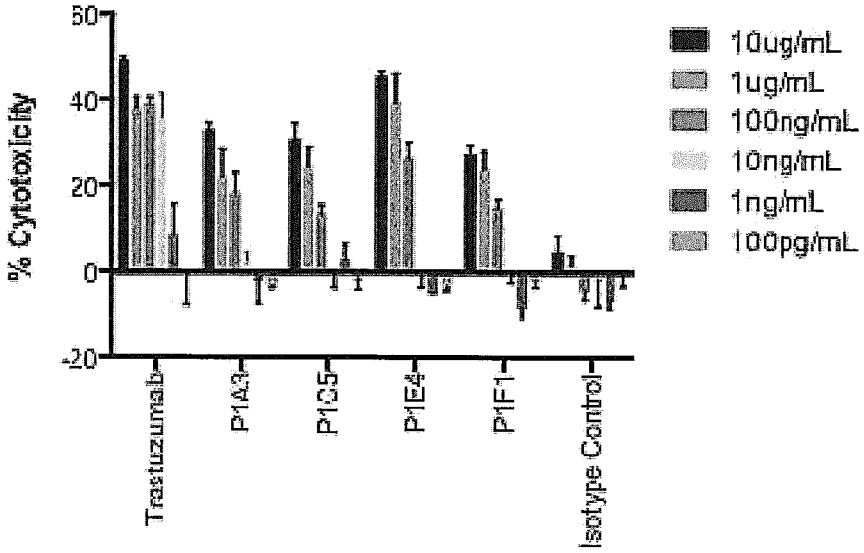

% Cytotoxicity
60
40
20
0
-20
Trastuzumab
P1A3
P1C5
P1E4
P1F1
Isotype Control
10ug/mL
1ug/mL
100ng/mL
10ng/mL
1ng/mL
100pg/mL

Figure 14

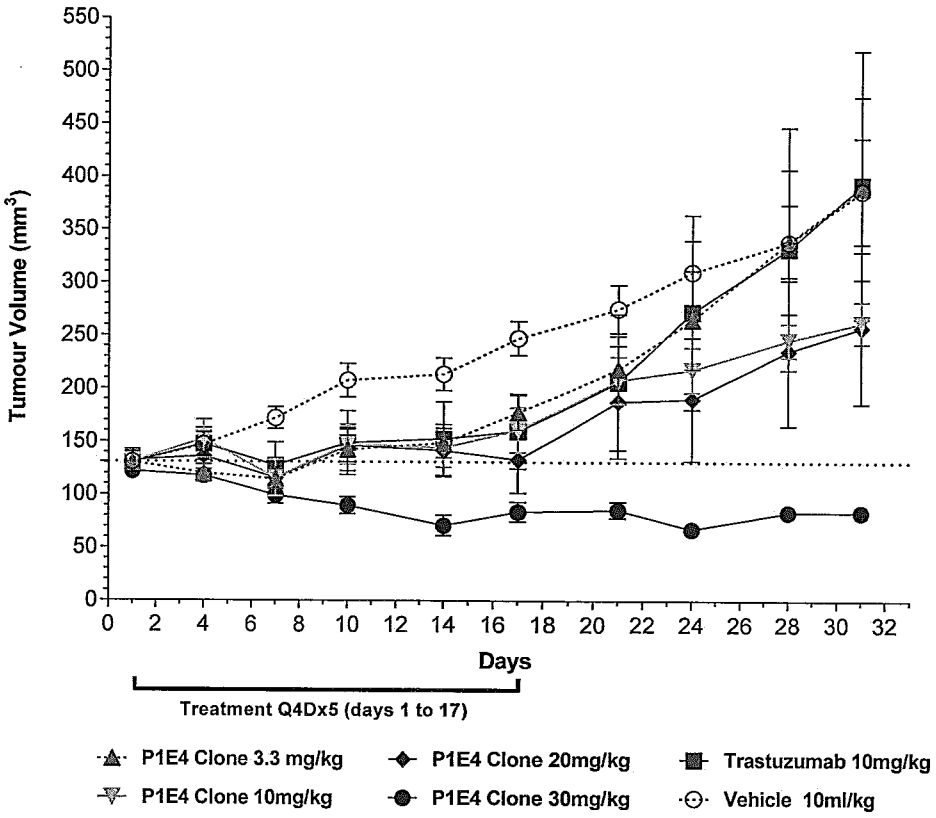

Tumour Volume (mm3)
550
500
450
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Days
Treatment Q4Dx5 (days 1 to 17)
P1E4 Clone 3.3 mg/kg
P1E4 Clone 20mg/kg
Trastuzumab 10mg/kg
P1E4 Clone 10mg/kg
P1E4 Clone 30mg/kg
Vehicle 10ml/kg

Figure 15

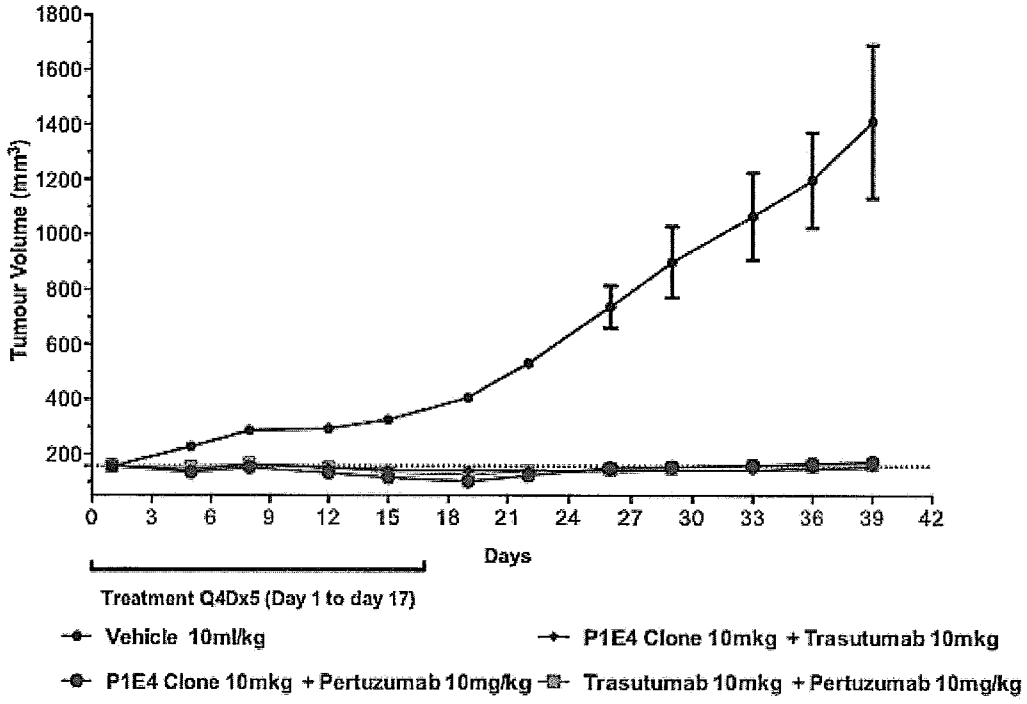

Tumour Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
Days
Treatment Q4Dx5 (Day 1 to day 17)
Vehicle 10ml/kg
P1E4 Clone 10mkg + Trasutumab 10mkg
P1E4 Clone 10mkg + Pertuzumab 10mg/kg
Trasutumab 10mkg + Pertuzumab 10mg/kg

Figure 16