FIG. 1

- **A19's binding to various ovarian cancer cell lines**

CLONE	Class																
		CaOV3	OVCAR3	OVCAR8	OV90	IGROV-1	OV17R	OVCA432	OVCA433	CH1	HEY	HEY C2	SKOV3	A2780	HEY A8	OVCAR10	TOV112D
A19	IgG1	2	4	4	0	1	0	1	0	0	2	0	4	1	1	0	0

- **A19's binding to various breast cancer cell lines**

CLONE	Class	Cell Lines						
		MCF7	T47D	CAMA1	MDA-MB-231	HS578T	hME1	MCF10A
A19	IgG1	3	4	4	3	2	0	0

- **A19's binding to various colorectal cancer cell lines**

CLONE	Class	Cell Lines														
		COLO205	SW620	HT29	HCT116	LS174T	HCT15	KM12	HCC2998	RKO	DLD1	LOVO	SW480	SKCO1	SW1116	CRL1541
A19	IgG1	4	0	3	2	1	3	0	1	3	4	3	0	1	0	2

- **A19's binding to NPC**

CLONE	Class	Cell Lines
		C666-1
A19	IgG1	3

Binding Via FACS

4 Binding > 80%
3 60% < Binding< 80%
2 40% < Binding < 60%
1 20% < Binding < 40%
0 Binding < 20%

FIG. 2

A

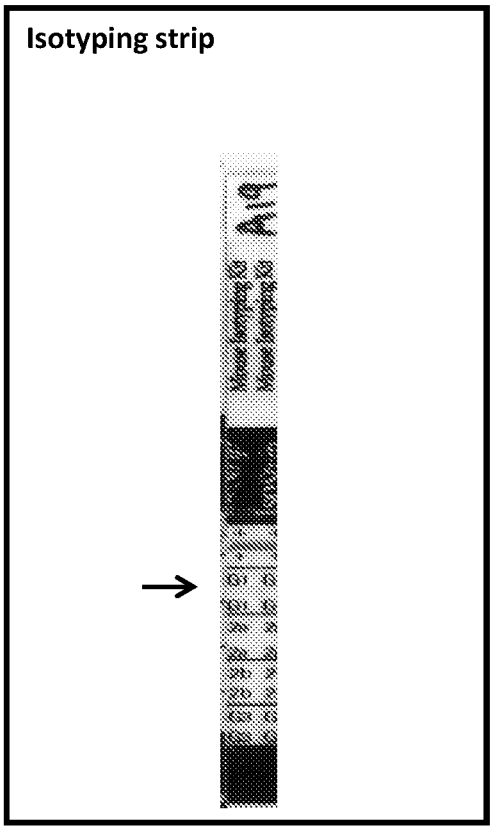

Isotyping strip
Anti-human IgG
Anti-human IgM
Anti-human IgA
Anti-human IgG1
Anti-human IgG2
Anti-human IgG3
Anti-human IgG4
Anti-human Kappa
Anti-human Lambda

FIG. 2

B

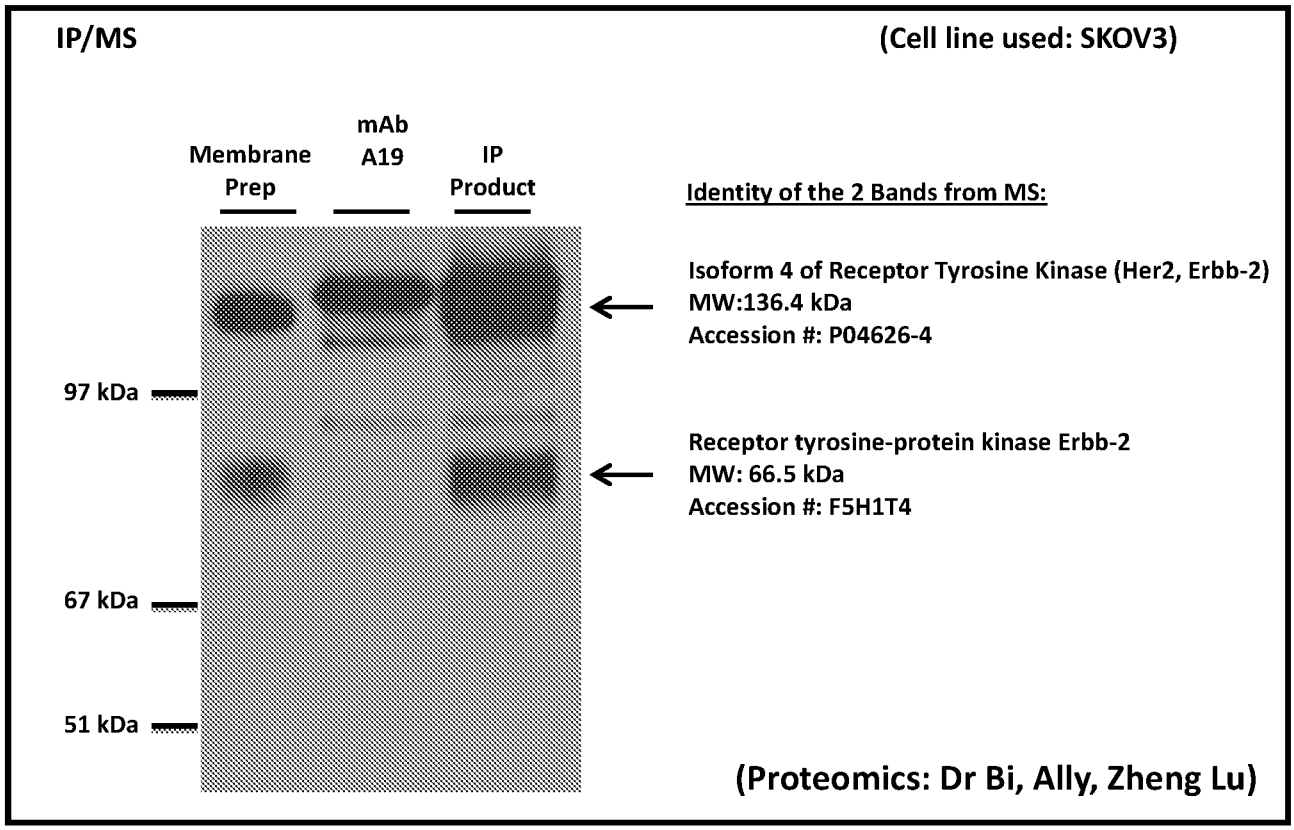
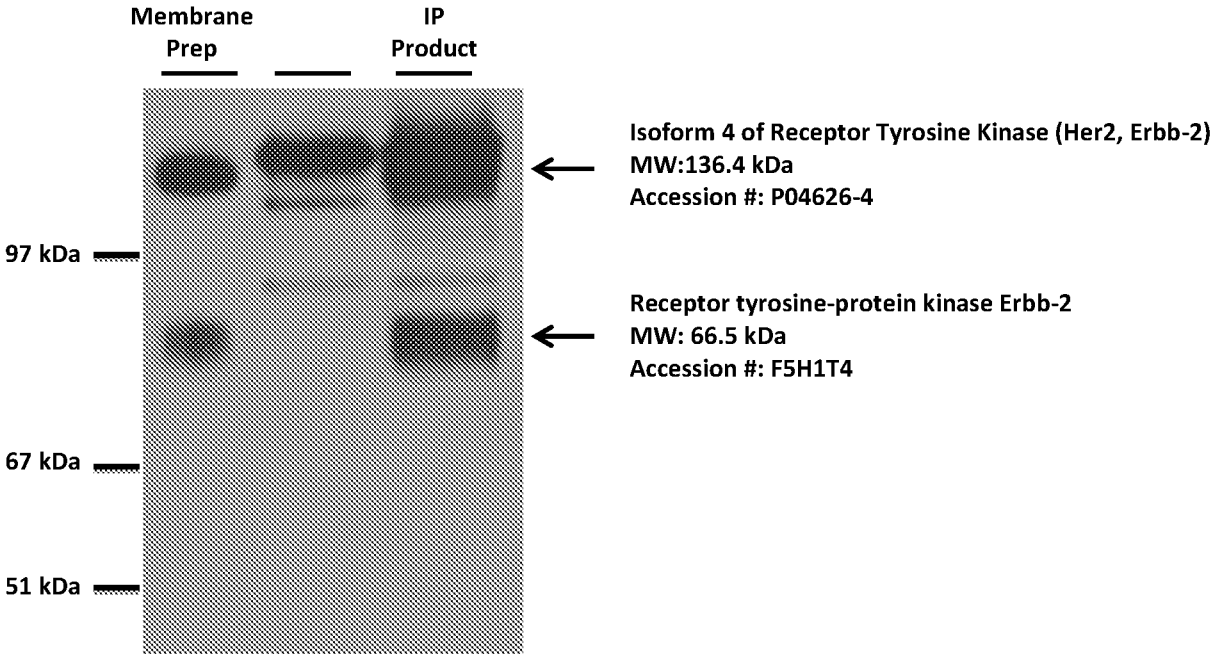

IP/MS
(Cell line used: SKOV3)
Membrane Prep
mAb A19
IP Product
97 kDa
67 kDa
51 kDa
Identity of the 2 Bands from MS:
Isoform 4 of Receptor Tyrosine Kinase (Her2, Erbb-2)
MW:136.4 kDa
Accession #: P04626-4
Receptor tyrosine-protein kinase Erbb-2
MW: 66.5 kDa
Accession #: F5H1T4
(Proteomics: Dr Bi, Ally, Zheng Lu)

FIG. 3

(SEQ ID NO: 11)

MPRGSWKPQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQ
VRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQ
DTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGPLPTDCCHEQ
CAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLV
CPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTA
PLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGL
ALIHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQE
CVEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIW
KFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRR
LLQETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKAN
KEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDV
RLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGVTVWEL
MTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVVI
QNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLE
PSEEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYV
NQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPEYLTPQGGAAPQPHPPPAFSP
AFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV

a.a. length = 1240
(n=5, 38.5% coverage)



MKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQ
LFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALT
LIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHF
NHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKC
SKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYL
YISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLF
RNPHQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLP
CHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHS

a.a. length = 603
(n=3, 14.3% coverage)

(SEQ ID NO: 12)

Fig. 4

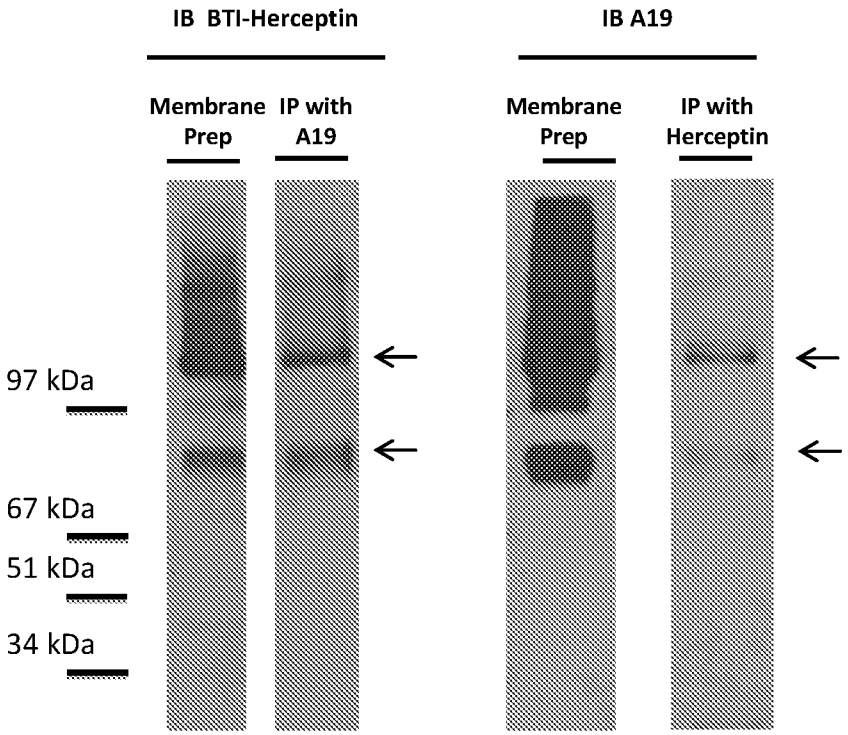
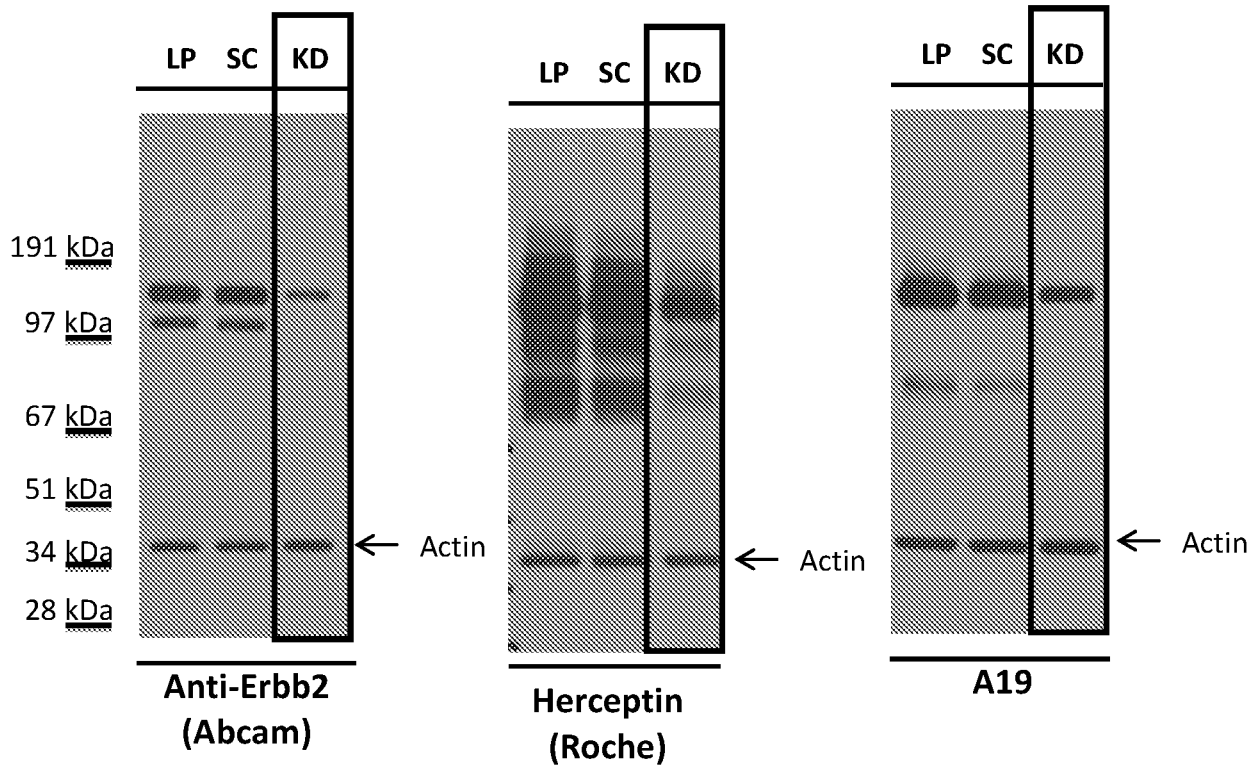

IB BTI-Herceptin
IB A19
Membrane Prep
IP with A19
Membrane Prep
IP with Herceptin
97 kDa
67 kDa
51 kDa
34 kDa

Fig. 5



Legend
LP: Lipofectamine
SC: Scramble
KD: Knocked Down

FIG. 6

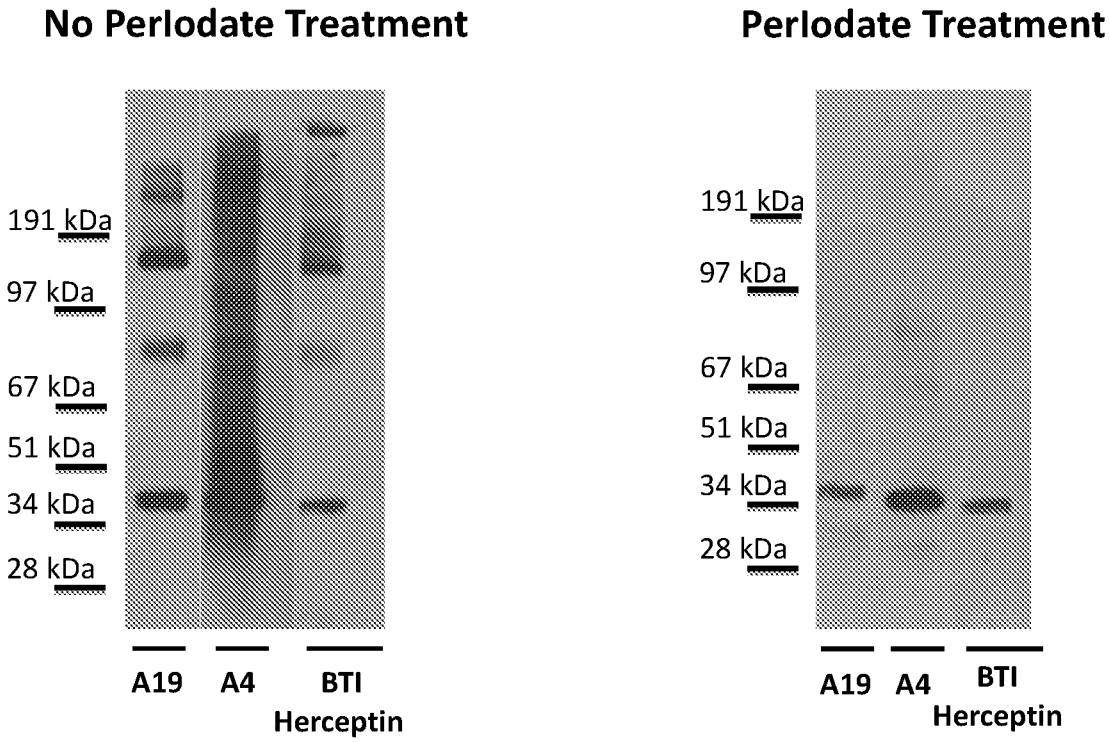

No Perlodate Treatment
191 kDa
97 kDa
67 kDa
51 kDa
34 kDa
28 kDa
A19
A4
BTI
Herceptin
Perlodate Treatment
191 kDa
97 kDa
67 kDa
51 kDa
34 kDa
28 kDa
A19
A4
BTI
Herceptin

FIG. 7

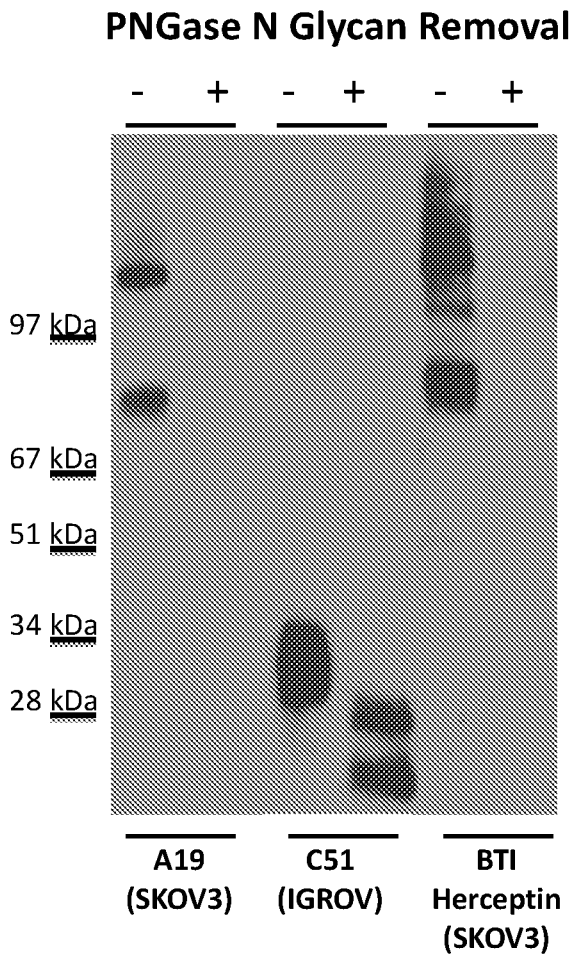

PNGase N Glycan Removal
- + - + - +
97 kDa
67 kDa
51 kDa
34 kDa
28 kDa
A19 (SKOV3)
C51 (IGROV)
BTI Herceptin (SKOV3)

Fig. 8

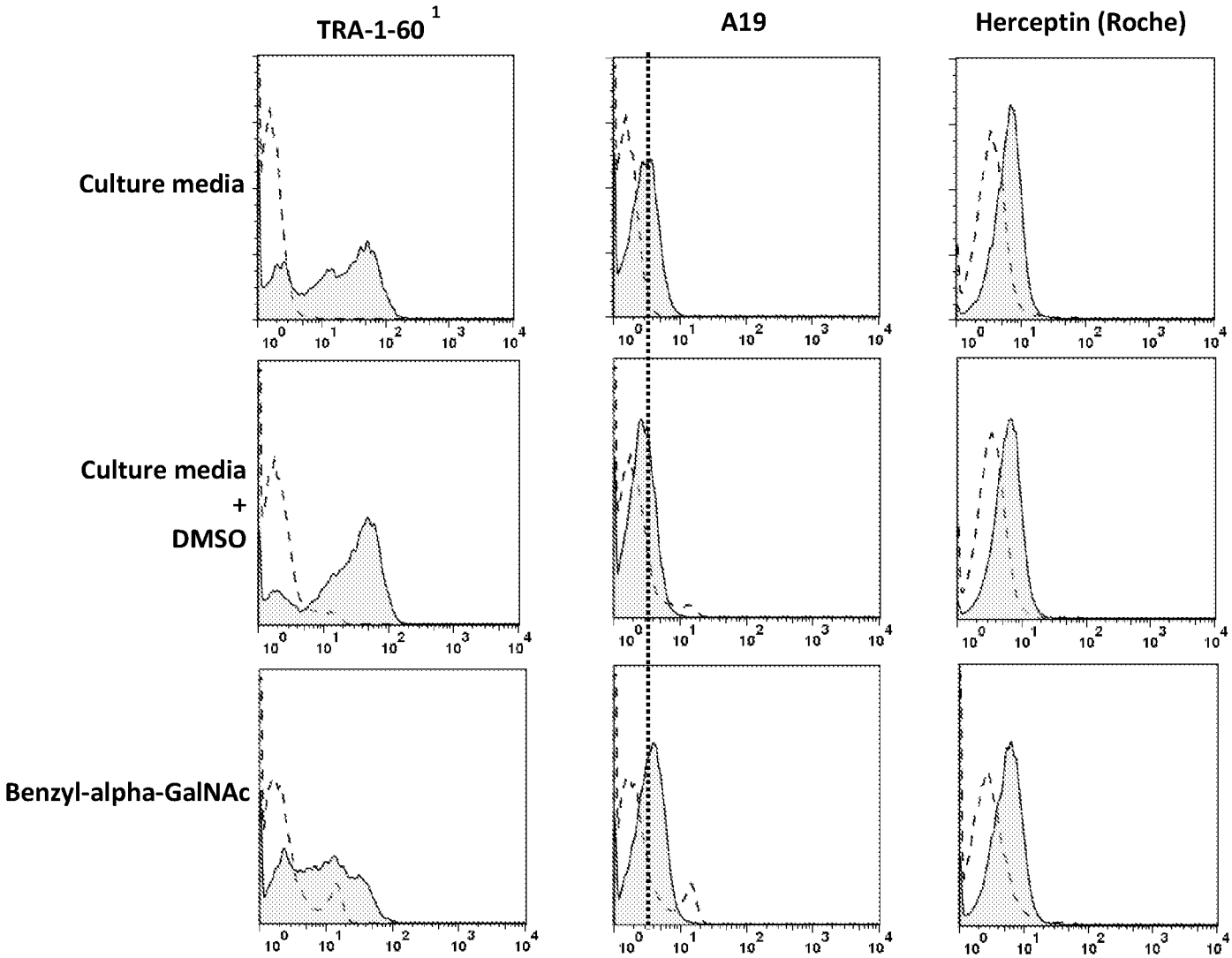

TRA-1-60 1
A19
Herceptin (Roche)
Culture media
Culture media + DMSO
Benzyl-alpha-GalNAc

Fig. 9

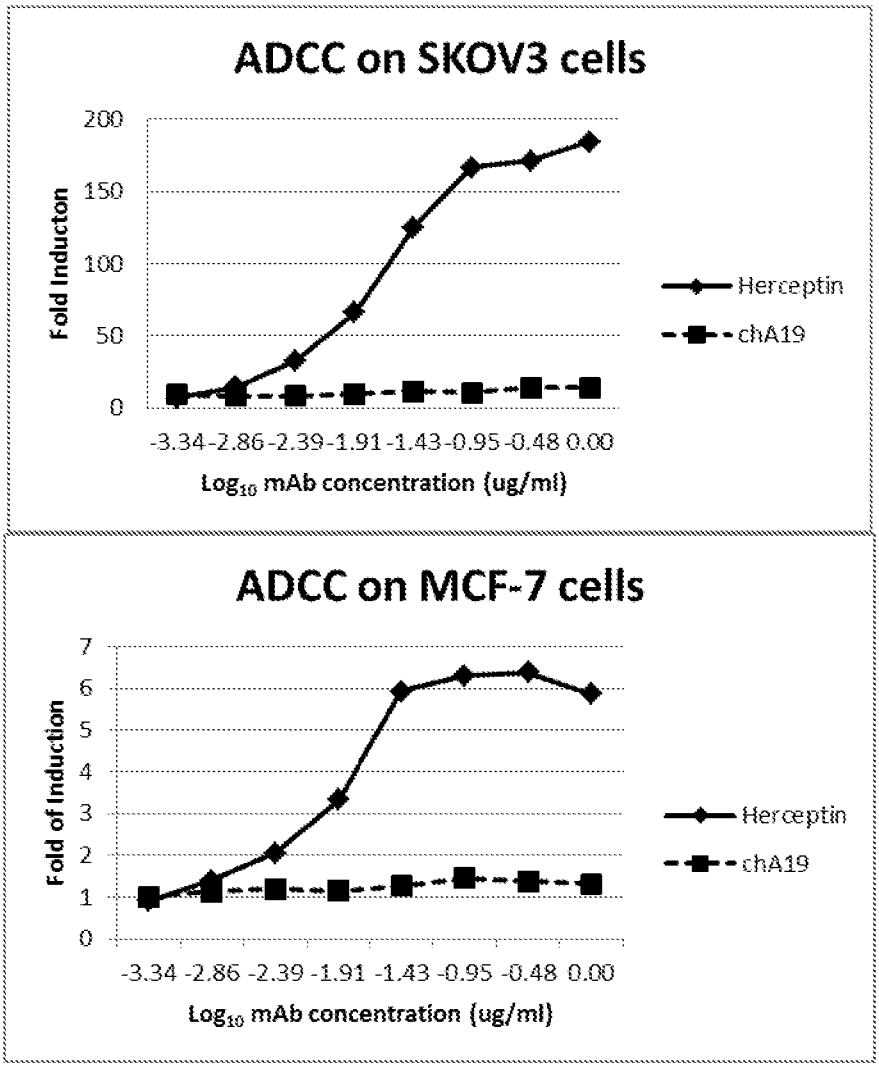

ADCC on SKOV3 cells
Fold Induction
200
150
100
50
0
-3.34 -2.86 -2.39 -1.91 -1.43 -0.95 -0.48 0.00
Log10 mAb concentration (ug/ml)
Herceptin
chA19
ADCC on MCF-7 cells
Fold of Induction
7
6
5
4
3
2
1
0
-3.34 -2.86 -2.39 -1.91 -1.43 -0.95 -0.48 0.00
Log10 mAb concentration (ug/ml)
Herceptin
chA19

Fig. 10

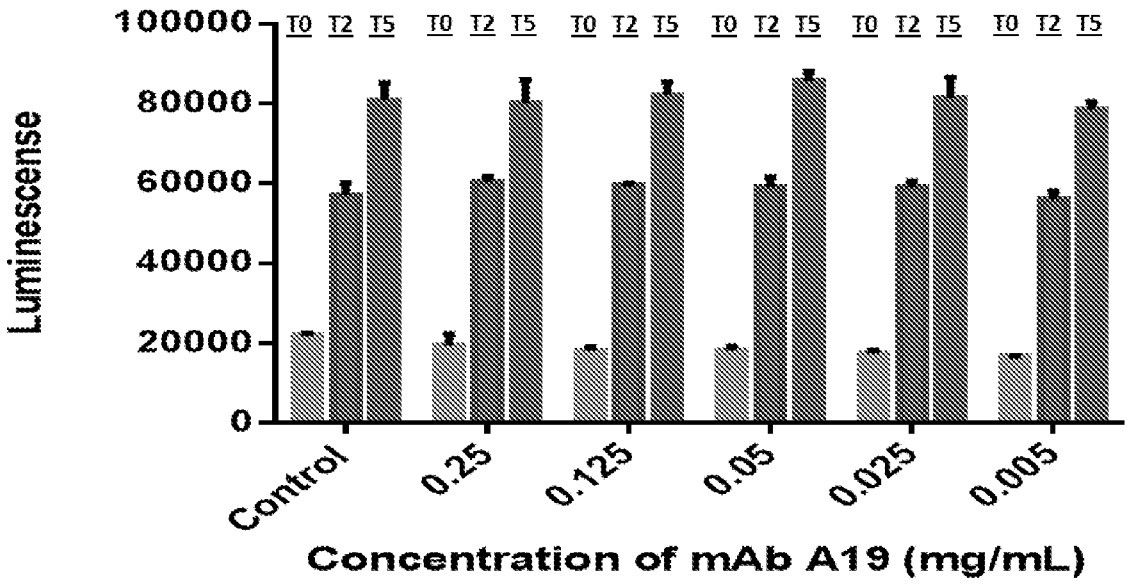

100000
80000
60000
40000
20000
0
Luminescense
T0 T2 T5 T0 T2 T5 T0 T2 T5 T0 T2 T5 T0 T2 T5 T0 T2 T5
Control
0.25
0.125
0.05
0.025
0.005
Concentration of mAb A19 (mg/mL)

Fig. 11

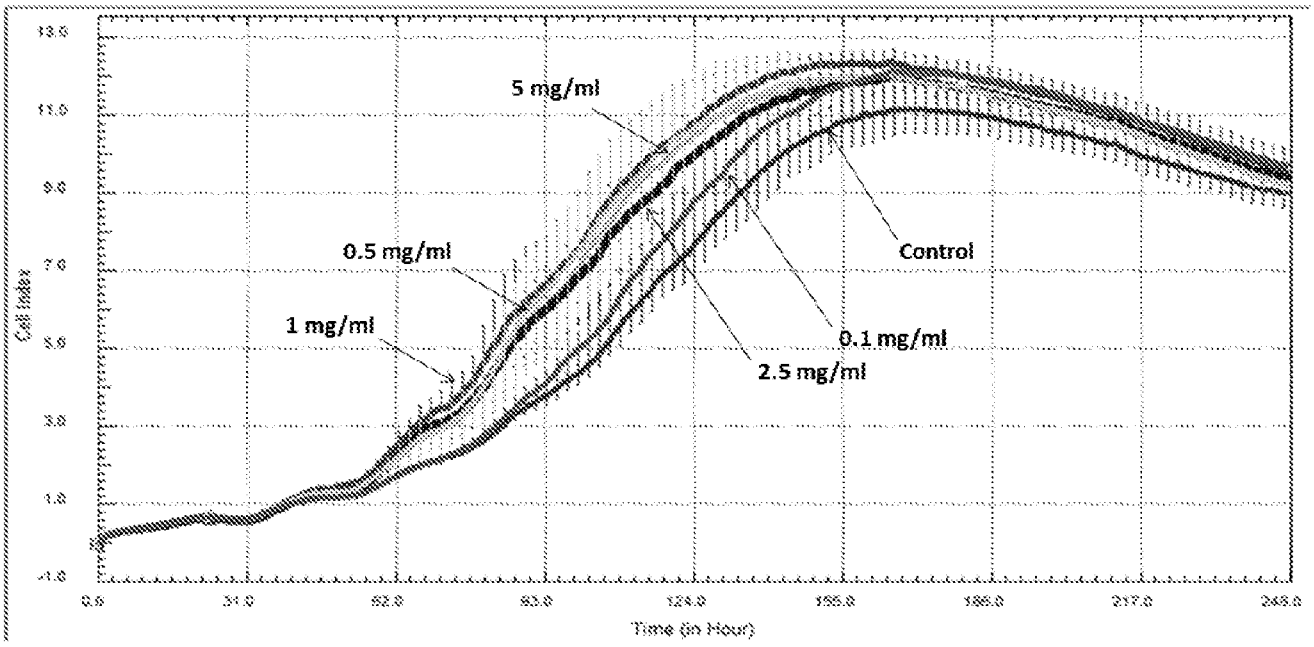

5 mg/ml
0.5 mg/ml
1 mg/ml
Control
0.1 mg/ml
2.5 mg/ml
Cell Index
Time (in Hour)

Fig. 12

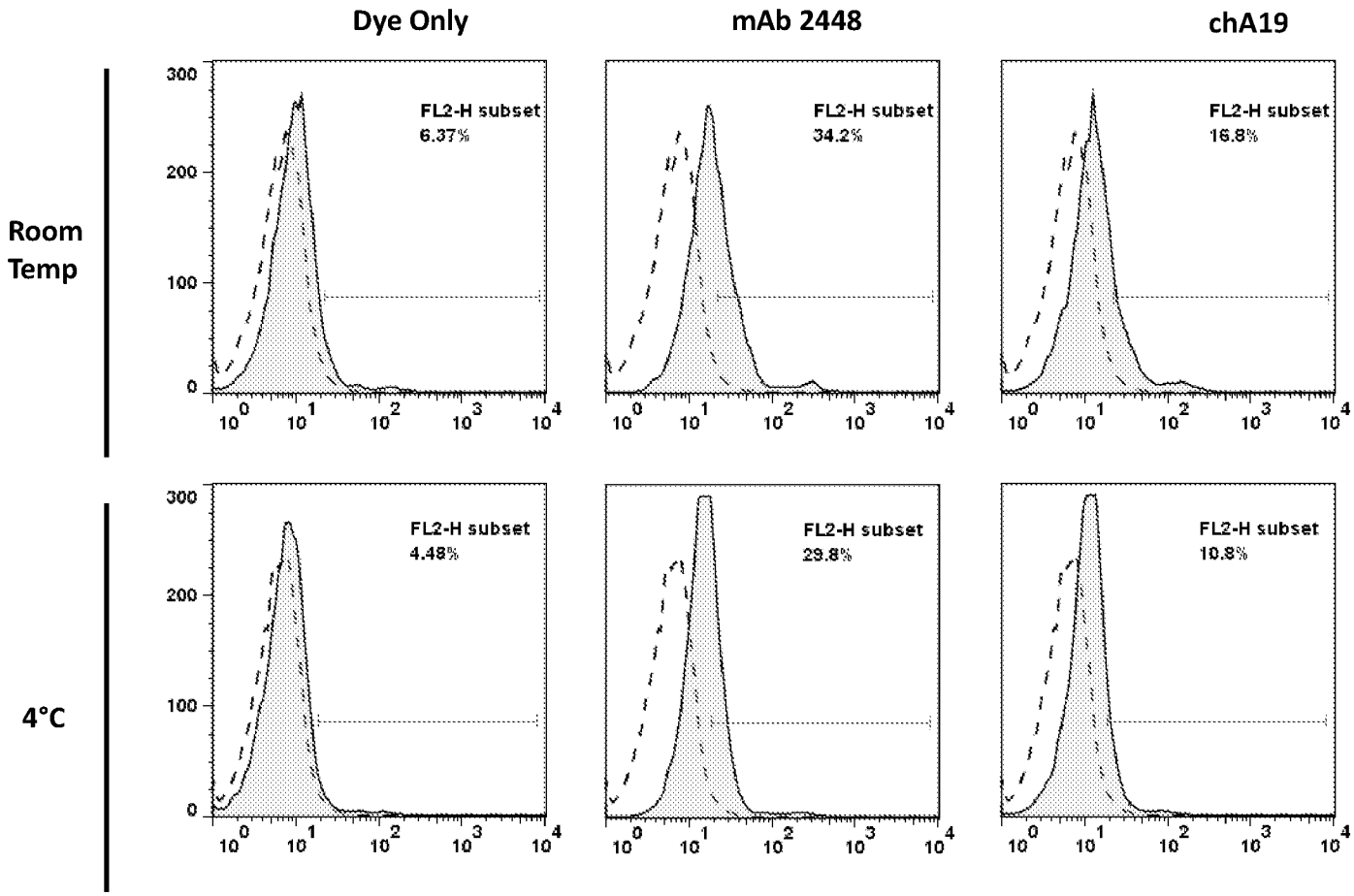

Dye Only
mAb 2448
chA19
Room Temp
4°C
FL2-H subset
6.37%
FL2-H subset
34.2%
FL2-H subset
16.8%
FL2-H subset
4.48%
FL2-H subset
29.8%
FL2-H subset
10.8%
300
200
100
0
10^0 10^1 10^2 10^3 10^4

Fig. 13

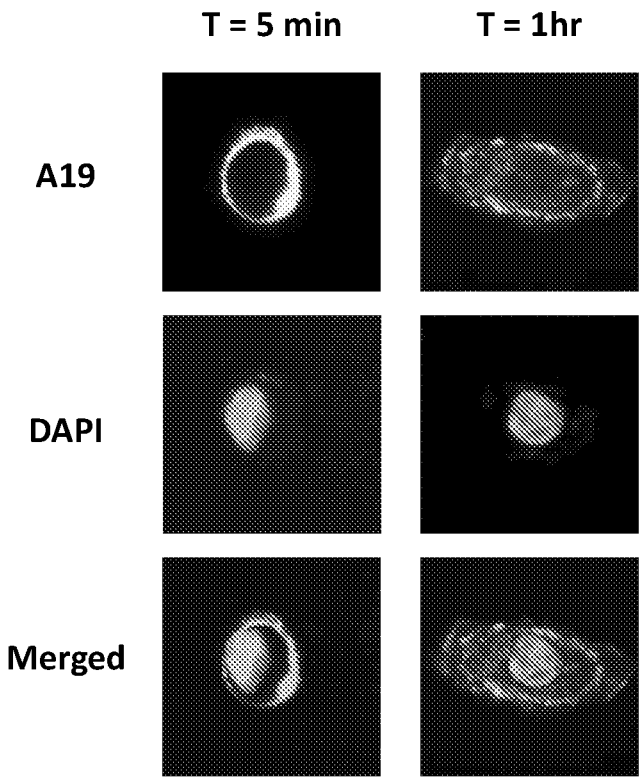
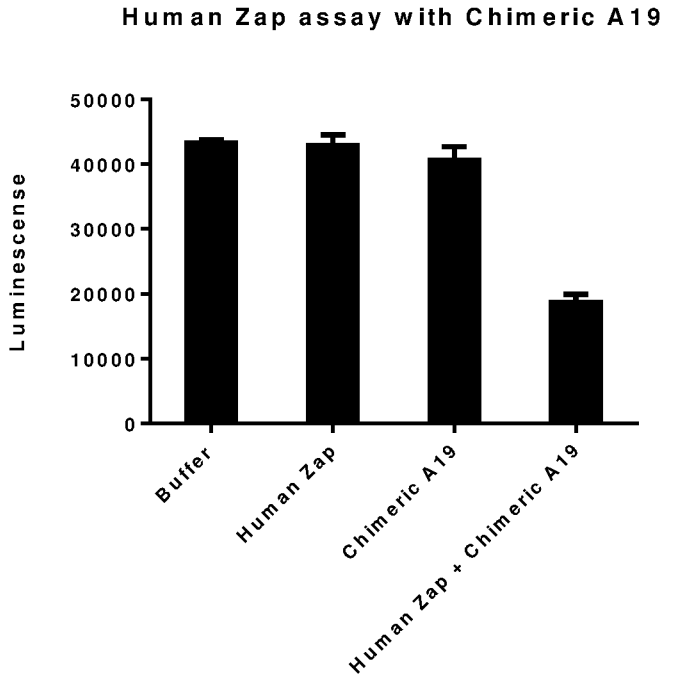


Fig. 14


Human Zap assay with Chimeric A19
Luminescense
50000
40000
30000
20000
10000
0
Buffer
Human Zap
Chimeric A19
Human Zap + Chimeric A19

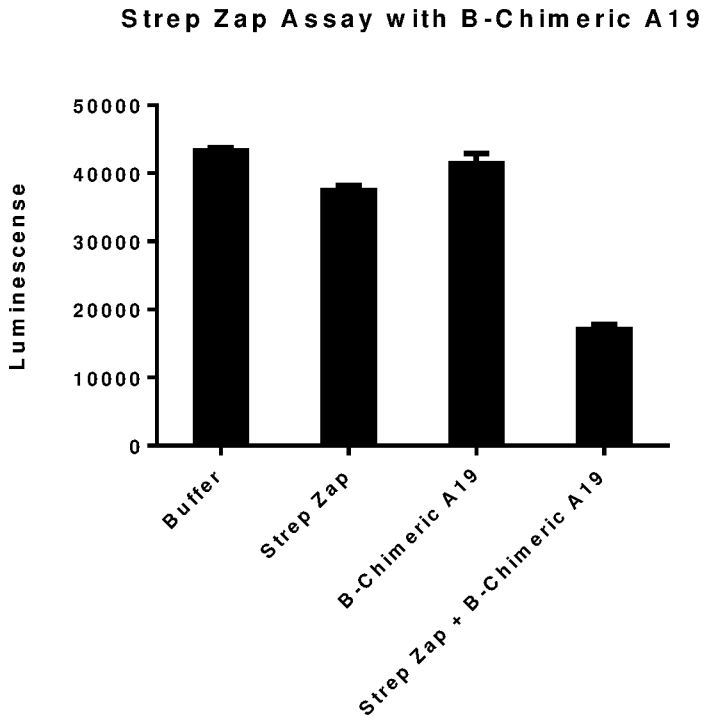

Strep Zap Assay with B-Chimeric A19
Luminescense
50000
40000
30000
20000
10000
0
Buffer
Strep Zap
B-Chimeric A19
Strep Zap + B-Chimeric A19

Fig. 15

mA19 binding to various cancers

SKOV3
(Ovarian Cancer)

SKBR3
(Breast Cancer)

BT474
(Breast Cancer)

CAMA1
(Breast Cancer)

MCF7 (R10)
(Breast Cancer)

COLO205
(Colorectal Cancer)

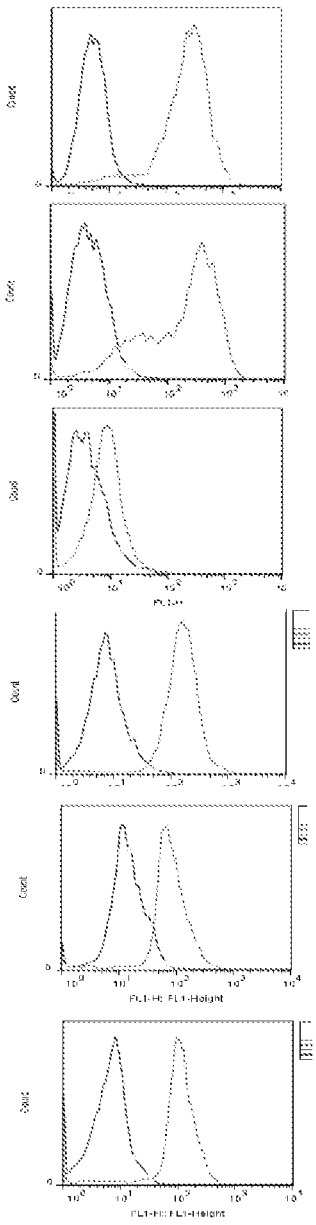


Fig. 15

(cont'd)

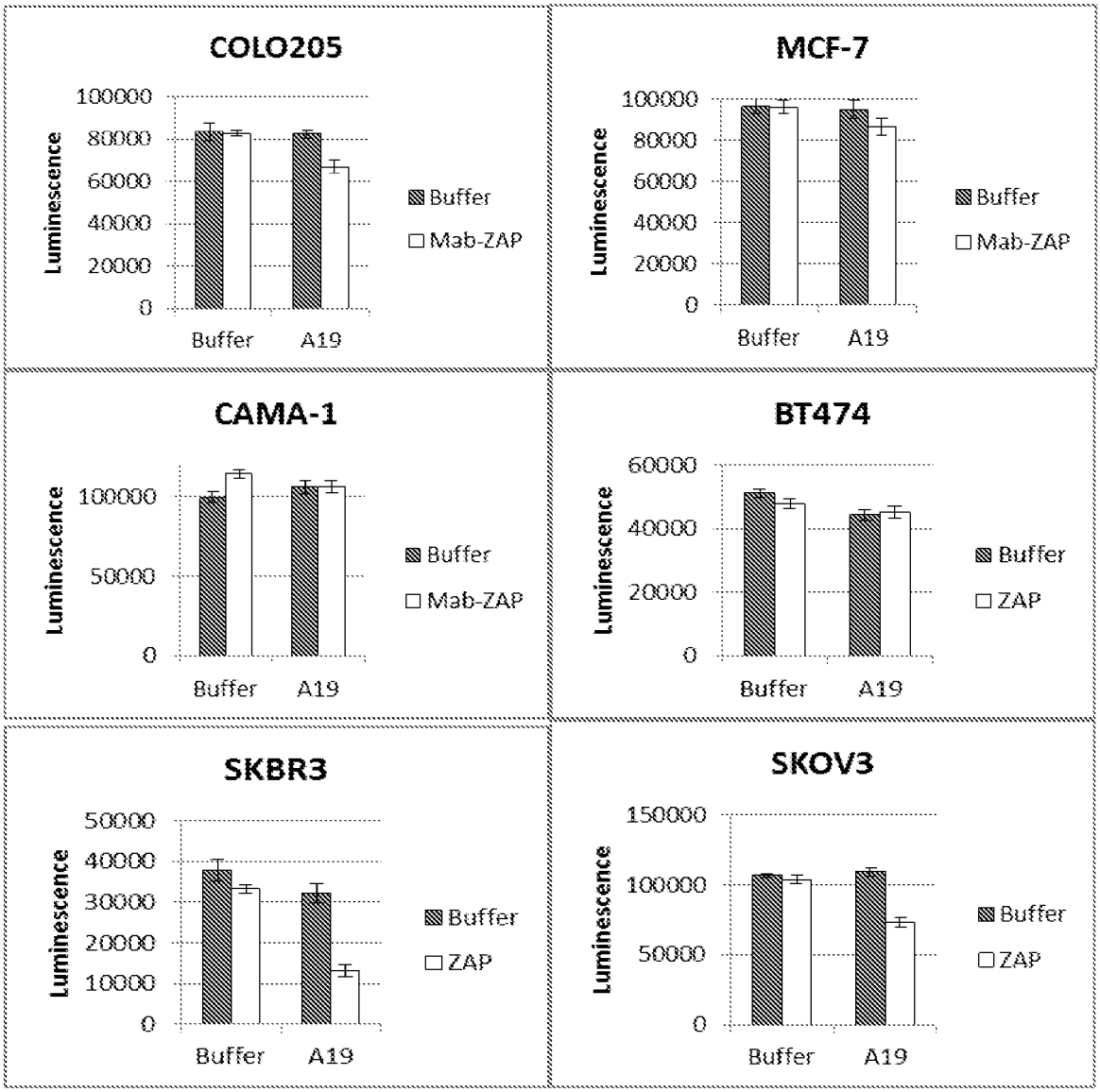

COLO205
Luminescence
100000
80000
60000
40000
20000
0
Buffer
A19
Buffer
Mab-ZAP
MCF-7
Luminescence
100000
80000
60000
40000
20000
0
Buffer
A19
Buffer
Mab-ZAP
CAMA-1
Luminescence
100000
50000
0
Buffer
A19
Buffer
Mab-ZAP
BT474
Luminescence
60000
40000
20000
0
Buffer
A19
Buffer
ZAP
SKBR3
Luminescence
50000
40000
30000
20000
10000
0
Buffer
A19
Buffer
ZAP
SKOV3
Luminescence
150000
100000
50000
0
Buffer
A19
Buffer
ZAP

Fig. 16

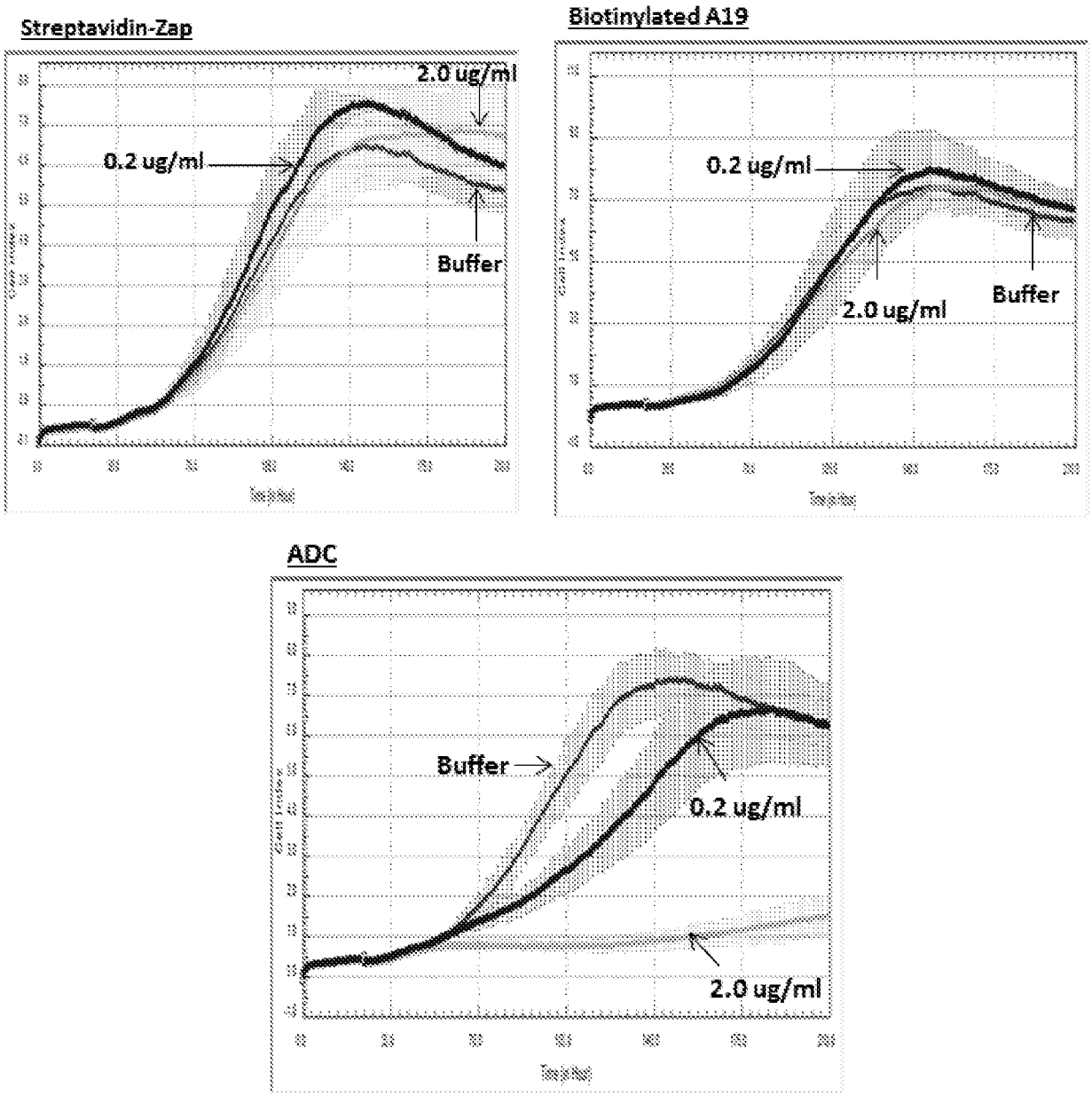

Streptavidin-Zap
2.0 ug/ml
0.2 ug/ml
Buffer
Biotinylated A19
0.2 ug/ml
2.0 ug/ml
Buffer
ADC
Buffer
0.2 ug/ml
2.0 ug/ml

Fig. 17

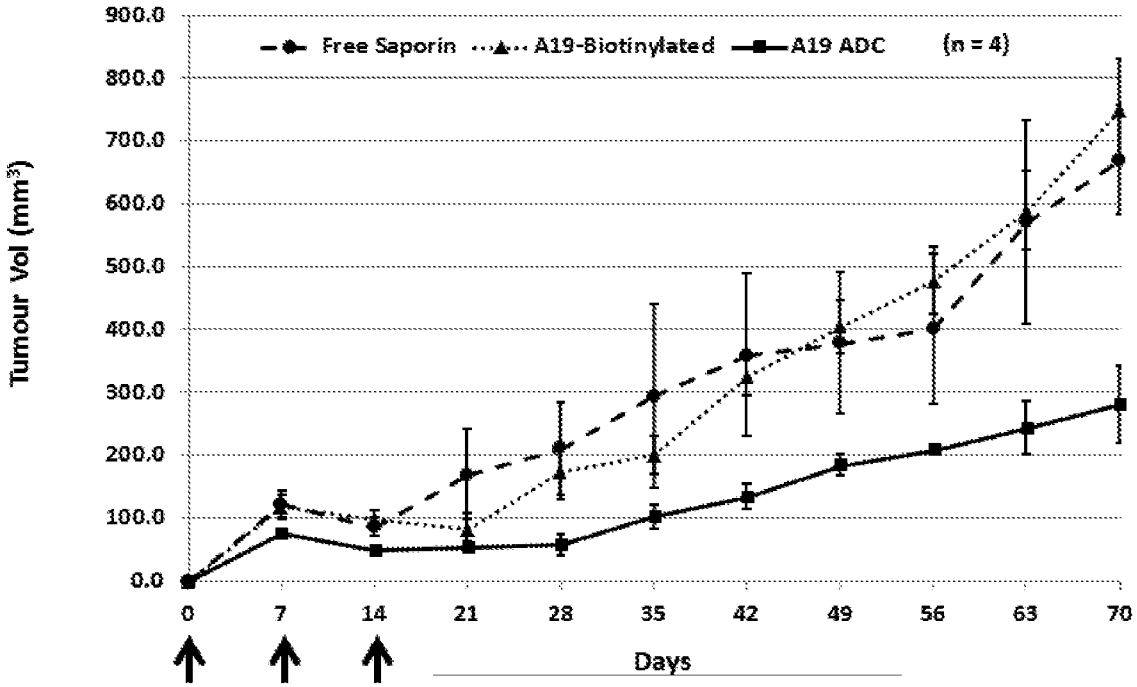

Free Saporin
A19-Biotinylated
A19 ADC
(n = 4)
Tumour Vol (mm3)
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
0
7
14
21
28
35
42
49
56
63
70
Days

Fig. 18

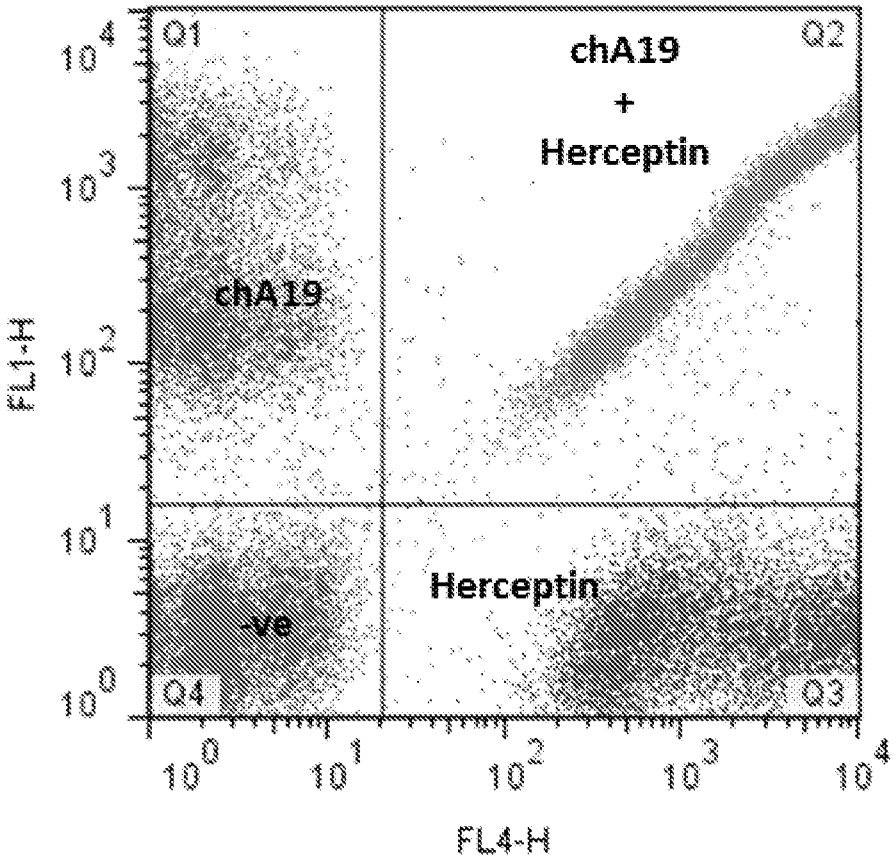
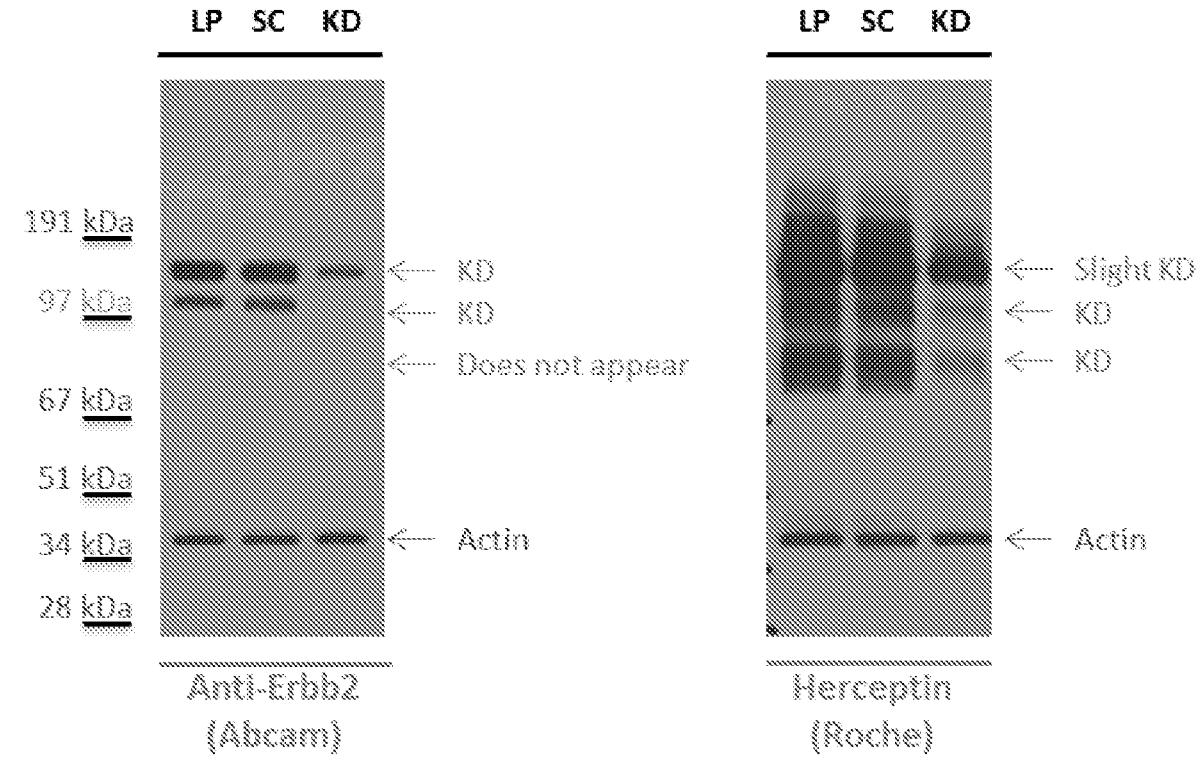
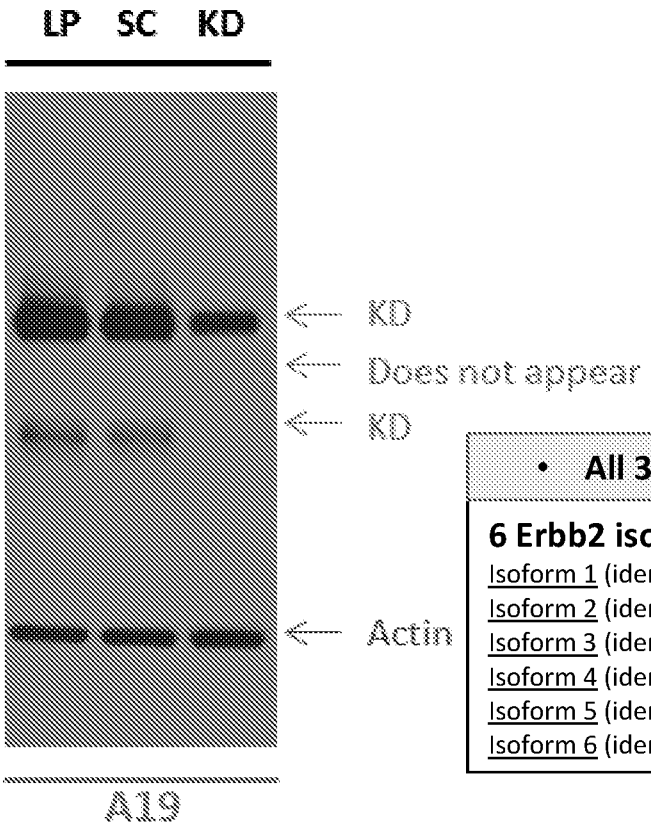

Q1
Q2
chA19
+
Herceptin
chA19
Herceptin
-ve
Q4
Q3
FL1-H
FL4-H
10^0
10^1
10^2
10^3
10^4

Fig. 19





Legend
LP: Lipofectamine
SC: Scramble
KD: Knocked Down

- **All 3 mAbs detect different isoforms**

6 Erbb2 isoforms:

Isoform 1 (identifier: P04626-1) Length:1,255 / Mass (Da):137,910
Isoform 2 (identifier: P04626-2) Length:645 / Mass (Da):70,917
Isoform 3 (identifier: P04626-3) Length:569 / Mass (Da):62,568
Isoform 4 (identifier: P04626-4) Length:1,240 /Mass (Da):136,501
Isoform 5 (identifier: P04626-5) Length:1,225 / Mass (Da):134,856
Isoform 6 (identifier: P04626-6) Length:887 / Mass (Da):97,253

Fig. 20

Variable Gene Sequence

A19 Heavy Chain

Gene Sequence (SEQ ID NO: 9)

CAGGTGAAGCTGCAGGAGTCTGGAGCTGAGCTGATGAAGCCTGGGGCCTCAGTGAAGATATCCTG
CAAGGCTACTGGCTACACATTCAGTAACTACTGGATAGAGTGGGTAAAGCAGAGGCCTGGACATGG
CCTTGAGTGGATTGGAGAGATTTTACCTGGAAGTGATAGTACTAACTACAATGAGAAGTTCAAGGG
CAAGGCCACATTCACTGCAGATACATCCTCCAACACAGCCTACATGCAACTCAGCAGCCTGACATCT
GAGGACTCTGCCGTCTATTACTGTGCAAGAGGAGGGTCGAACTACGGGTACTACTTTGACTACTGG
GGCCAAGGGACCACGGTCACCGTCTCCTCA

A19 Light Chain

Gene Sequence (SEQ ID NO: 10)

GACATTCTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCATCCC
CTGCAAGGCCAGTCAGGATGTGGGTACTGCTGTAGCCTGGTATCAACAAAAACCAGGGCAATCTC
CTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGAGTCCCTGATCGCTTCACAGGCAGT
GGATCTGGGACAGATTTCACTCTCTCCATTAGCAATGTGCAGTCTGAAGACTTGGCAGATTATTTC
TGTCAGCAATATAGCAGCTATCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGG

Protein Sequence

A19 Heavy Chain

Protein Sequence (SEQ ID NO: 1)

QVKLQESGAELMKPGASVKISCKATGYTFSNYWIEWVKQRPGHGLEWIGEILPGSDSTNYNEKF
KGKATFTADTSSNTAYMQLSSLTSEDSAVYYCARGGSNYGYYFDYWGQGTTVTVSS

A19 Light Chain

Protein Sequence (SEQ ID NO: 2)

DILMTQSHKFMSTSVGDRVSIPCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSG
TDFTLSISNVQSEDLADYFCQQYSSYRTFGGGTKLEIKR