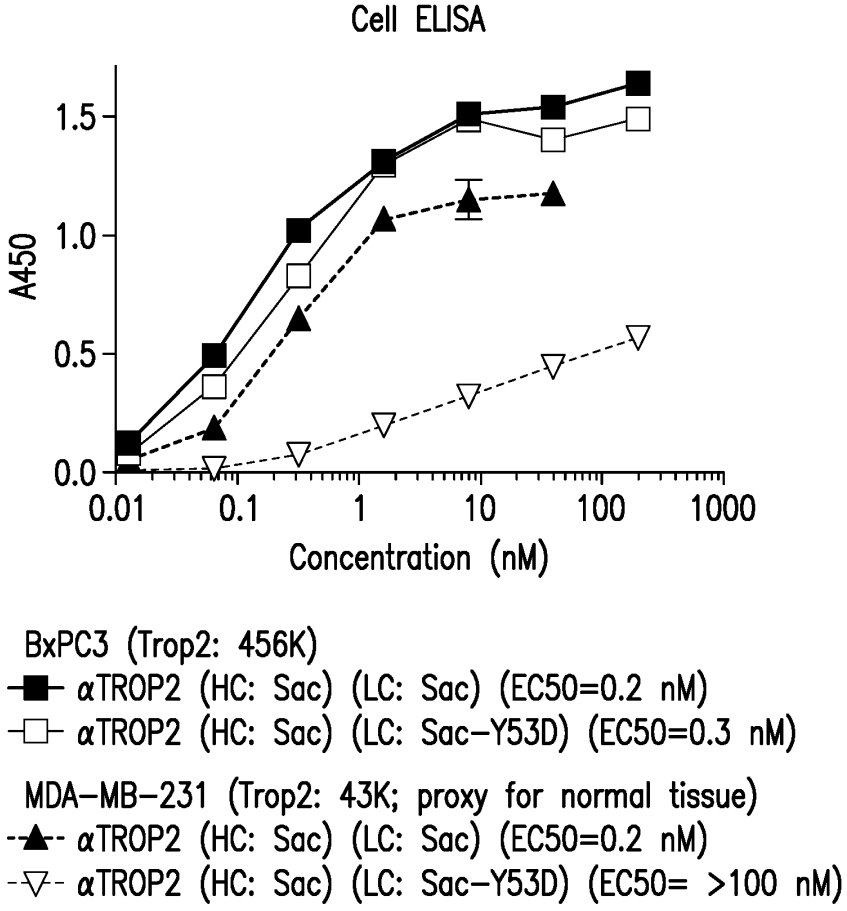
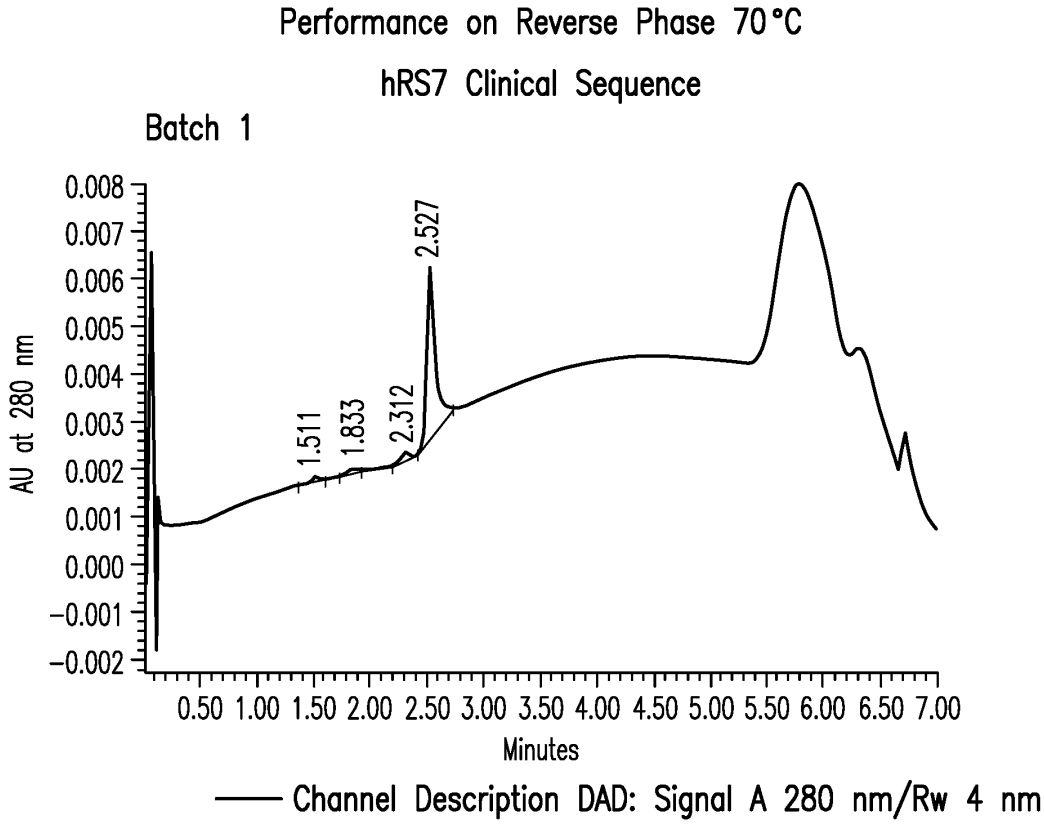

Cell ELISA
A450
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
Concentration (nM)
BxPC3 (Trop2: 456K)
αTROP2 (HC: Sac) (LC: Sac) (EC50=0.2 nM)
αTROP2 (HC: Sac) (LC: Sac-Y53D) (EC50=0.3 nM)
MDA-MB-231 (Trop2: 43K; proxy for normal tissue)
αTROP2 (HC: Sac) (LC: Sac) (EC50=0.2 nM)
αTROP2 (HC: Sac) (LC: Sac-Y53D) (EC50= >100 nM)

FIG. 1


Performance on Reverse Phase 70°C
hRS7 Clinical Sequence
Batch 1
AU at 280 nm
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
−0.001
−0.002
1.511
1.833
2.312
2.527
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00
Minutes
Channel Description DAD: Signal A 280 nm/Rw 4 nm

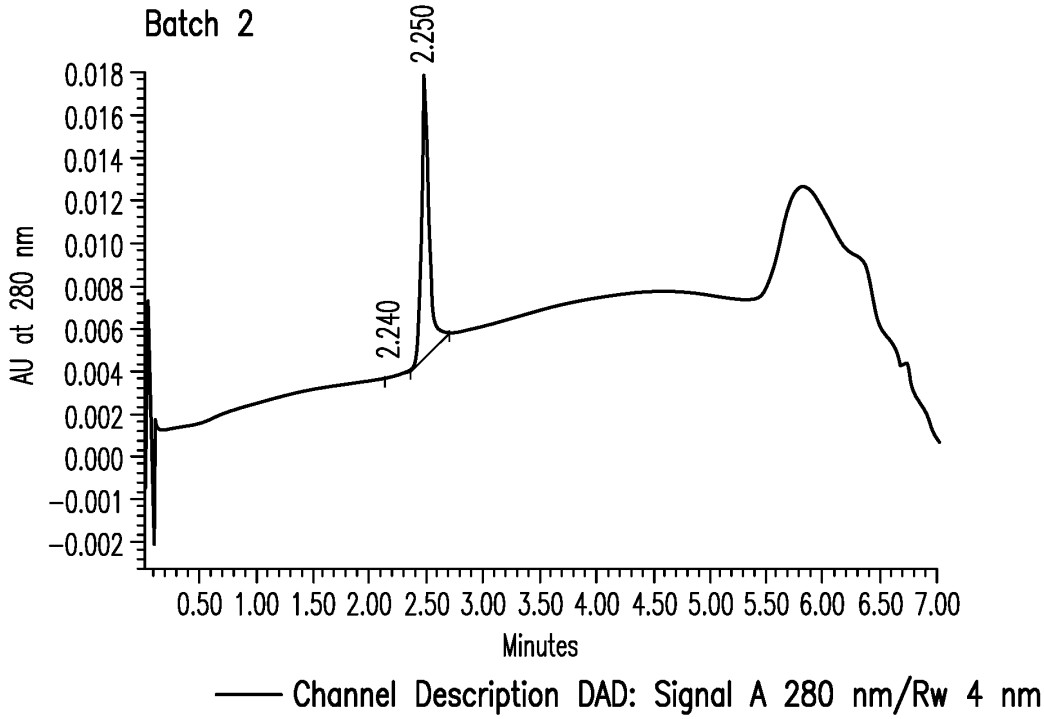

Batch 2
AU at 280 nm
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
−0.001
−0.002
2.240
2.250
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00
Minutes
Channel Description DAD: Signal A 280 nm/Rw 4 nm

FIG.2

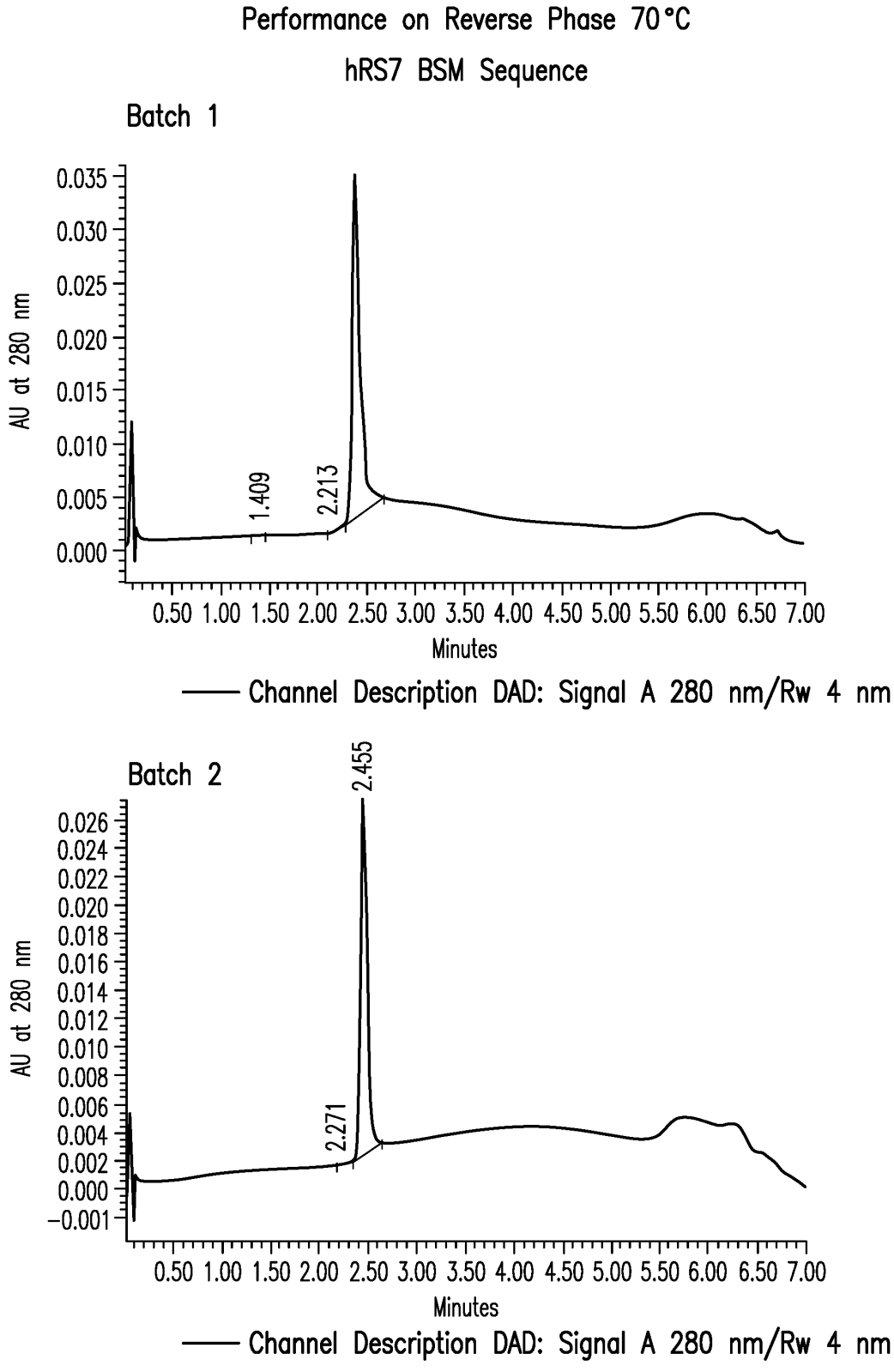

Performance on Reverse Phase 70°C
hRS7 BSM Sequence
Batch 1
AU at 280 nm
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1.409
2.213
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00
Minutes
Channel Description DAD: Signal A 280 nm/Rw 4 nm
Batch 2
2.455
2.271
AU at 280 nm
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
−0.001
0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00
Minutes
Channel Description DAD: Signal A 280 nm/Rw 4 nm

FIG.2-1

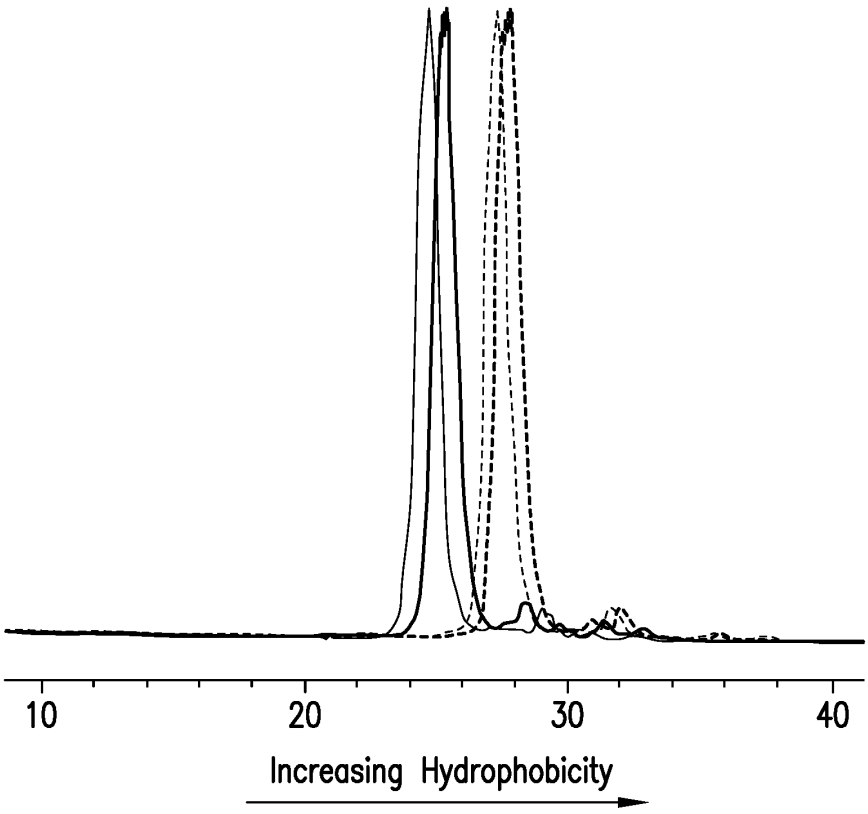

10
20
30
40
Increasing Hydrophobicity
αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-???????)
αTROP2 (HC: BSM-S375C) (LC: BSM-Y105S)

FIG.3

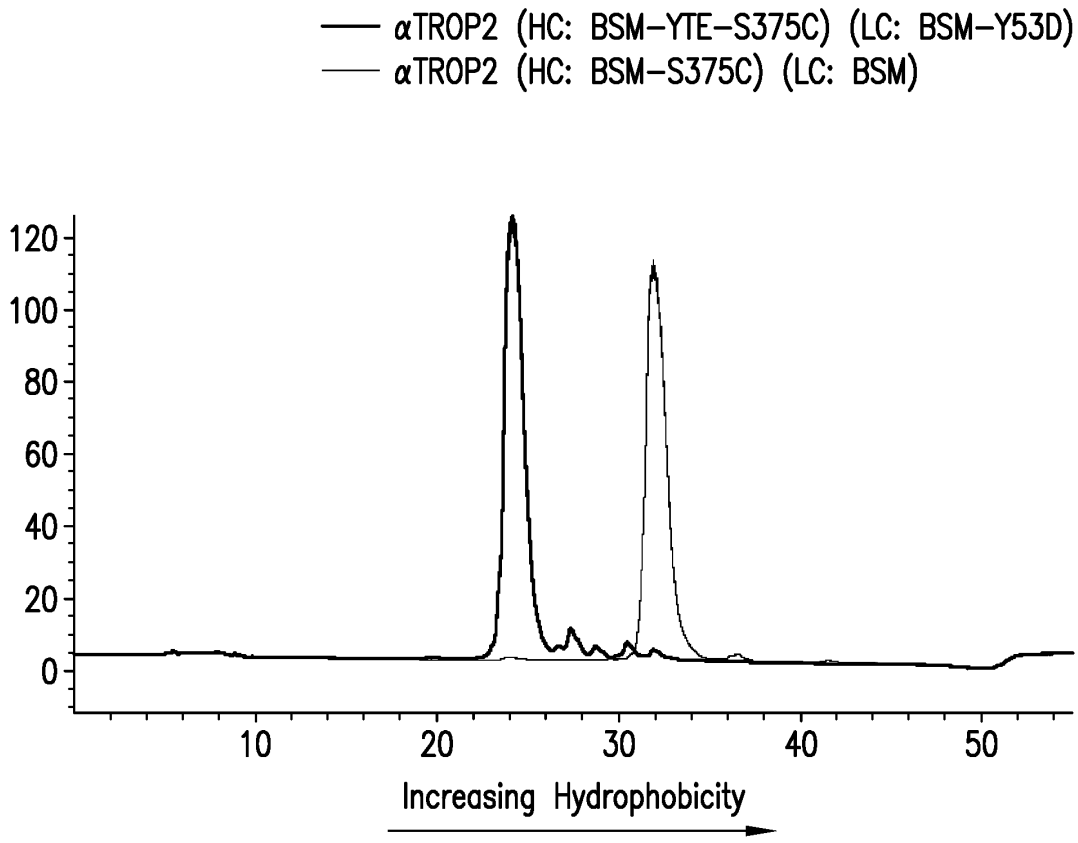

αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-S375C) (LC: BSM)
120
100
80
60
40
20
0
10
20
30
40
50
Increasing Hydrophobicity

FIG.4

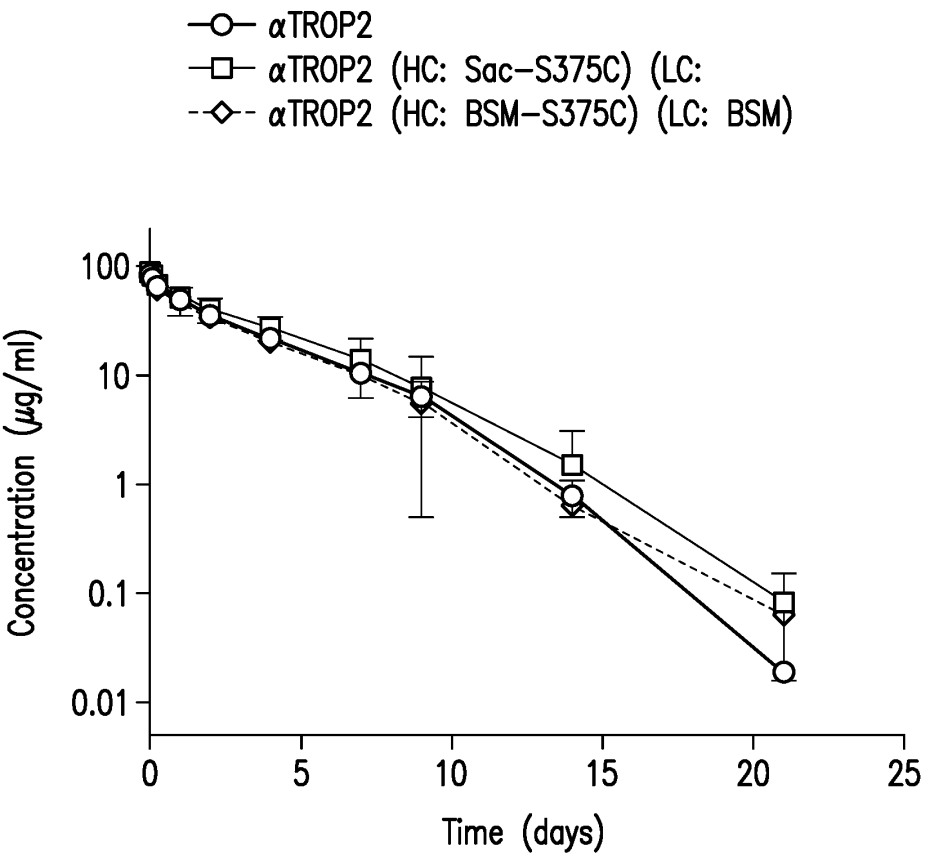

αTROP2
αTROP2 (HC: Sac-S375C) (LC:
αTROP2 (HC: BSM-S375C) (LC: BSM)
Concentration (μg/ml)
100
10
1
0.1
0.01
0
5
10
15
20
25
Time (days)

FIG.5

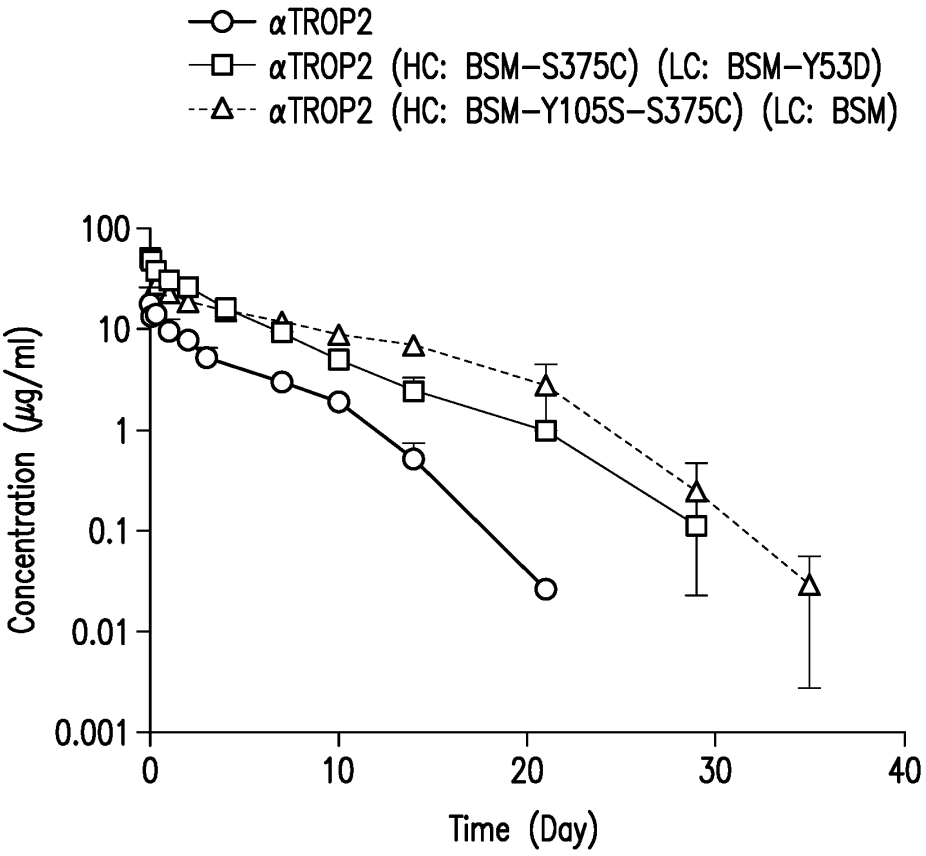

αTROP2
αTROP2 (HC: BSM-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-Y105S-S375C) (LC: BSM)
Concentration (μg/ml)
100
10
1
0.1
0.01
0.001
0
10
20
30
40
Time (Day)

FIG.6

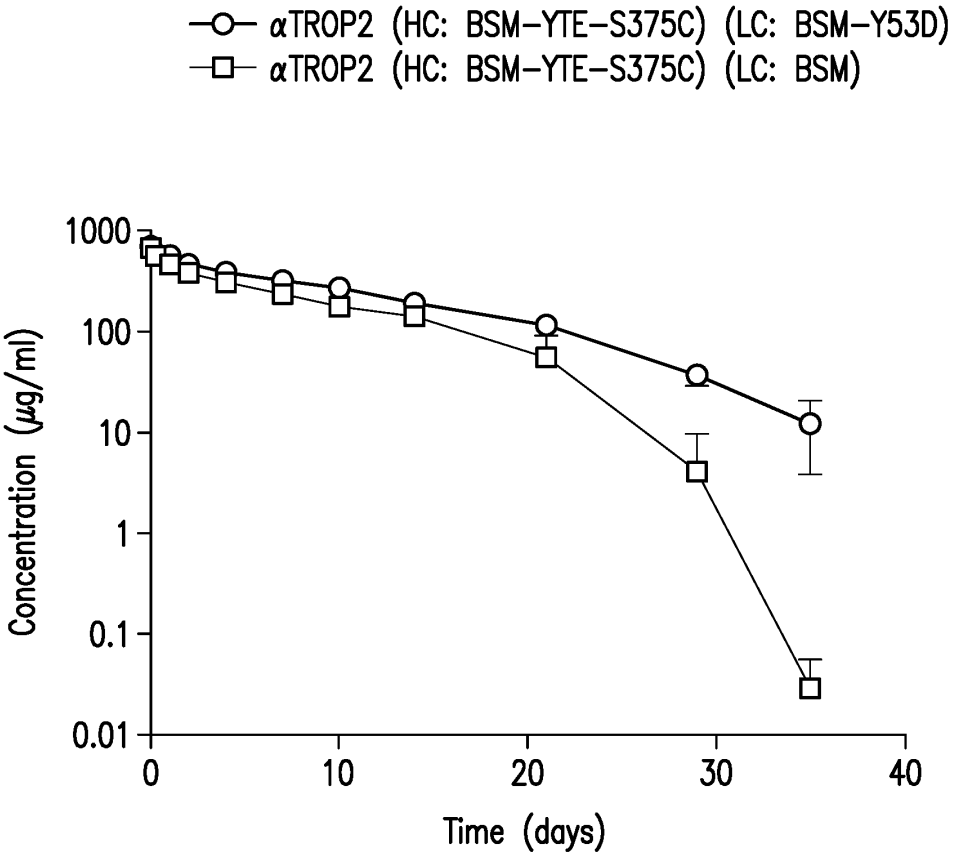

αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-YTE-S375C) (LC: BSM)
Concentration (μg/ml)
1000
100
10
1
0.1
0.01
0
10
20
30
40
Time (days)

FIG.7

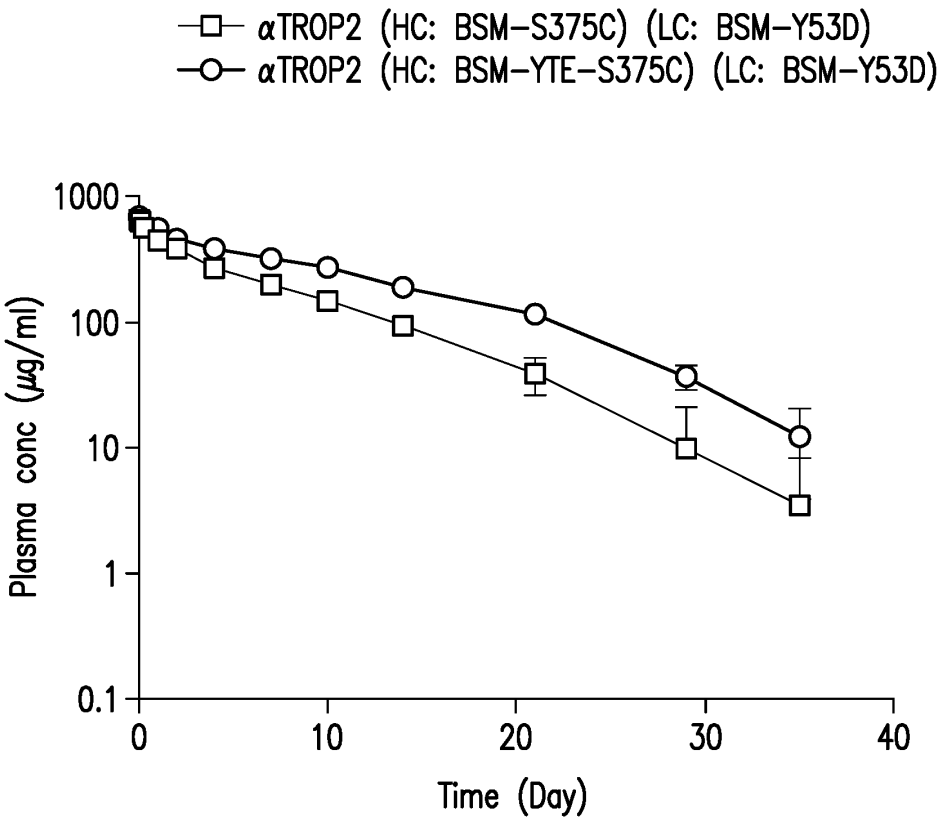

αTROP2 (HC: BSM-S375C) (LC: BSM-Y53D)
αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-Y53D)
Plasma conc (μg/ml)
1000
100
10
1
0.1
0
10
20
30
40
Time (Day)

FIG.8

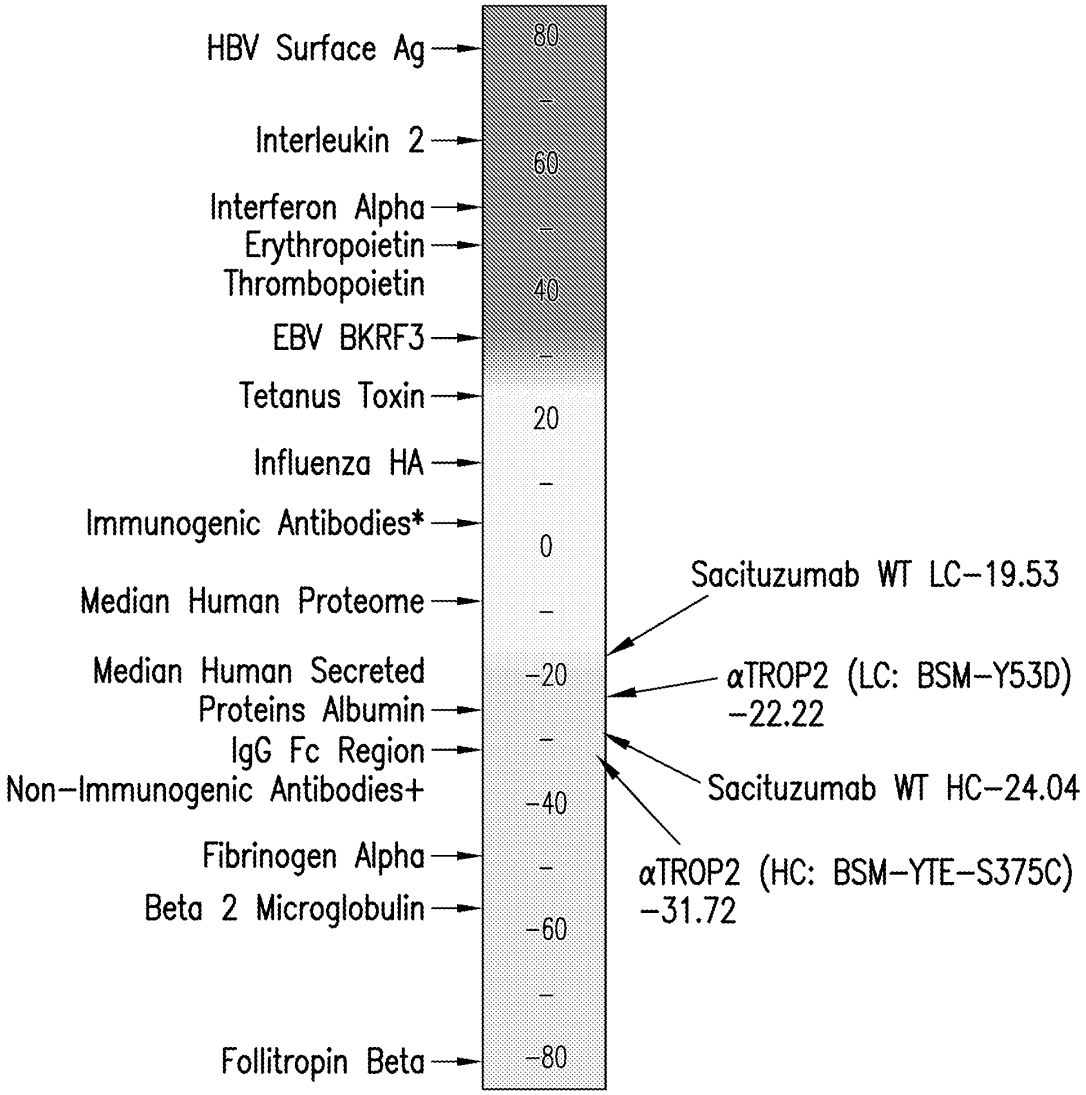

HBV Surface Ag
Interleukin 2
Interferon Alpha
Erythropoietin
Thrombopoietin
EBV BKRF3
Tetanus Toxin
Influenza HA
Immunogenic Antibodies*
Median Human Proteome
Median Human Secreted Proteins Albumin
IgG Fc Region
Non-Immunogenic Antibodies+
Fibrinogen Alpha
Beta 2 Microglobulin
Follitropin Beta
80
60
40
20
0
-20
-40
-60
-80
Sacituzumab WT LC-19.53
αTROP2 (LC: BSM-Y53D) -22.22
Sacituzumab WT HC-24.04
αTROP2 (HC: BSM-YTE-S375C) -31.72

FIG.9

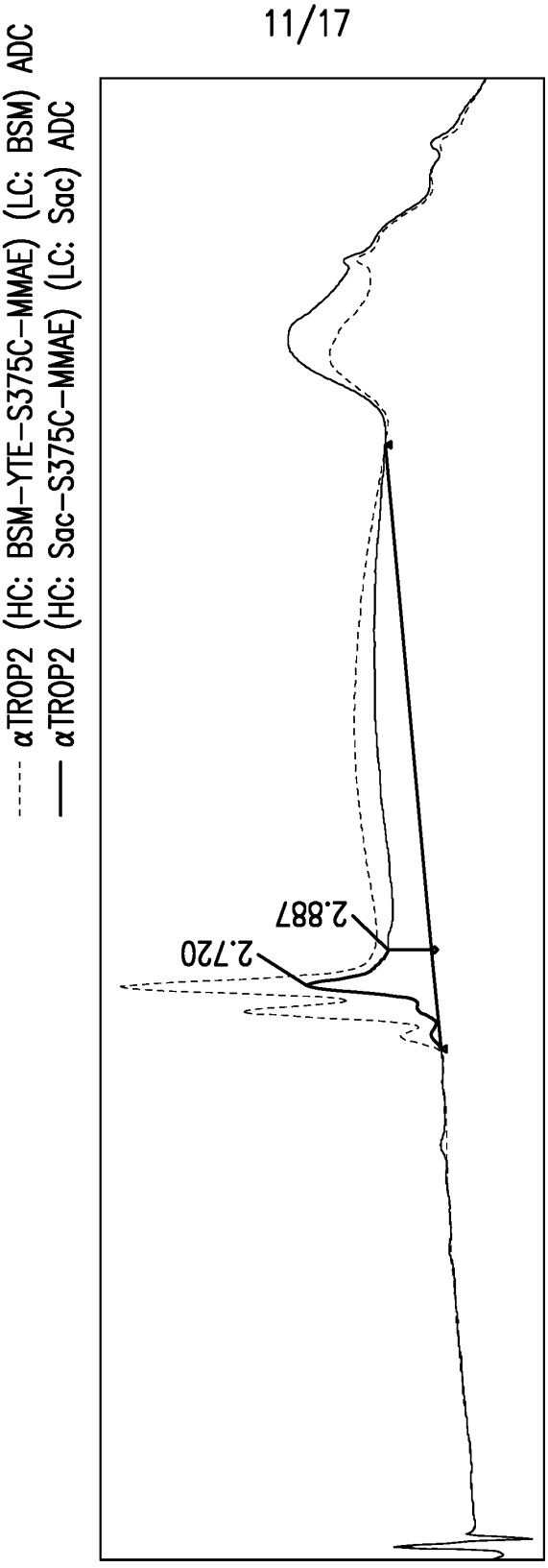

αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM) ADC
αTROP2 (HC: Sac-S375C-MMAE) (LC: Sac) ADC
2.887
2.720

FIG.10

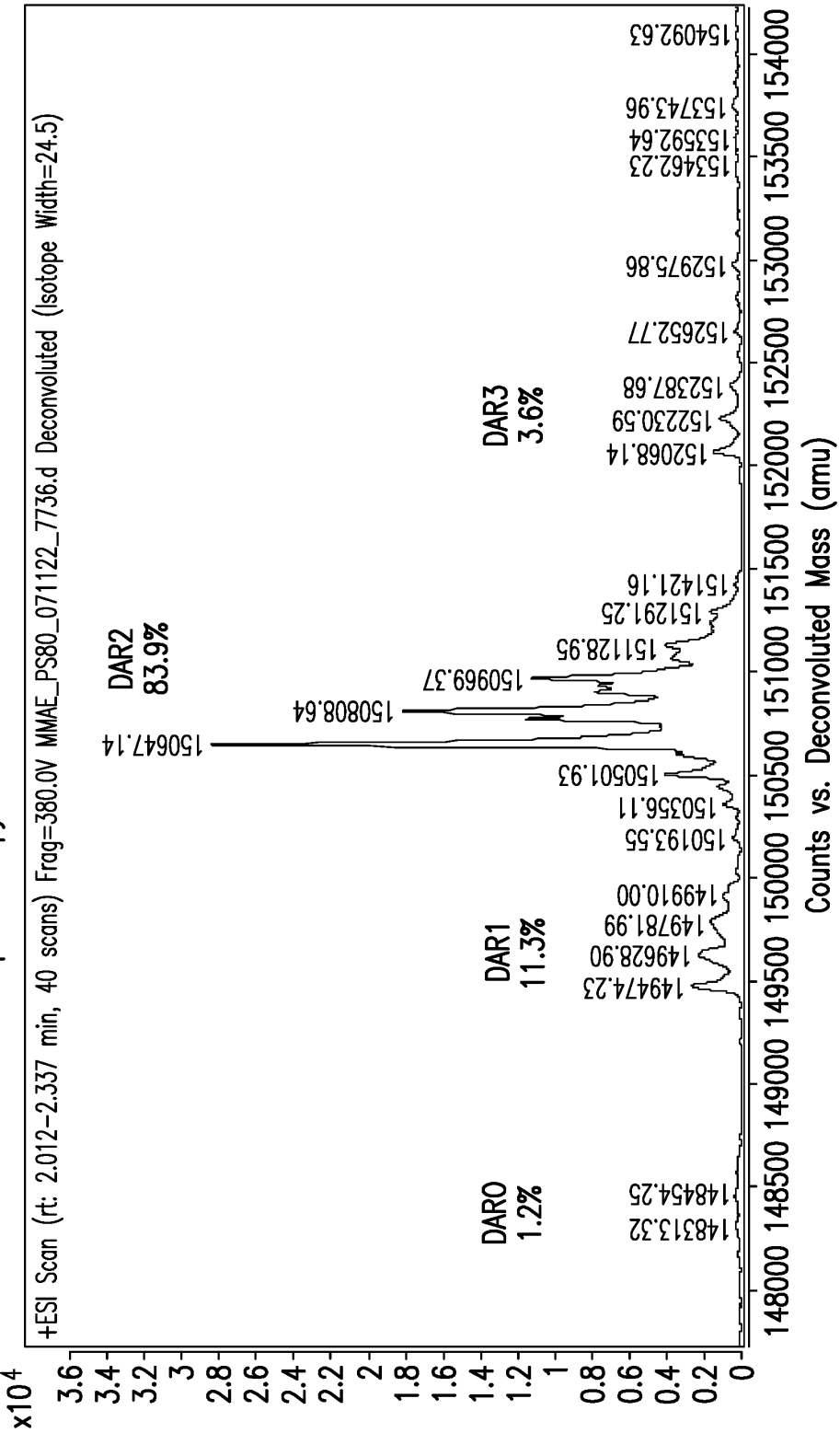

Mass Spectroscopy Profile of αTROP2-MP-AA-PABC-MMAE
+ESI Scan (rt: 2.012-2.337 min, 40 scans) Frag=380.0V MMAE_PS80_071122_7736.d Deconvoluted (Isotope Width=24.5)
x10^4
Counts vs. Deconvoluted Mass (amu)
DAR0 1.2%
DAR1 11.3%
DAR2 83.9%
DAR3 3.6%
148313.32
148454.25
149474.23
149628.90
149781.99
149910.00
150193.55
150356.11
150501.93
150647.14
150808.64
150969.37
151128.95
151291.25
151421.16
152068.14
152230.59
152387.68
152652.77
152975.86
153462.23
153592.64
153743.96
154092.63

FIG. 11

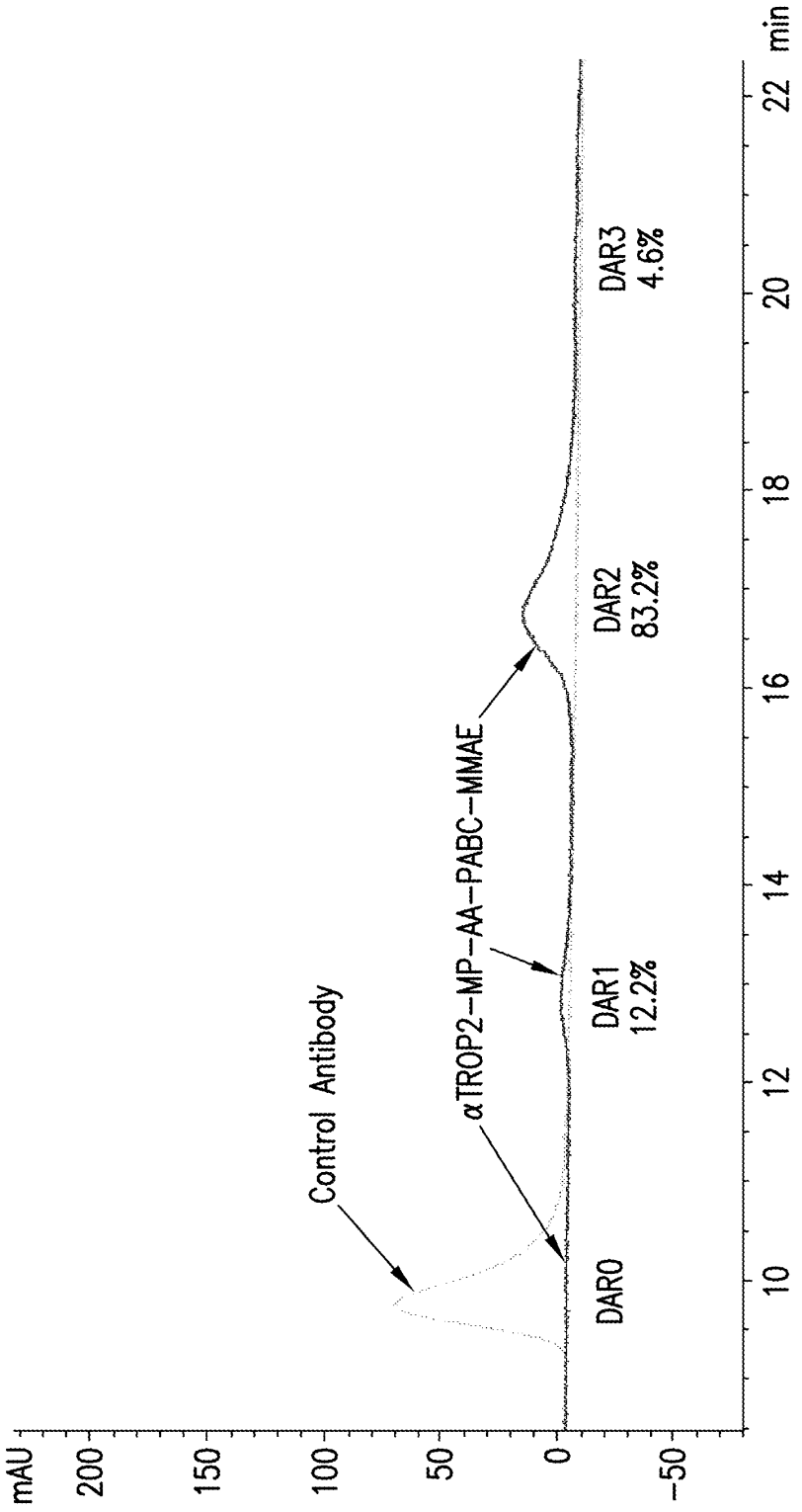
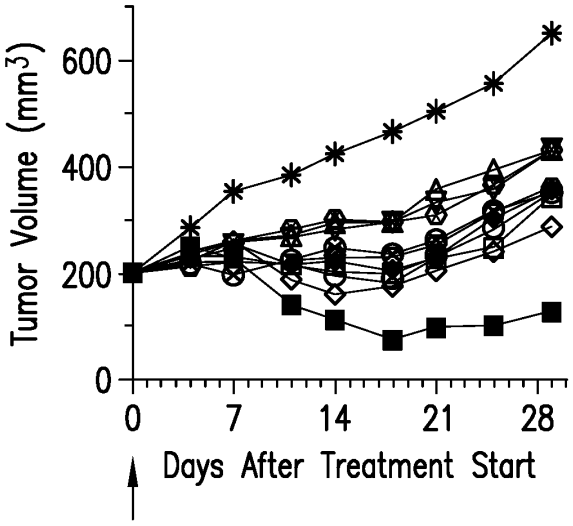
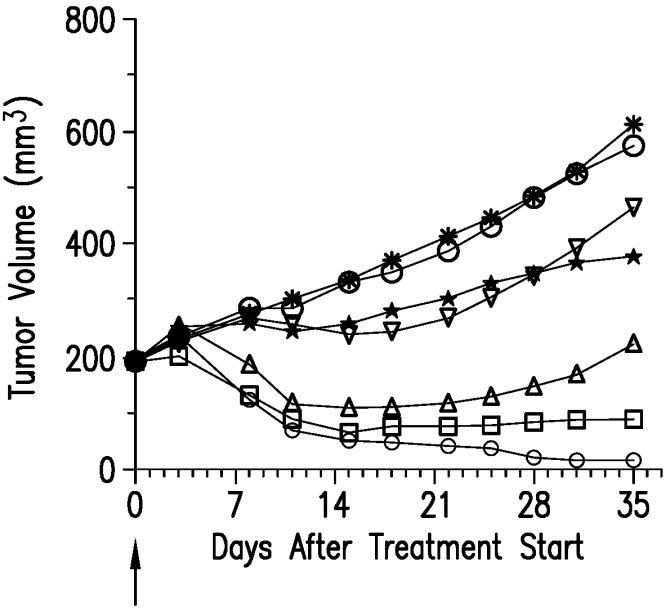

HIC Profile of αTROP2-MP-AA-PABC-MMAE
mAU
200
150
100
50
0
-50
10
12
14
16
18
20
22
min
Control Antibody
αTROP2-MP-AA-PABC-MMAE
DAR0
DAR1
12.2%
DAR2
83.2%
DAR3
4.6%

FIG.12



- PBS
- αTROP2 (HC: S375C–MMAE)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM–S30R)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM–I39K)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM–Y53D)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM–Y53N)
- αTROP2 (HC: BSM–YTE–S375C–MMAE) (LC: BSM–Y92N)
- αTROP2 (HC: BSM–YTE–S104D–S375C–MMAE) (LC: BSM)
- αTROP2 (HC: BSM–YTE–F101E–S375C–MMAE) (LC: BSM)
- αTROP2 (HC: BSM–YTE–Y105S–S375C–MMAE) (LC: BSM)

FIG.13



- PBS
- Control MAb (HC: S375C-MMAE) 10 mpk
- αTROP2 (HC: BSM-YTE-S375C) (LC: BSM-Y53D) 10 mpk
- αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM-Y53D) 1.25 mpk
- αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM-Y53D) 2.5 mpk
- αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM-Y53D) 5 mpk
- αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM-Y53D) 10 mpk

FIG.14

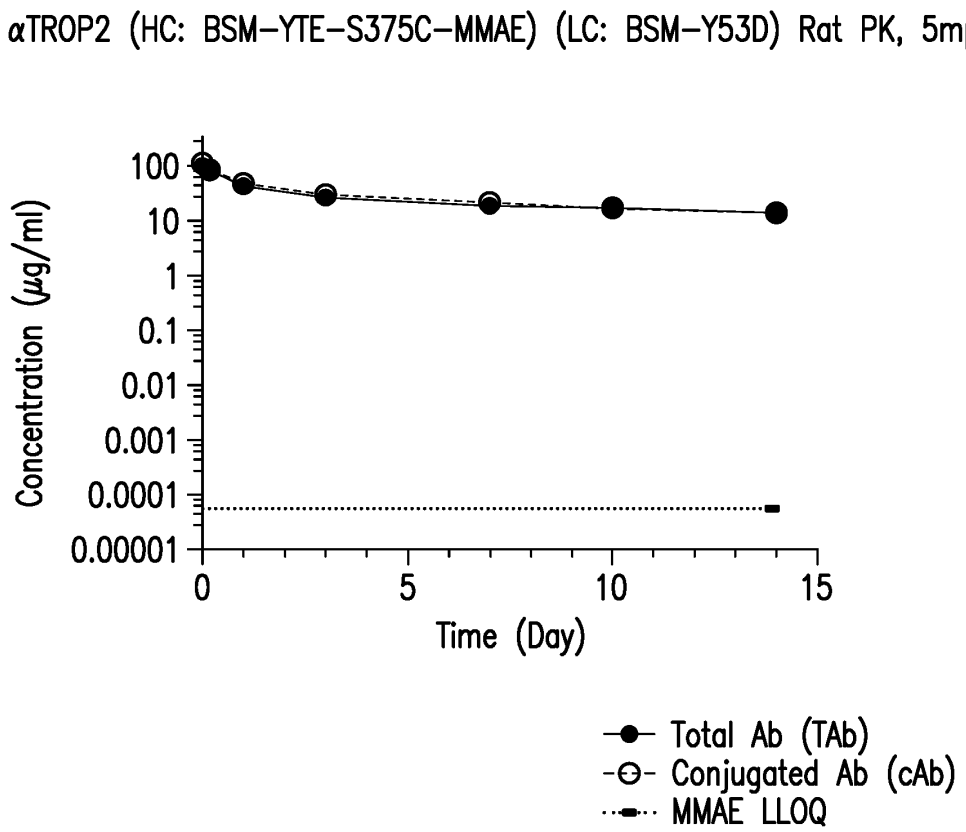

αTROP2 (HC: BSM-YTE-S375C-MMAE) (LC: BSM-Y53D) Rat PK, 5mpk
Concentration (μg/ml)
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0
5
10
15
Time (Day)
Total Ab (TAb)
Conjugated Ab (cAb)
MMAE LLOQ

FIG.15

V_H (SEQ ID NO: 14) amino acid positions defined according to Kabat

```
         10          20          30          40          50  a       60
70
QVQLLQSGSE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLKWMGW INTYTGEPTYT DDFKGRFSFS
         80  abc     90        100abcd       110
LDTSVSQAYL QISSLKAEDTAVY YCARGGFGSS YWYFDVWGQGTLVT VSS
```

V_H (SEQ ID NO: 14) amino acid positions defined according to sequential numbering

```
         10          20          30          40          50          60          70
QVQLLQSGSE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLKWMGW INTYTGEPTY TDDFKGRFSF
         80          90         100         110         120
SLDTSVSQAY LQISSLKAED TAVYYCARGG FGSSYWYFDV WGQGTLVTVS S
```

V_L (SEQ ID NO: 16) amino acid positions defined according to sequential numbering

```
         10          20          30          40          50          60          70
DIQLTQSPSS LSASVGDRVT ITCKASQDVS IAVAWYQQKP GKAPKLLIYS ASDRYTGVPS RFSGSGSGTD
         80          90         100     107
FTLTISSLQP EDFATYYCQQ HYITPLTFGP GTKVEIK
```

FIG.16