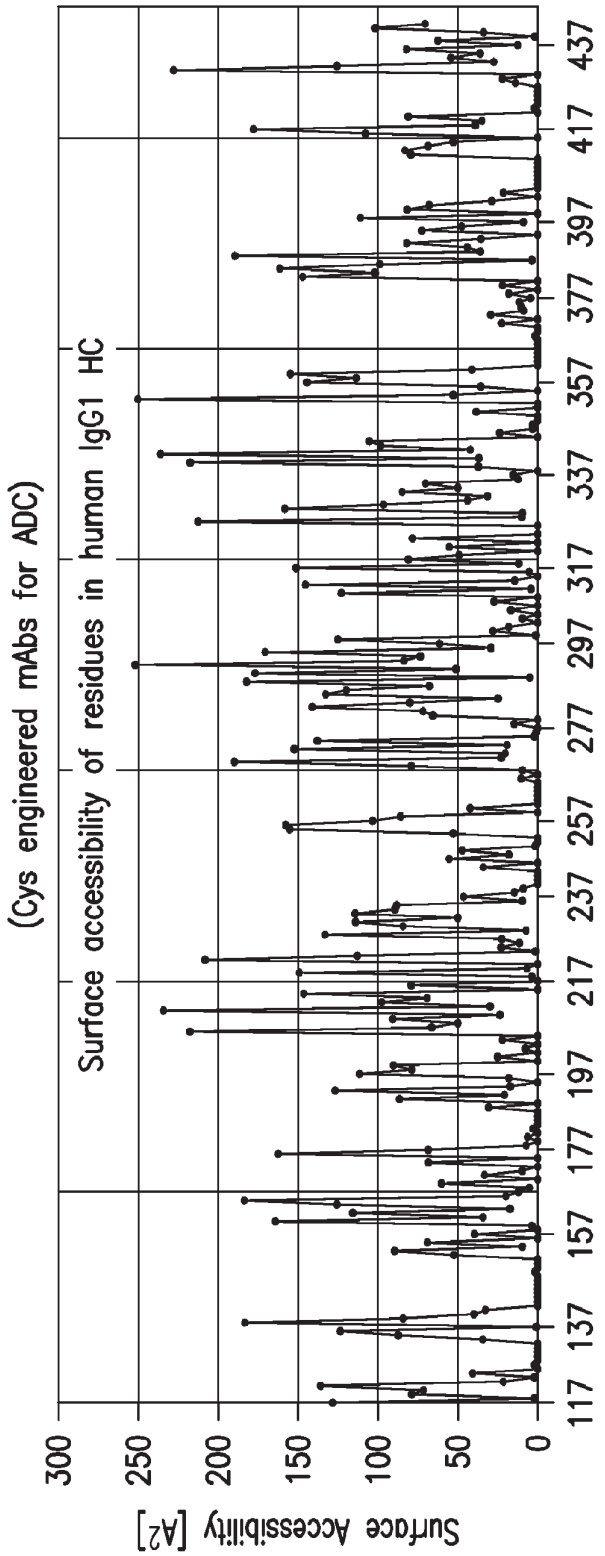

(Cys engineered mAbs for ADC)
Surface accessibility of residues in human IgG1 HC
Surface Accessibility [A2]
300
250
200
150
100
50
0
117 137 157 177 197 217 237 257 277 297 317 337 357 377 397 417 437
Eu Number

FIG.1A

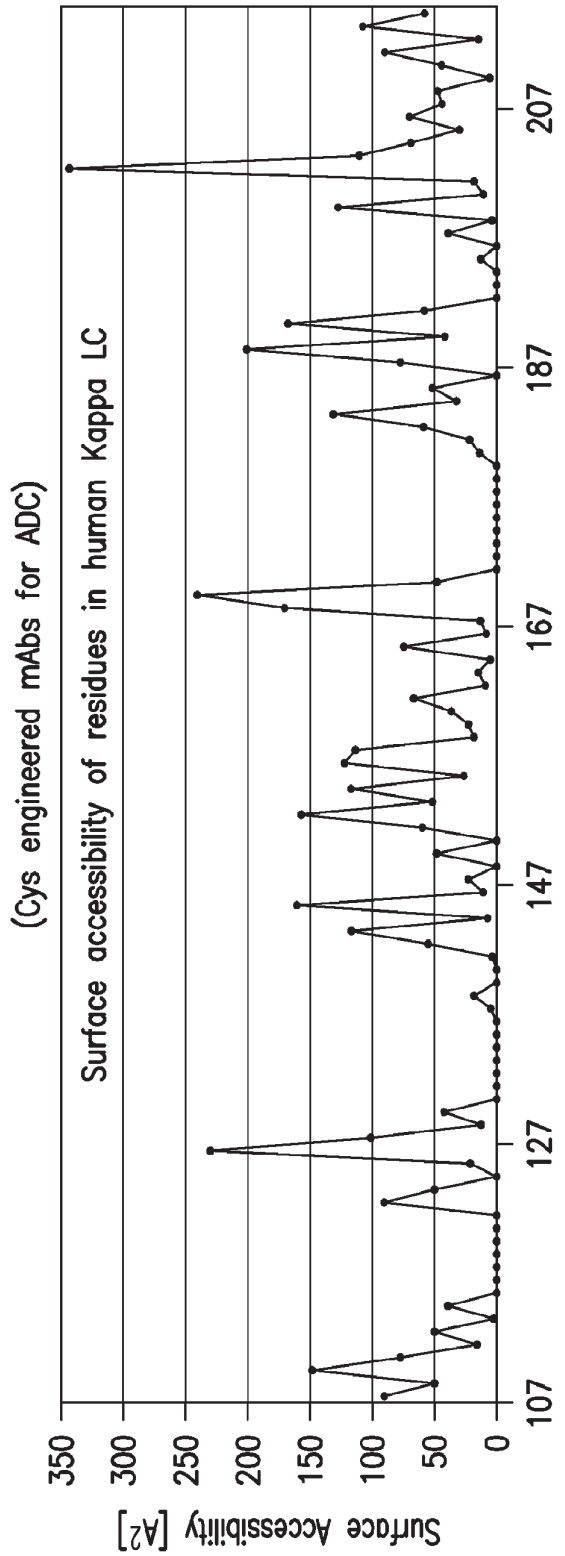

(Cys engineered mAbs for ADC)
Surface accessibility of residues in human Kappa LC
Surface Accessibility [A²]
350
300
250
200
150
100
50
0
107
127
147
167
187
207
Eu Number

FIG. 1B

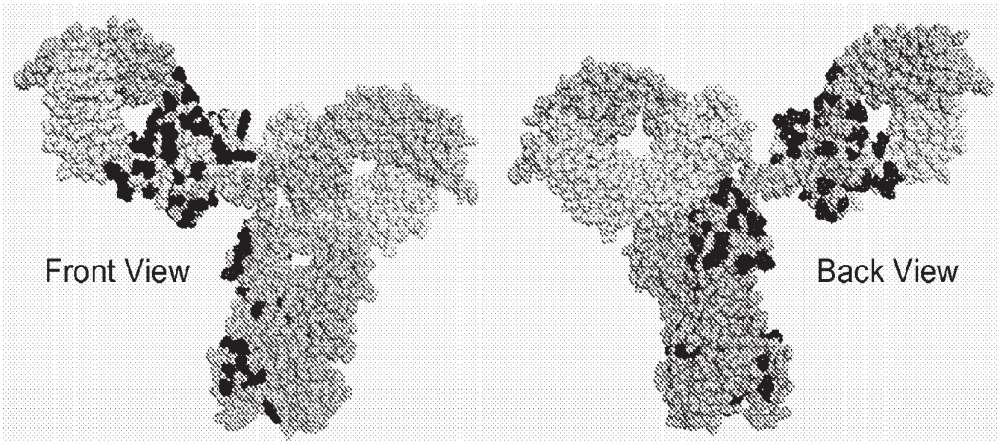


FIG.2

```
Tras  SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS 176
14090 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS 176
Tras  SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG 236
14090 SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG 236
Tras  GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY 296
14090 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY 296
Tras  NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE 356
14090 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE 356
Tras  EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR 416
14090 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR 416
Tras  WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 447
14090 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 447
```

FIG.3

```
trastuzumab, Ig gamma-1 chain C region                  119 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens  119 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01859[f-326], Ig gamma-2 chain C region, Homo sapiens  119 STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT 197
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens  119 STKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens  119 STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT 197

trastuzumab, Ig gamma-1 chain C region                  198 YICNVNHKPSNTKVDKKVE----------------------------------------------PKSCDKTHTCPPCP 230
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens  198 YICNVNHKPSNTKVDKKVE----------------------------------------------PKSCDKTHTCPPCP 230
P01859[f-326], Ig gamma-2 chain C region, Homo sapiens  198 YICNVDHKPSNTKVDKTVER--------------------------------------------------KCCVECPPCP 227
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens  198 YTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCP 277
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens  198 YTCNVDHKPSNTKVDKRVES----------------------------------------------KYGPPCPSCP 227

trastuzumab, Ig gamma-1 chain C region                  231 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH 310
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens  231 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH 310
P01859[f-326], Ig gamma-2 chain C region, Homo sapiens  228 APPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVVH 306
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens  278 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH 357
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens  228 APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH 307

trastuzumab, Ig gamma-1 chain C region                  311 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 390
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens  311 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 390
P01859[f-326], Ig gamma-2 chain C region, Homo sapiens  307 QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENN 386
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens  358 QDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENN 437
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens  308 QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 387

trastuzumab, Ig gamma-1 chain C region                  391 YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 446
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens  391 YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 446
P01859[f-326], Ig gamma-2 chain C region, Homo sapiens  387 YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 442
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens  438 YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRYTQKSLSLSPG 493
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens  388 YKTTPPVLDSDGSFFLYSRLTVDKSRWQQENVFSCSVMHEALHNHYTQKSLSLSLG 443
```

FIG.4

```
Kappa   RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA-LQSGNSQESVTEQDSK
           AAPSV +FPPS E+L++ A++VCL+++FYP     V WK D++ +++G   E+ T
Lambda  QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAG--VETTTPSKQS
Kappa   DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
        ++ Y+ SS L+L+   ++ H+ Y+C+VTH+G  S V K+    EC
Lambda  NNKYAASSYLSLTPEQWKSHRSYSCQVTHEG--STVEKTVAPTECS
```

FIG.5A

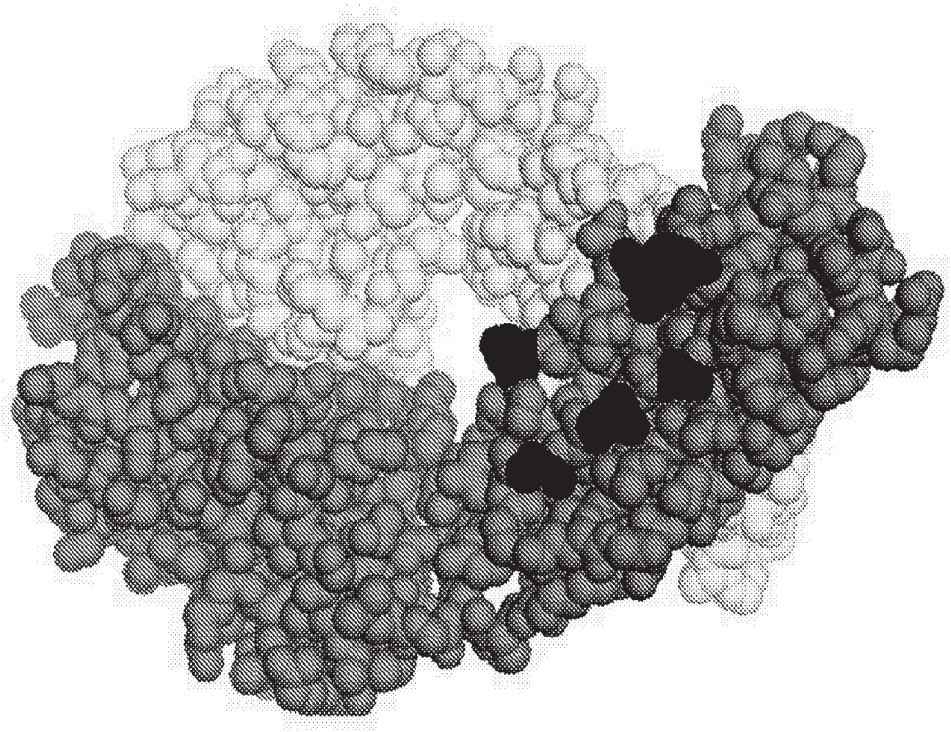


FIG.5B

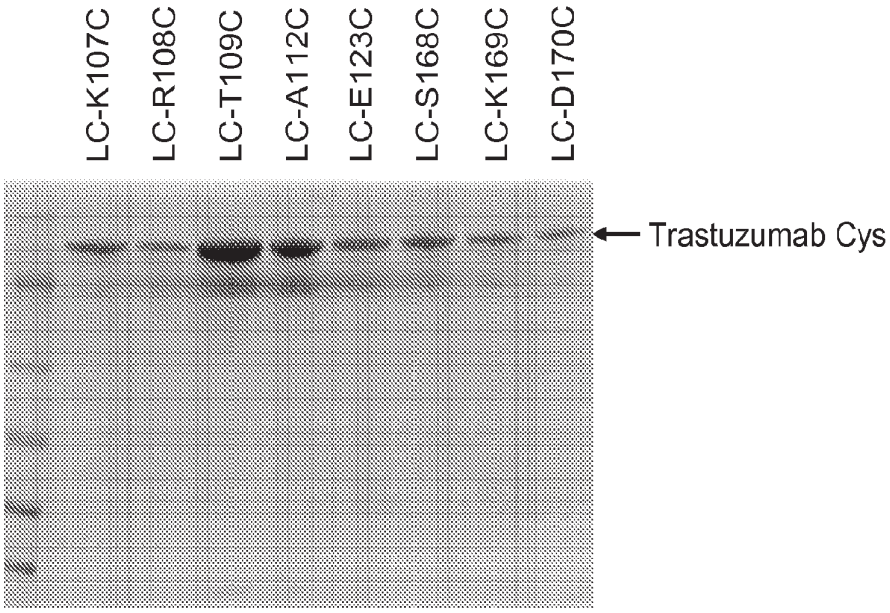

LC-K107C
LC-R108C
LC-T109C
LC-A112C
LC-E123C
LC-S168C
LC-K169C
LC-D170C
Trastuzumab Cys

FIG.6

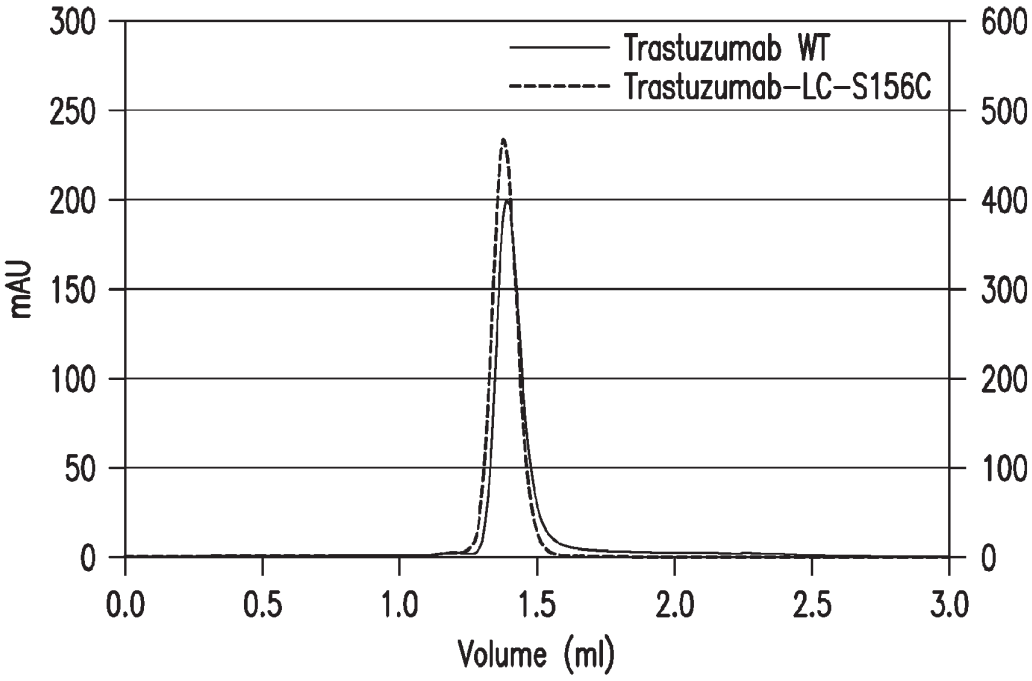

300
250
200
150
100
50
0
mAU
600
500
400
300
200
100
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Volume (ml)
Trastuzumab WT
Trastuzumab-LC-S156C

FIG.7

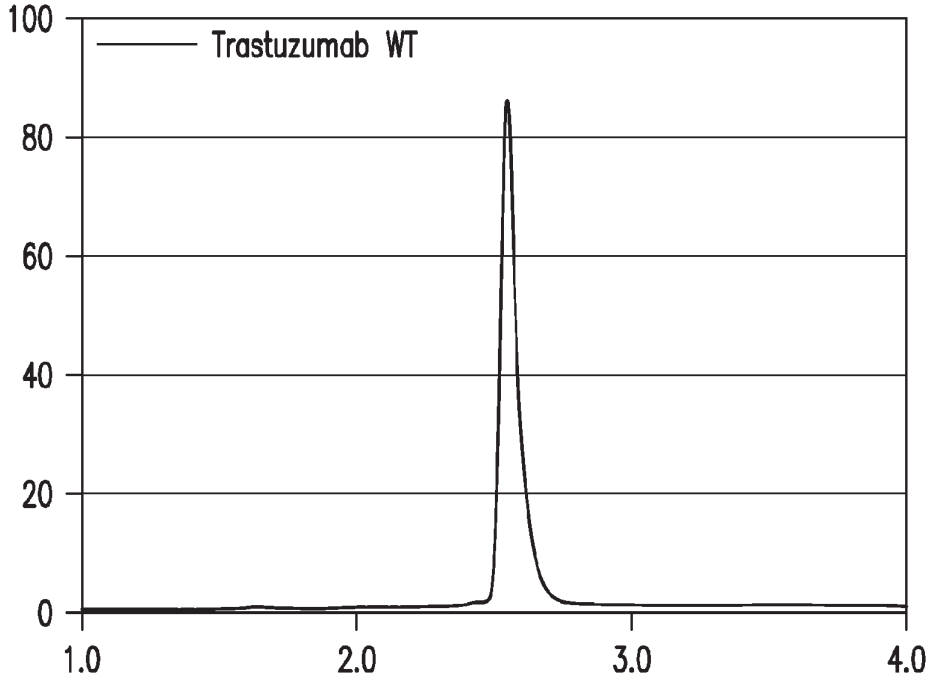

Trastuzumab WT
100
80
60
40
20
0
1.0
2.0
3.0
4.0

FIG.8A

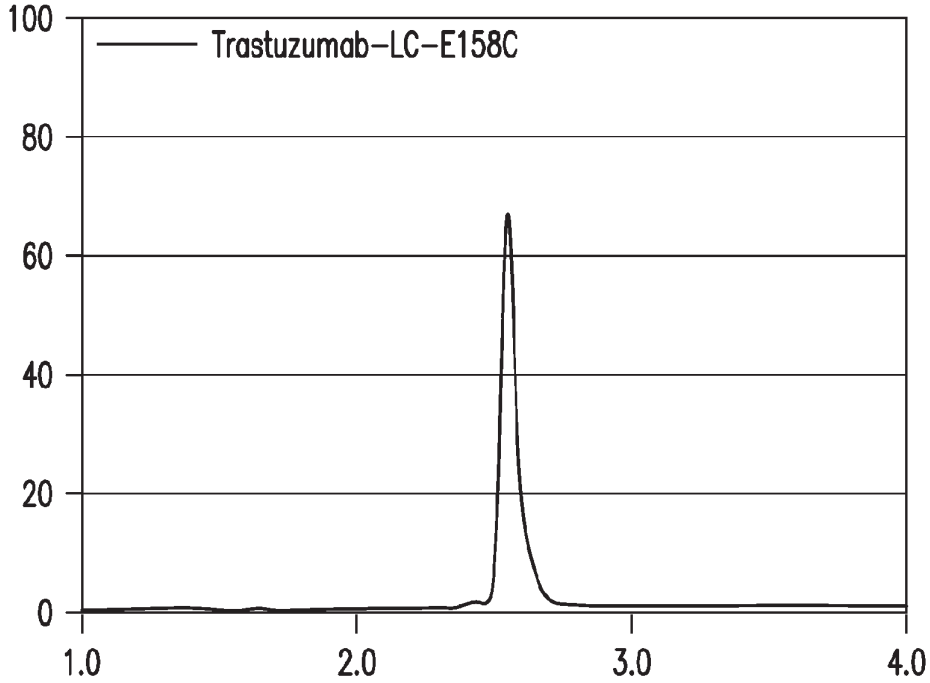

Trastuzumab-LC-E158C
100
80
60
40
20
0
1.0
2.0
3.0
4.0

FIG.8B

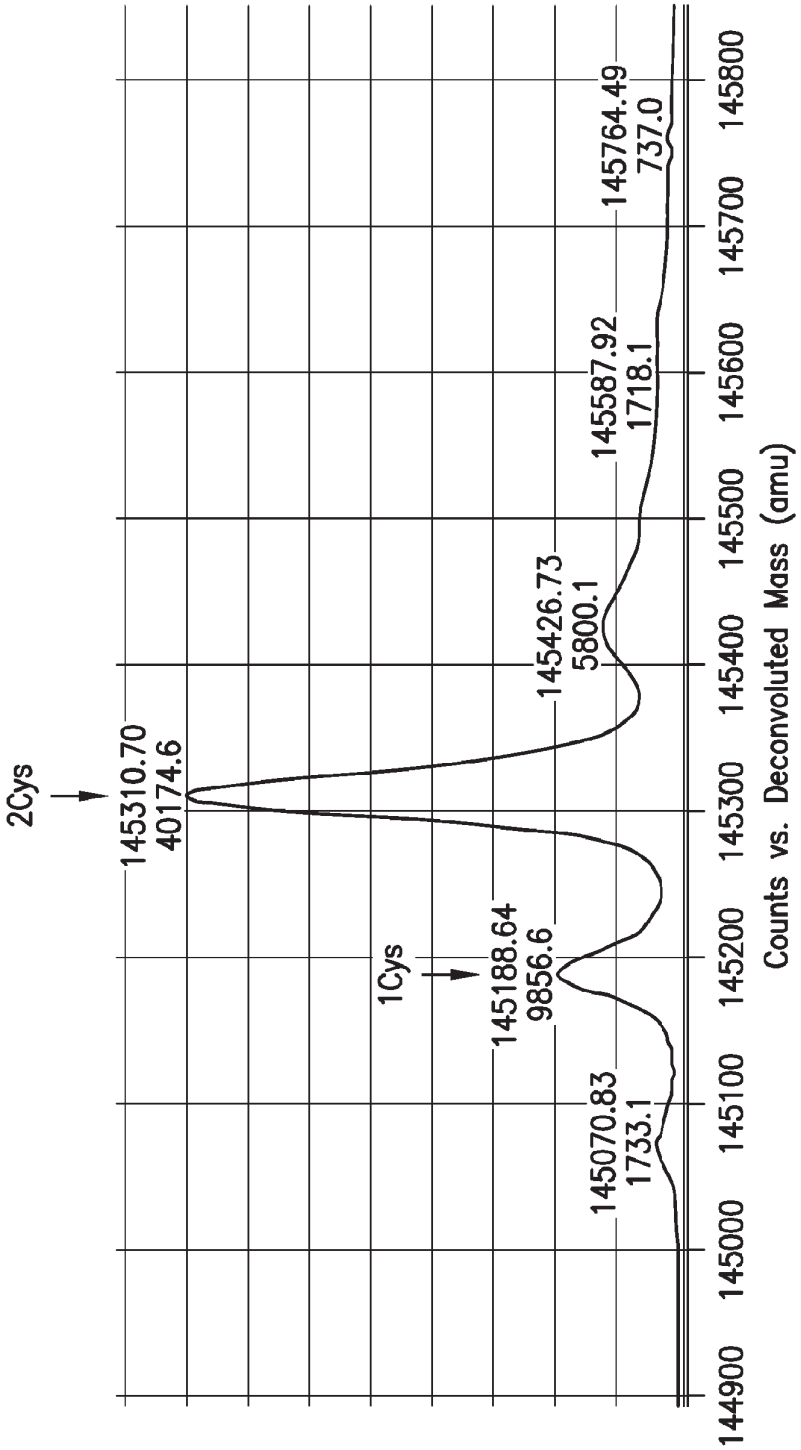

2Cys
145310.70
40174.6
1Cys
145188.64
9856.6
145070.83
1733.1
145426.73
5800.1
145587.92
1718.1
145764.49
737.0
144900
145000
145100
145200
145300
145400
145500
145600
145700
145800
Counts vs. Deconvoluted Mass (amu)

FIG.9

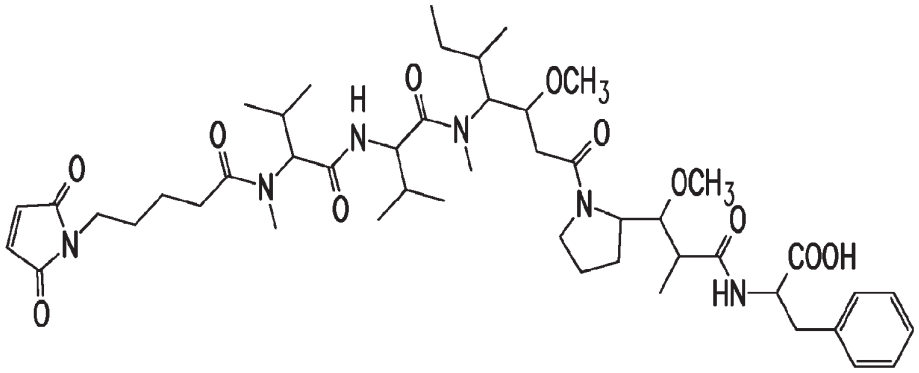


FIG.10

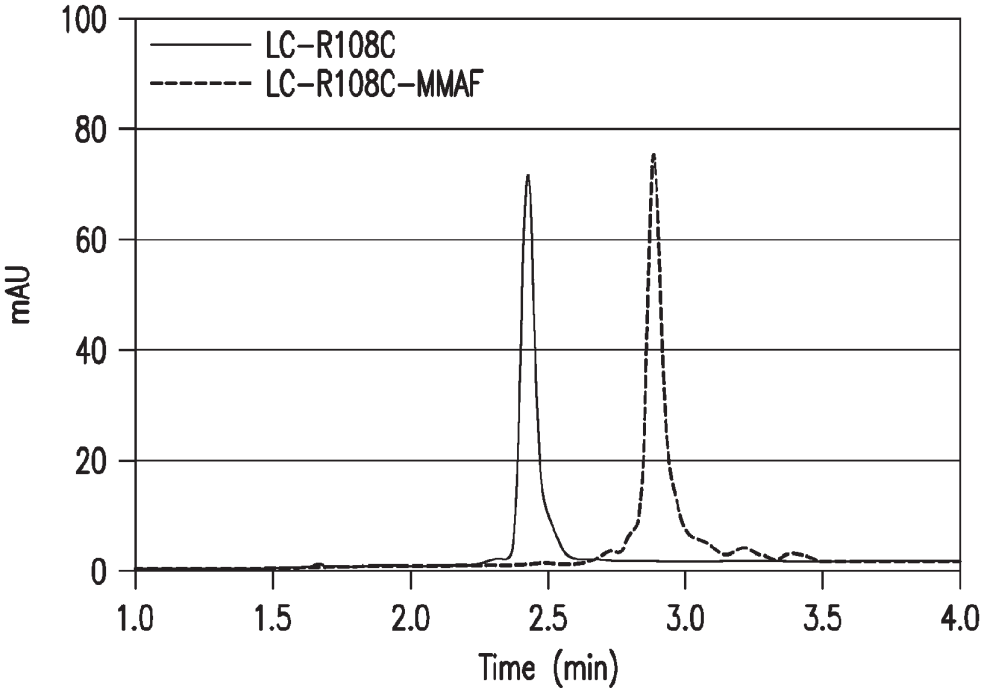


FIG.11A

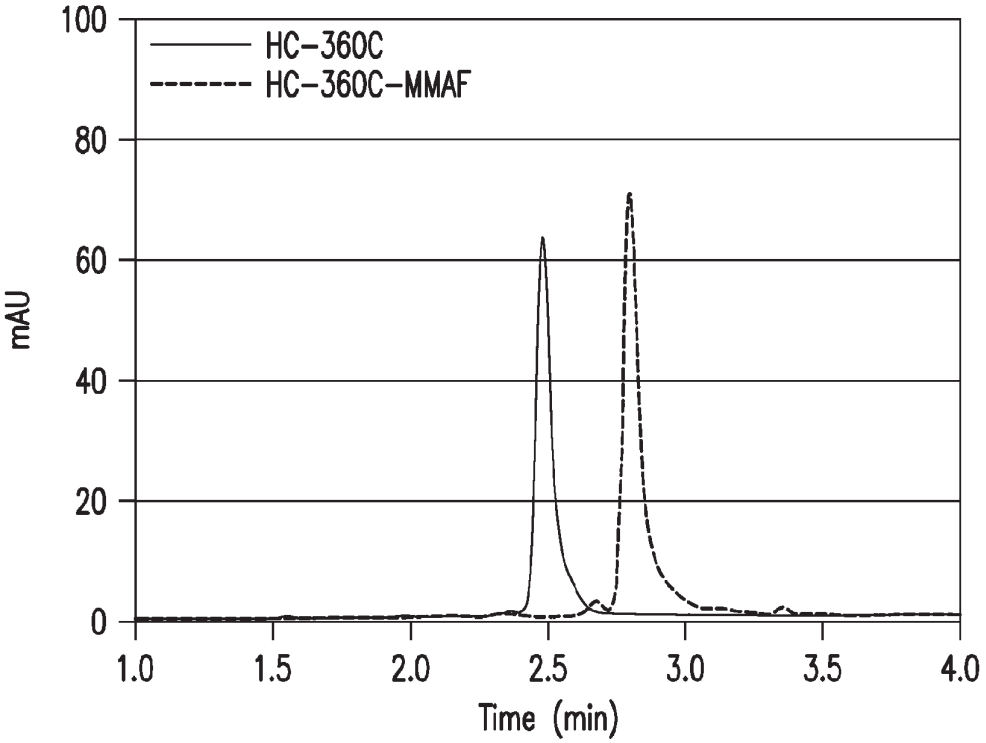


FIG.11B

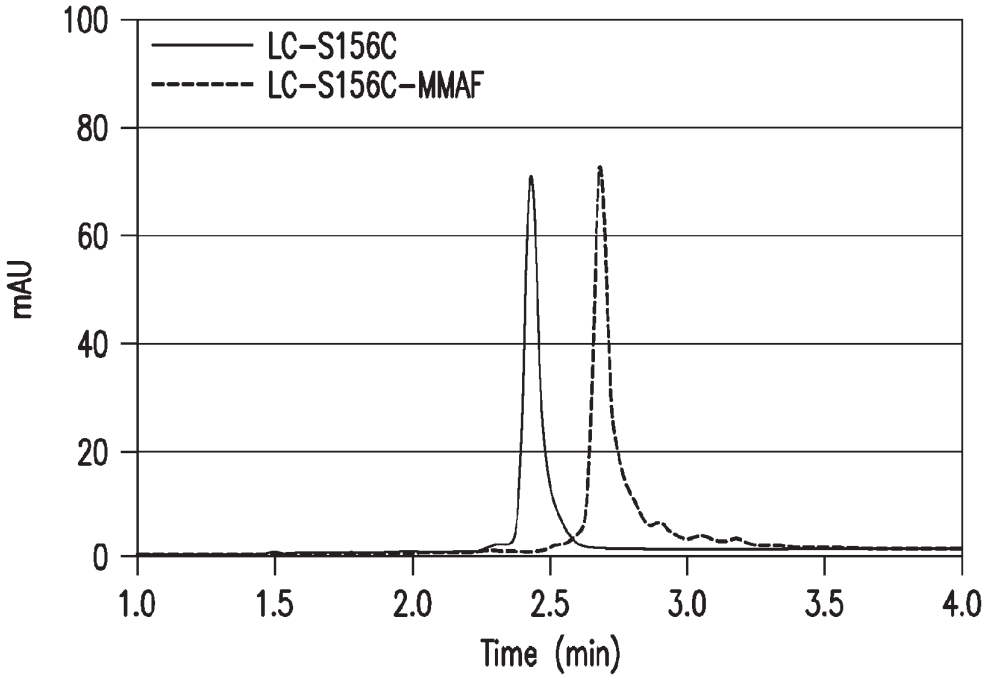


FIG.11C

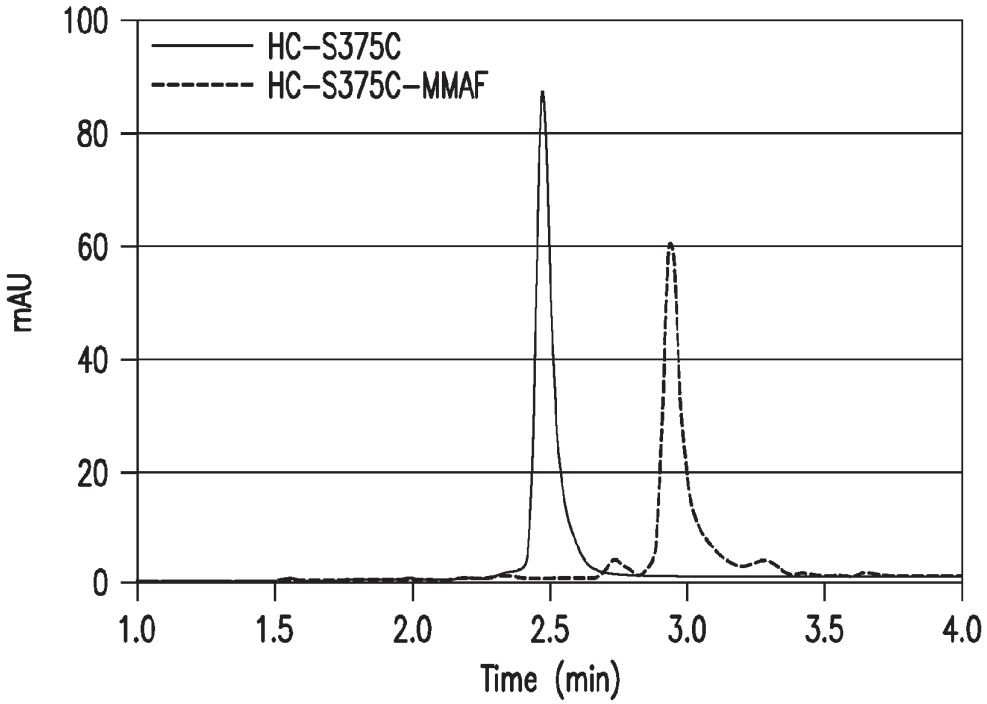


FIG.11D

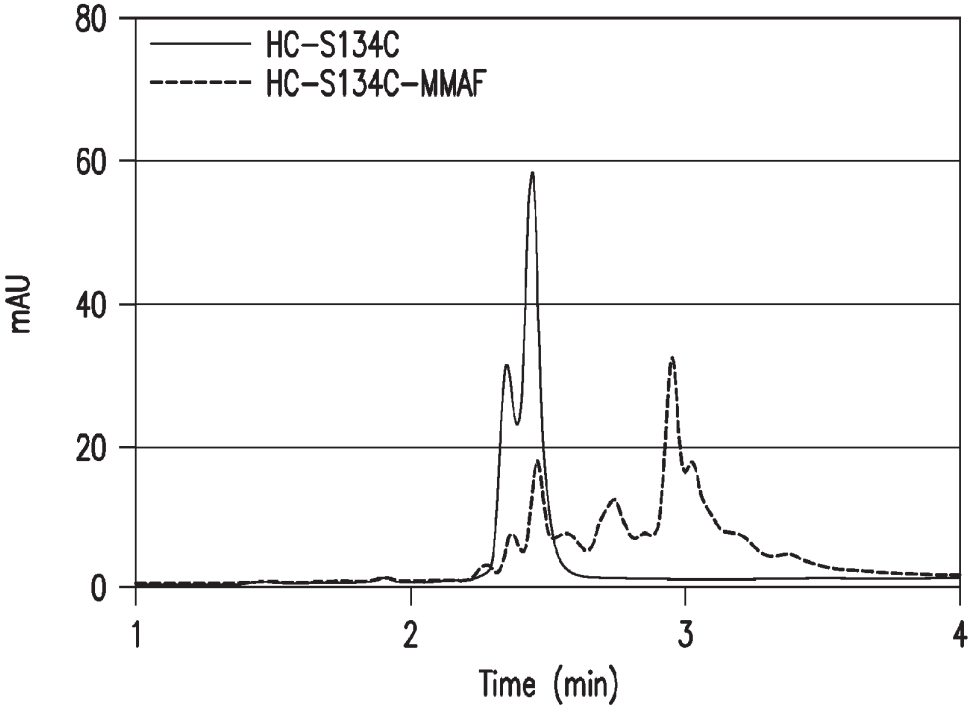


FIG.12A

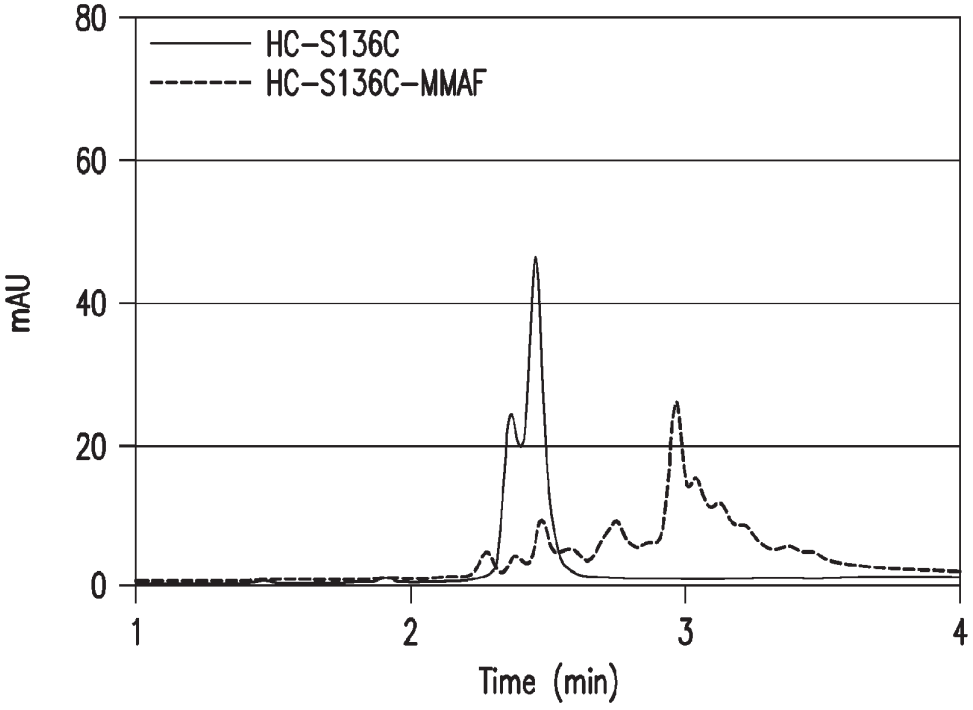


FIG.12B

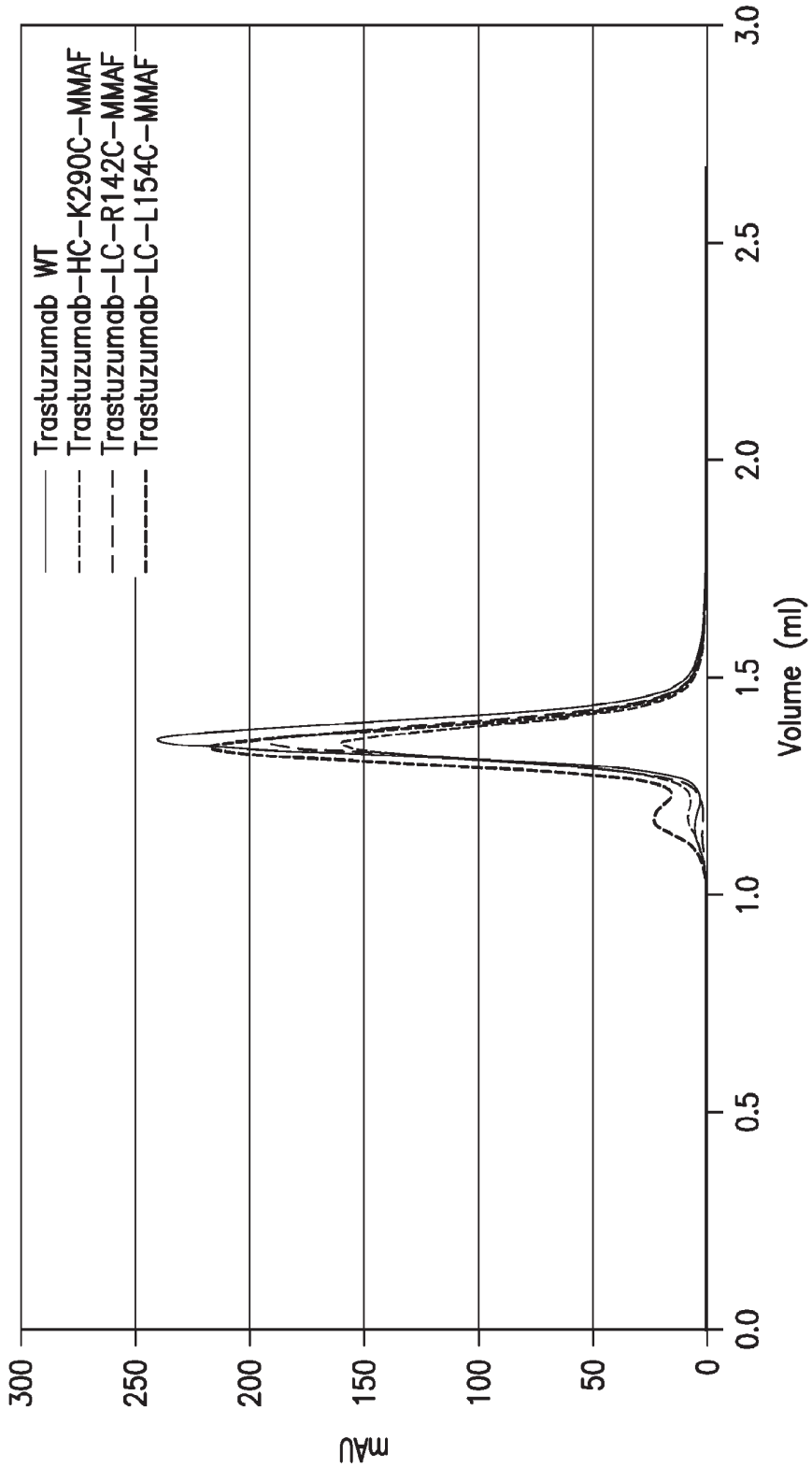

Trastuzumab WT
Trastuzumab-HC-K290C-MMAF
Trastuzumab-LC-R142C-MMAF
Trastuzumab-LC-L154C-MMAF
mAU
300
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Volume (ml)

FIG.13

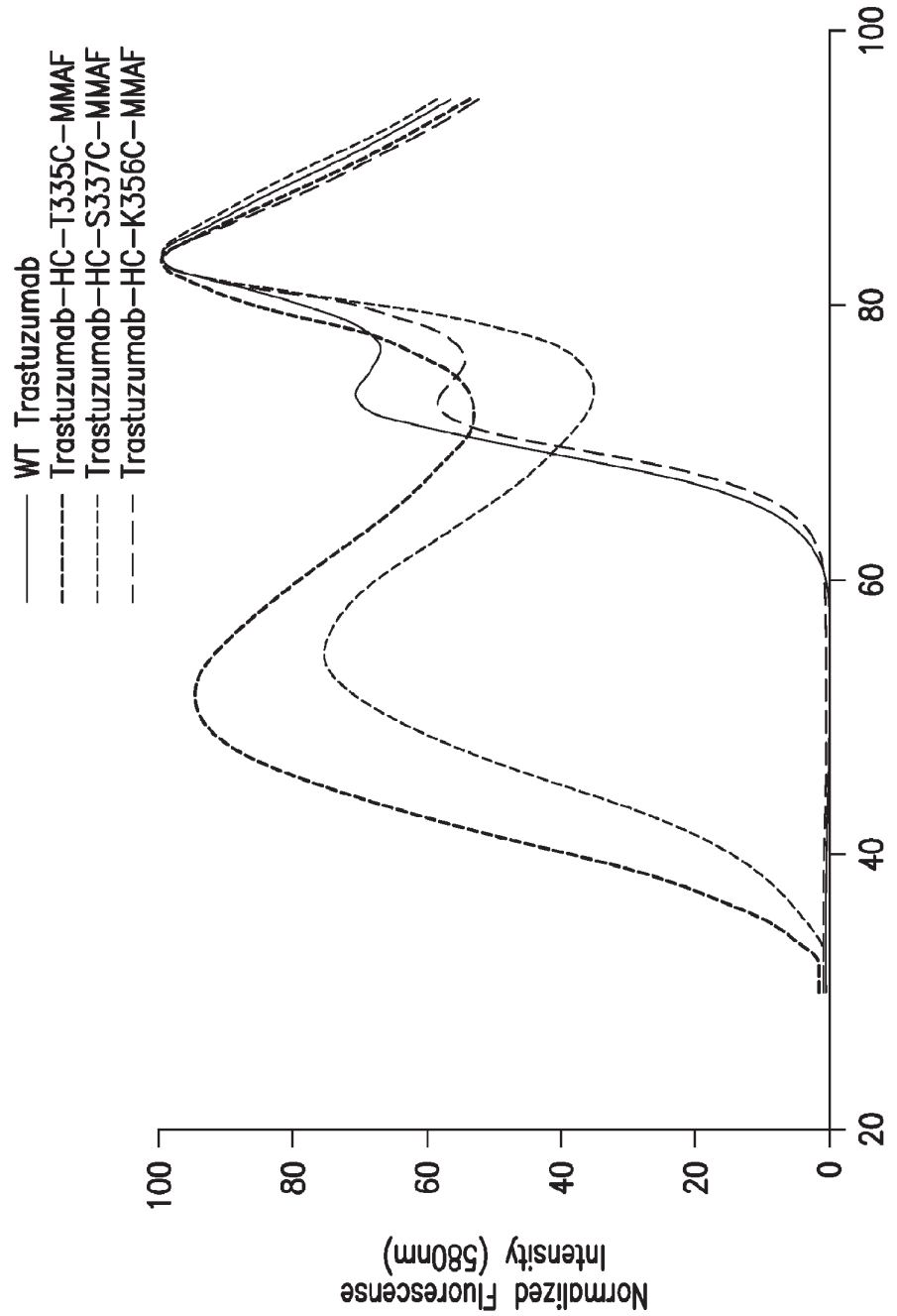

WT Trastuzumab
Trastuzumab-HC-T335C-MMAF
Trastuzumab-HC-S337C-MMAF
Trastuzumab-HC-K356C-MMAF
Normalized Fluorescense Intensity (580nm)
0
20
40
60
80
100
20
40
60
80
100
Temperature (°C)

FIG. 14

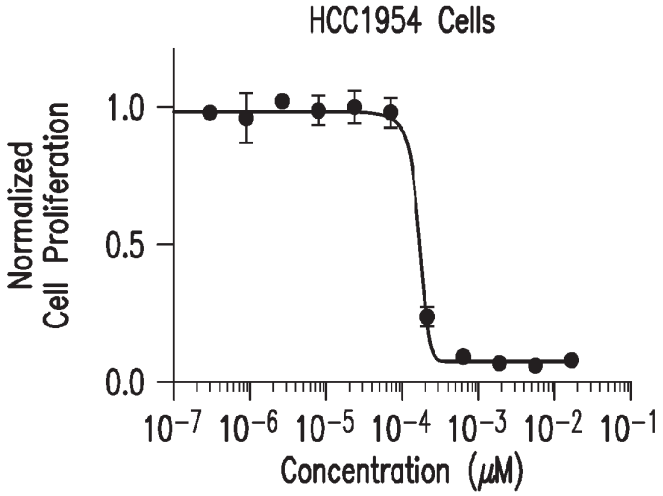


FIG.15A

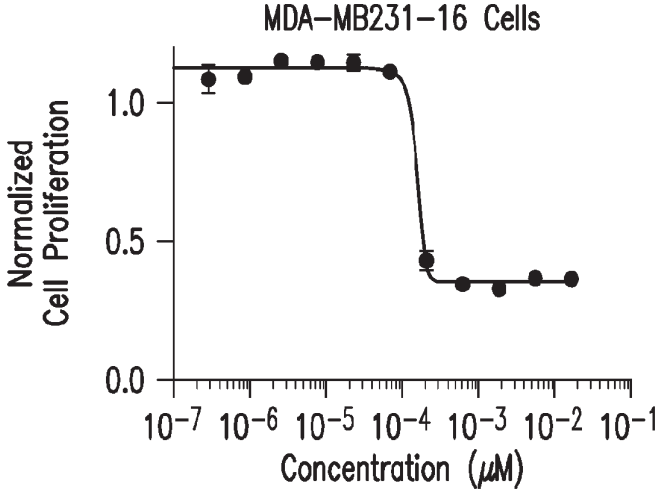


FIG.15B

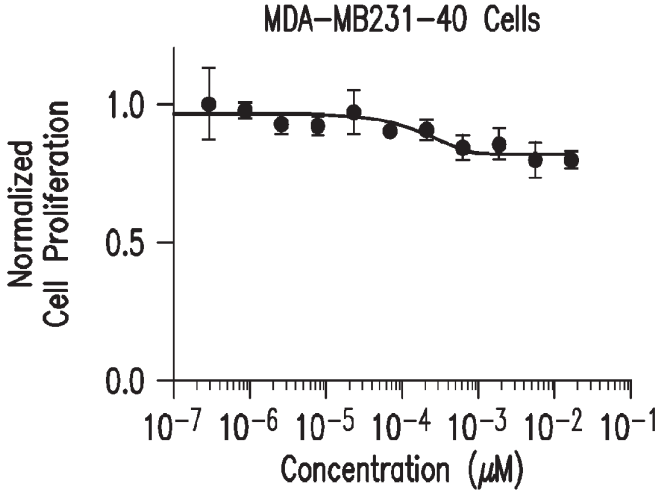


FIG.15C

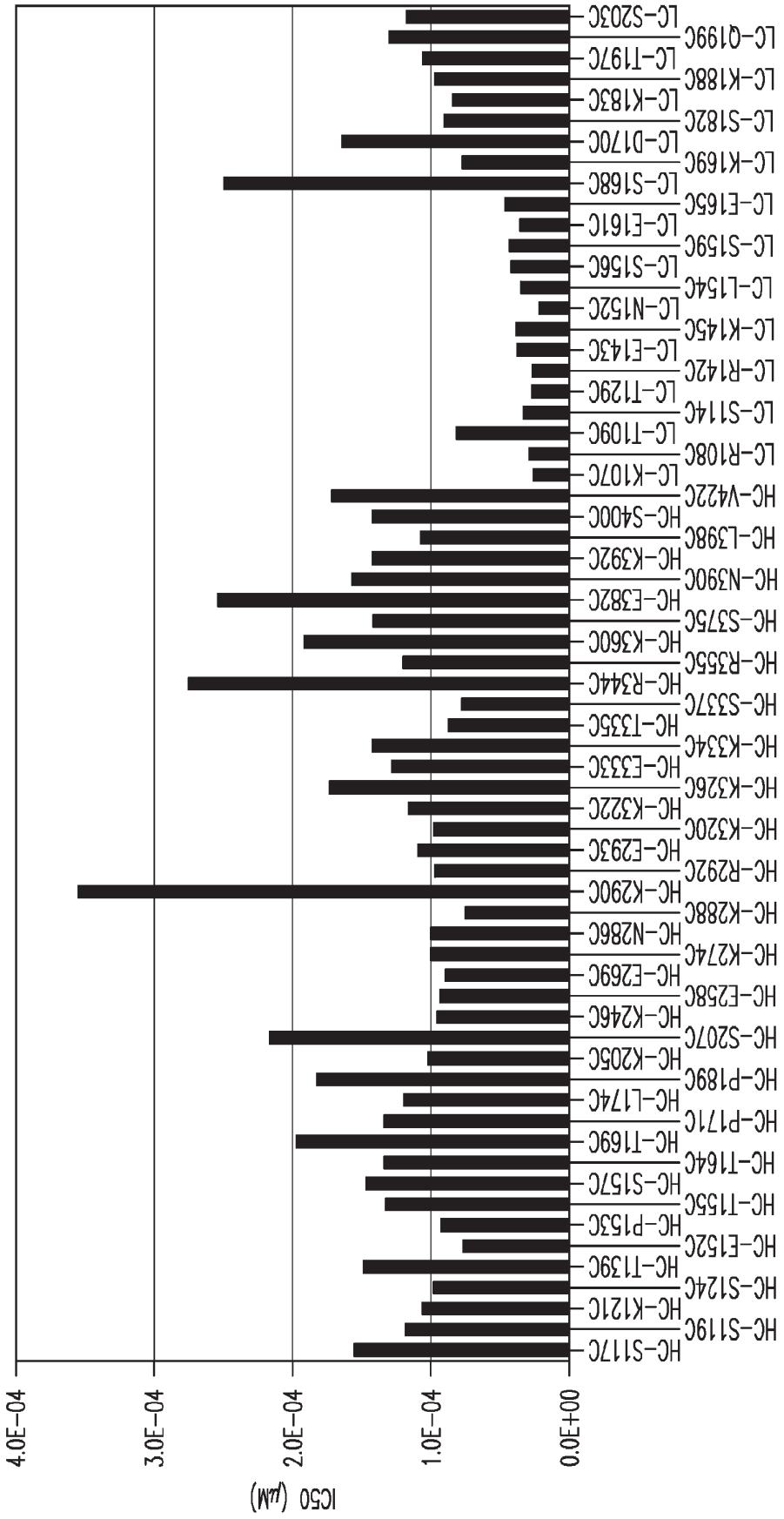

IC50 (μM)
4.0E−04
3.0E−04
2.0E−04
1.0E−04
0.0E+00
HC-S117C
HC-S119C
HC-K121C
HC-S124C
HC-T139C
HC-E152C
HC-P153C
HC-T155C
HC-S157C
HC-T164C
HC-T169C
HC-P171C
HC-L174C
HC-P189C
HC-K205C
HC-S207C
HC-K246C
HC-E258C
HC-E269C
HC-K274C
HC-N286C
HC-K288C
HC-K290C
HC-R292C
HC-E293C
HC-K320C
HC-K322C
HC-K326C
HC-K334C
HC-E335C
HC-T335C
HC-S337C
HC-R344C
HC-K360C
HC-R355C
HC-S375C
HC-E382C
HC-N390C
HC-K392C
HC-L398C
HC-S400C
HC-V422C
LC-K107C
LC-R108C
LC-T109C
LC-S114C
LC-T129C
LC-R142C
LC-E143C
LC-K145C
LC-N152C
LC-L154C
LC-S156C
LC-S159C
LC-E161C
LC-E165C
LC-S168C
LC-K169C
LC-D170C
LC-S182C
LC-K183C
LC-K188C
LC-T197C
LC-Q199C
LC-S203C

FIG.16

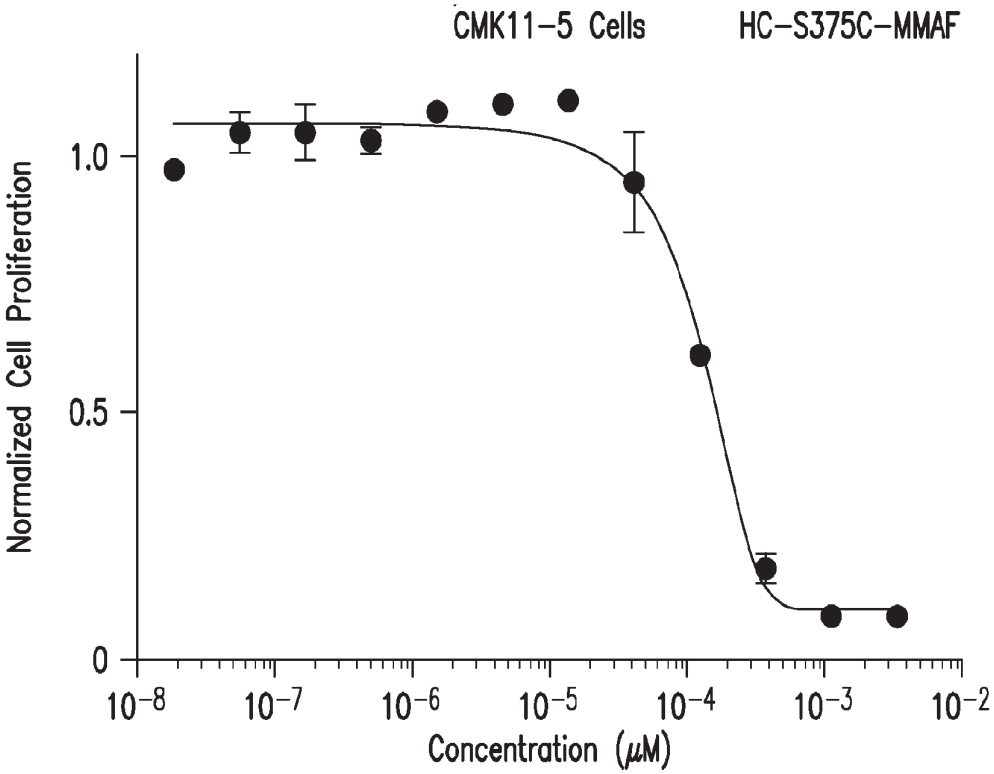


FIG.17A

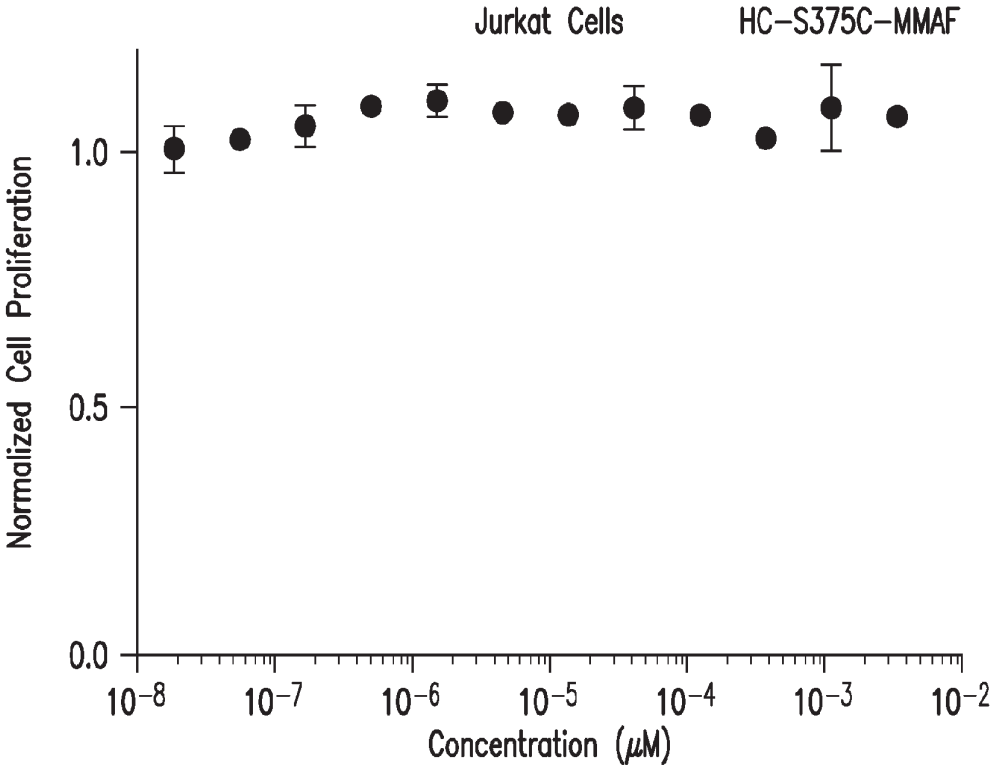


FIG.17B

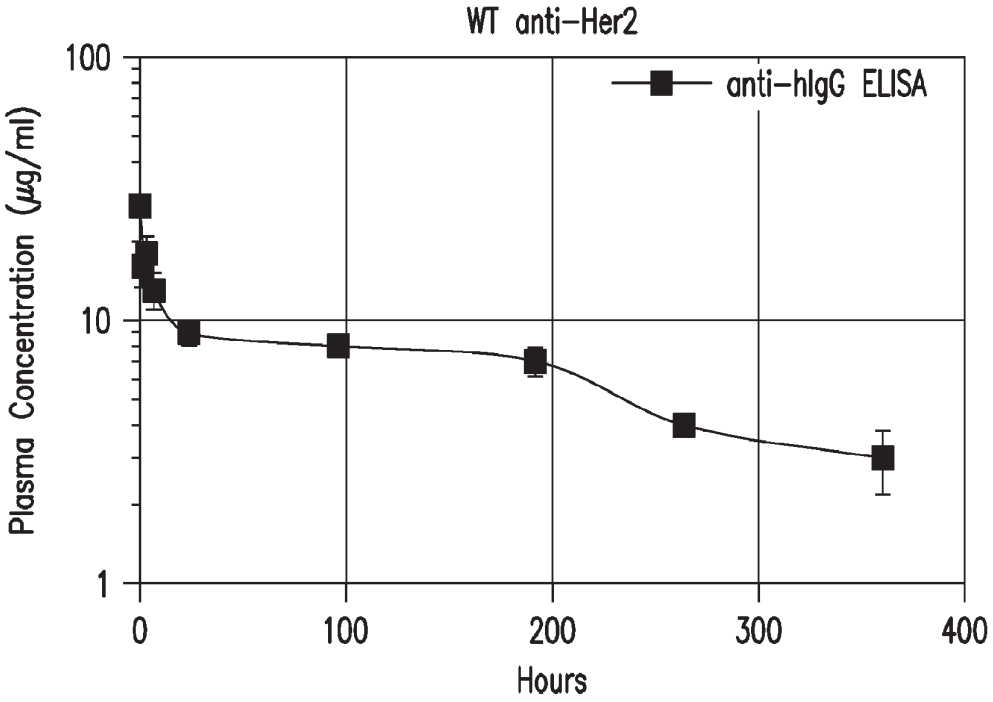


FIG.18A

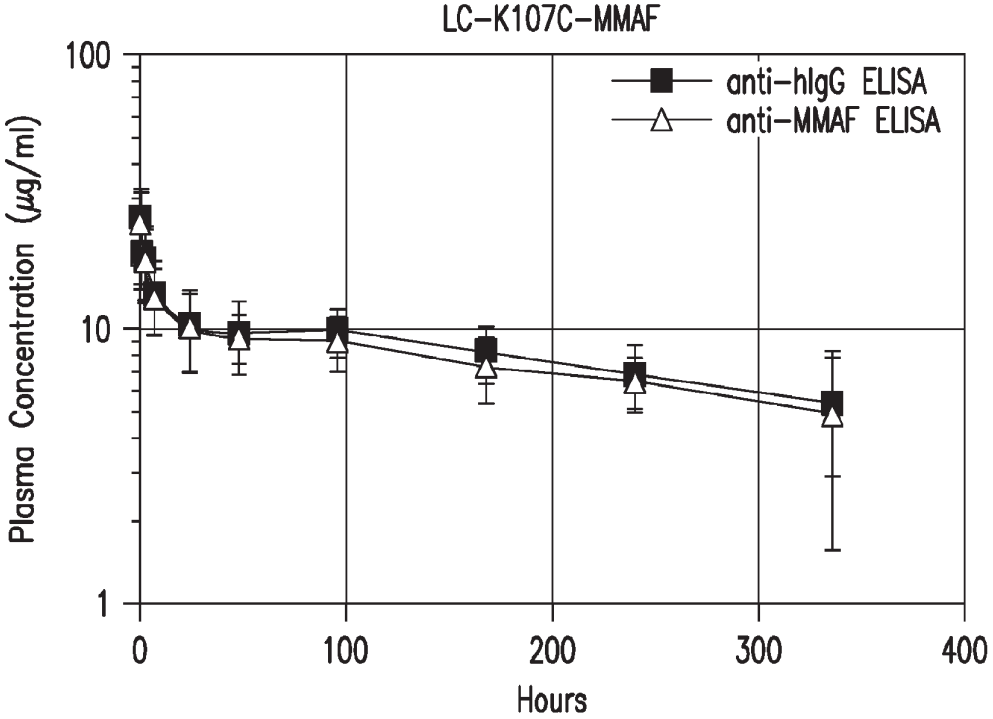


FIG.18B

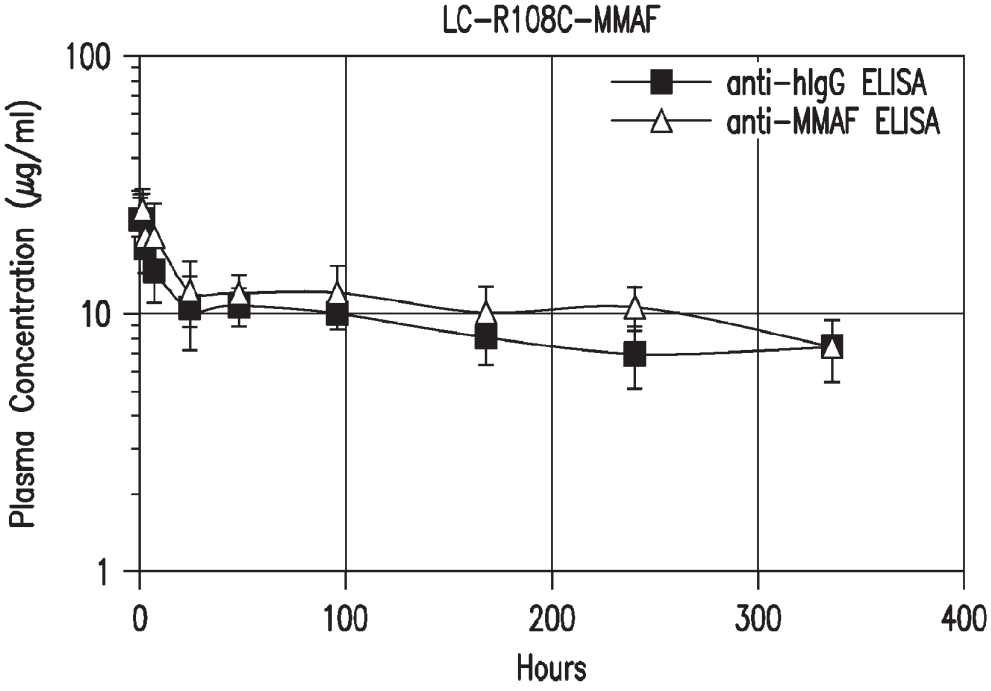


FIG.18C

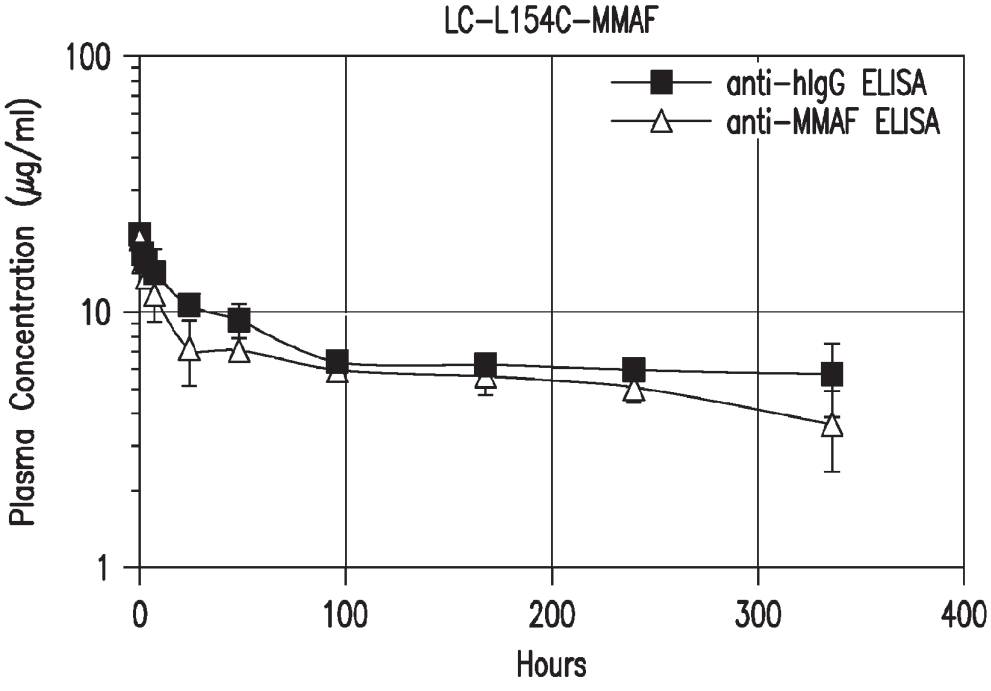


FIG.18D

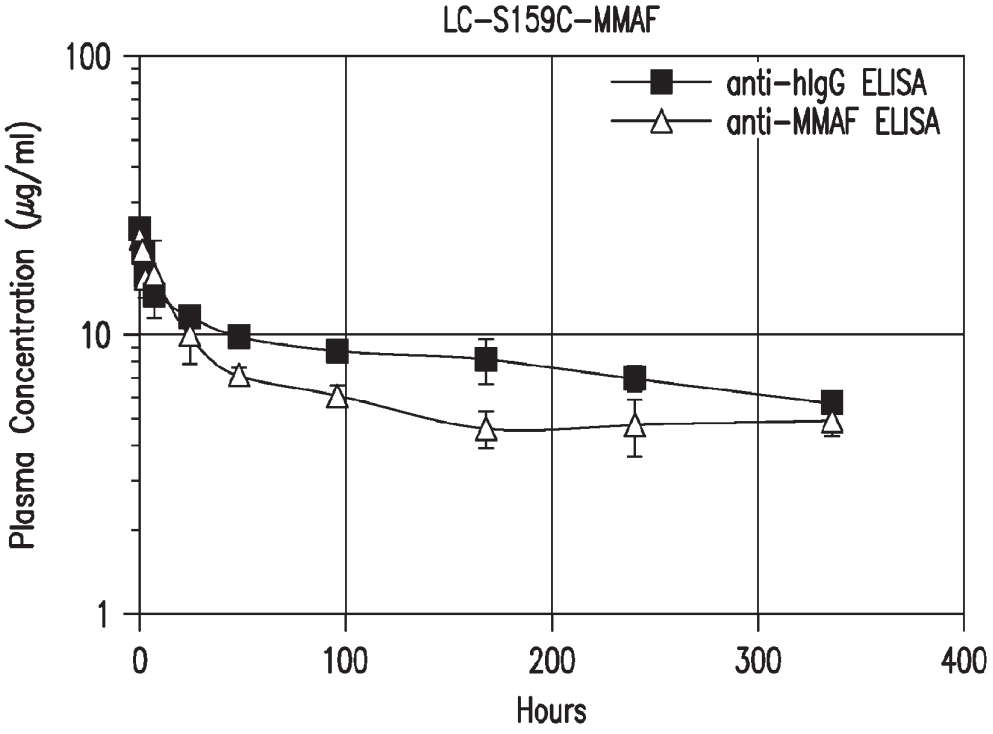

LC-S159C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
Hours

FIG.18E

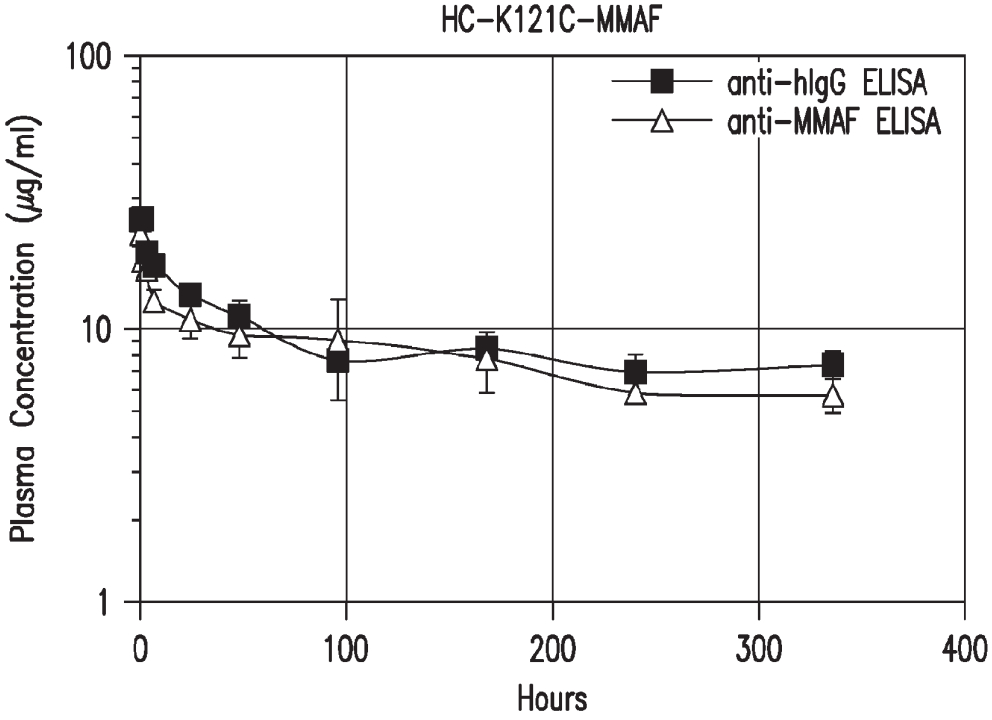


FIG.19A

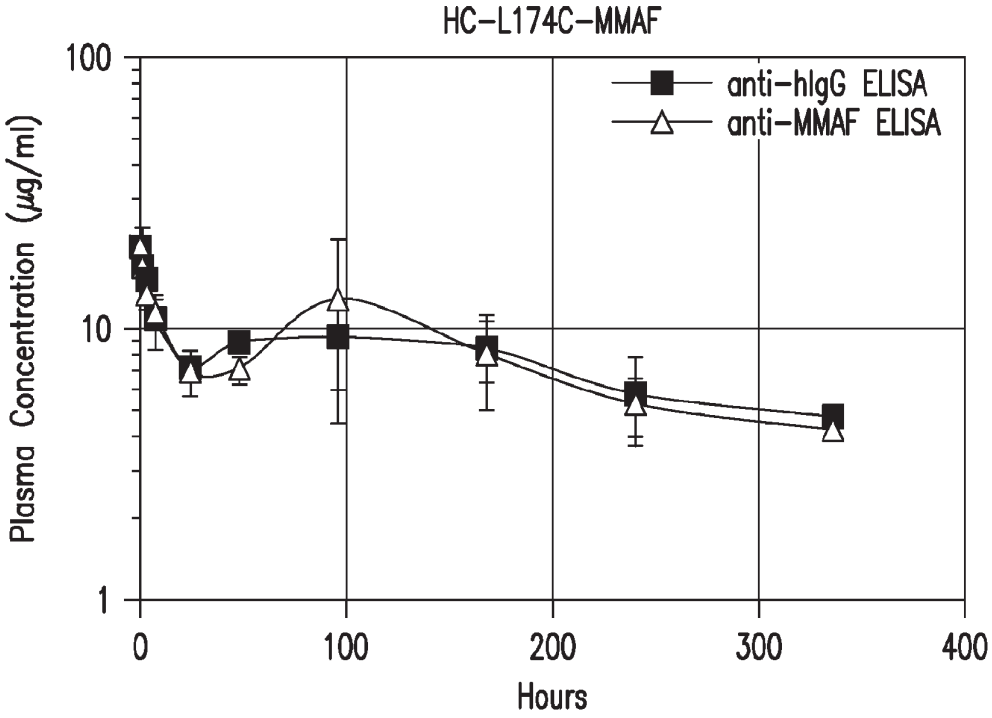


FIG.19B

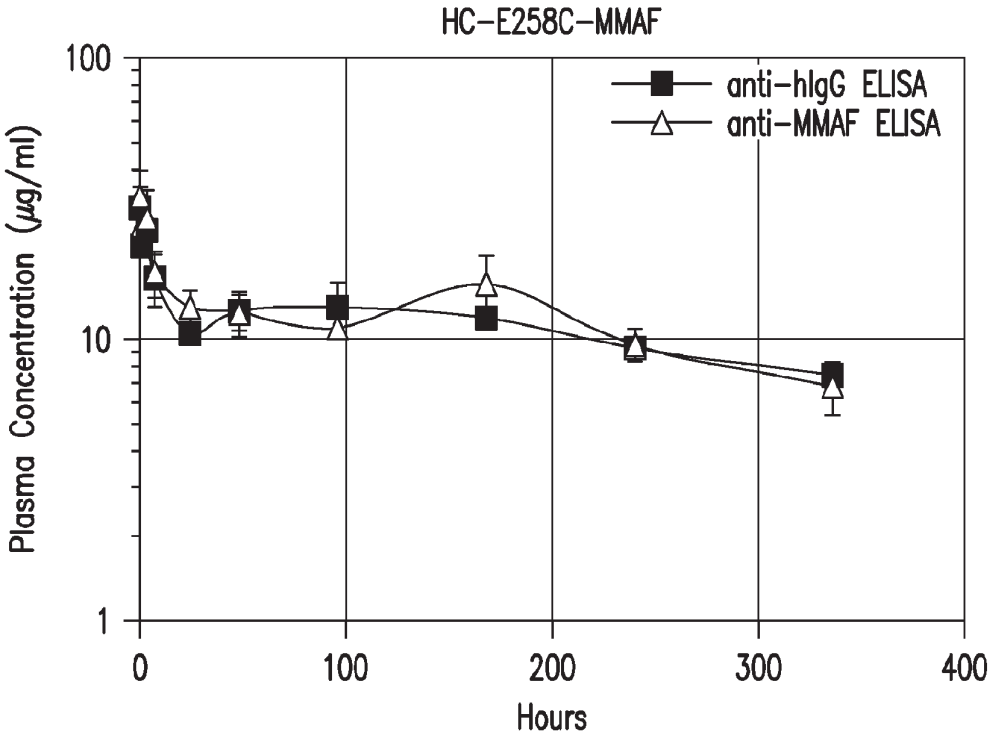


FIG.19C

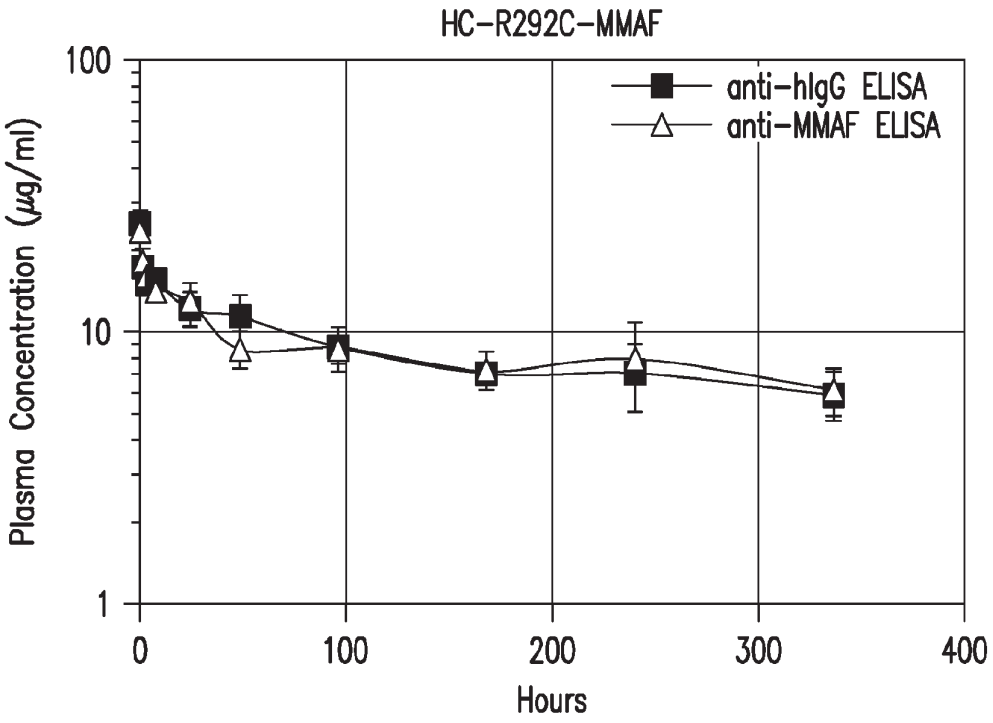


FIG.19D

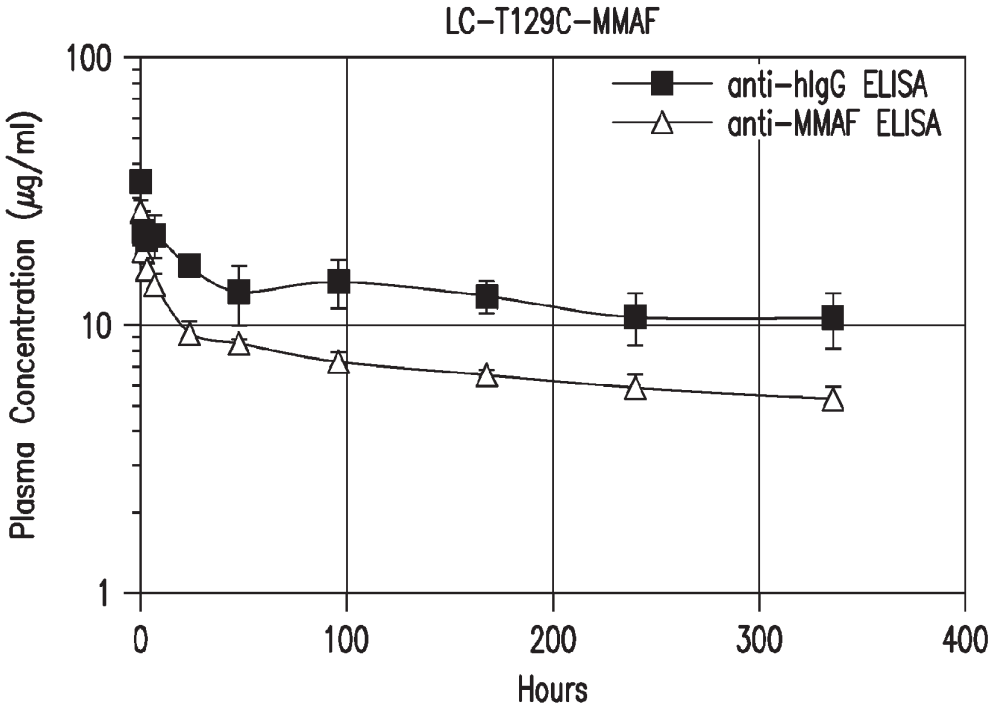


FIG.20A

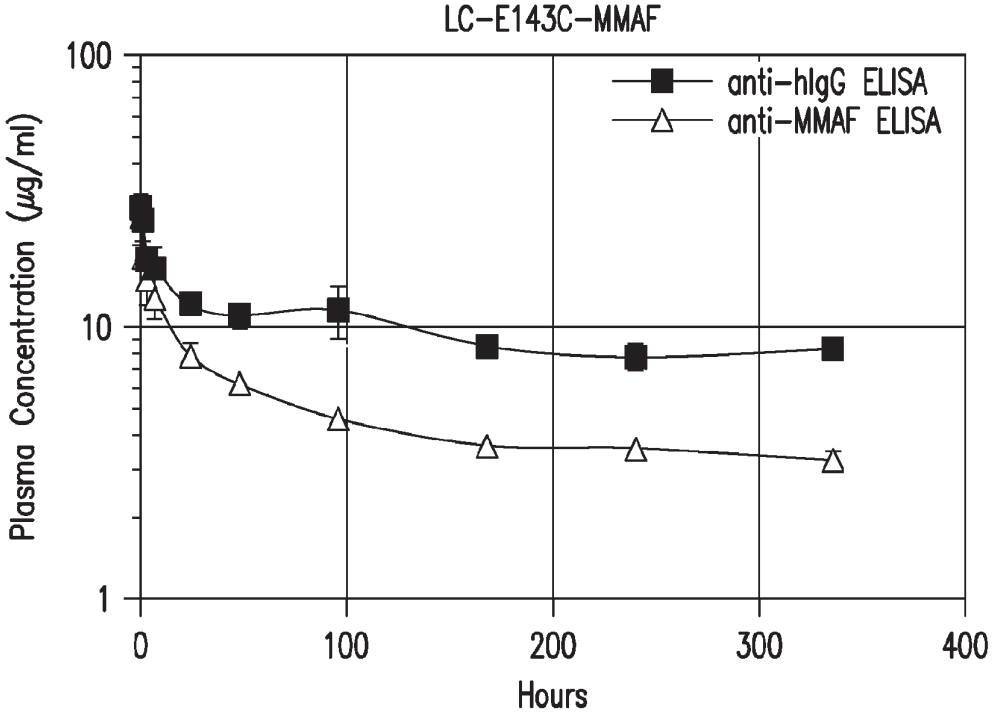


FIG.20B

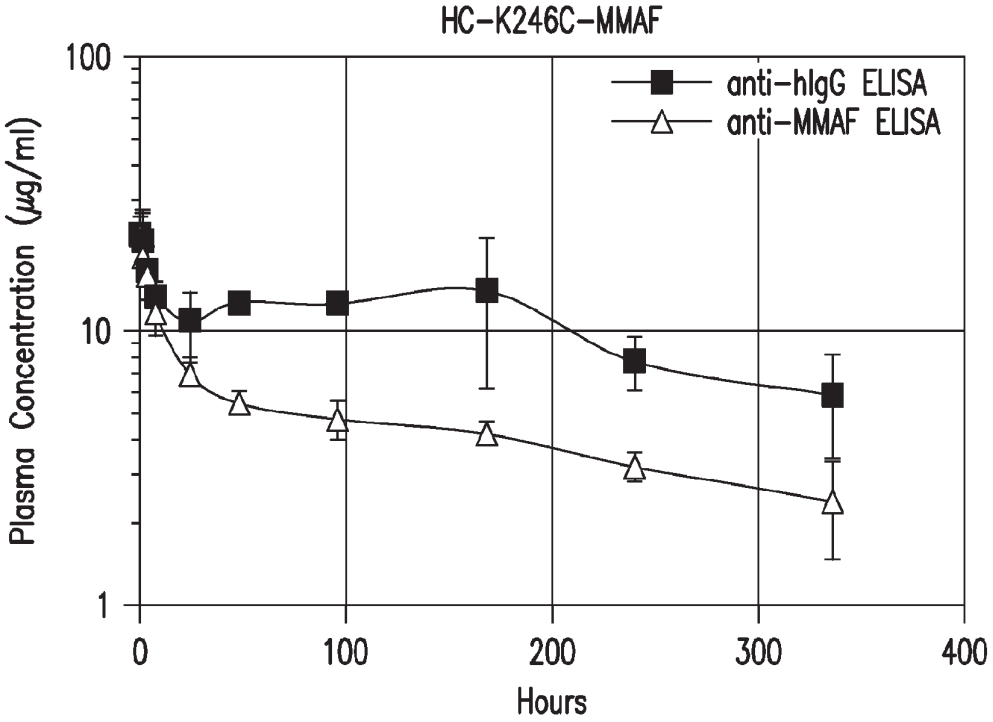


FIG.20C

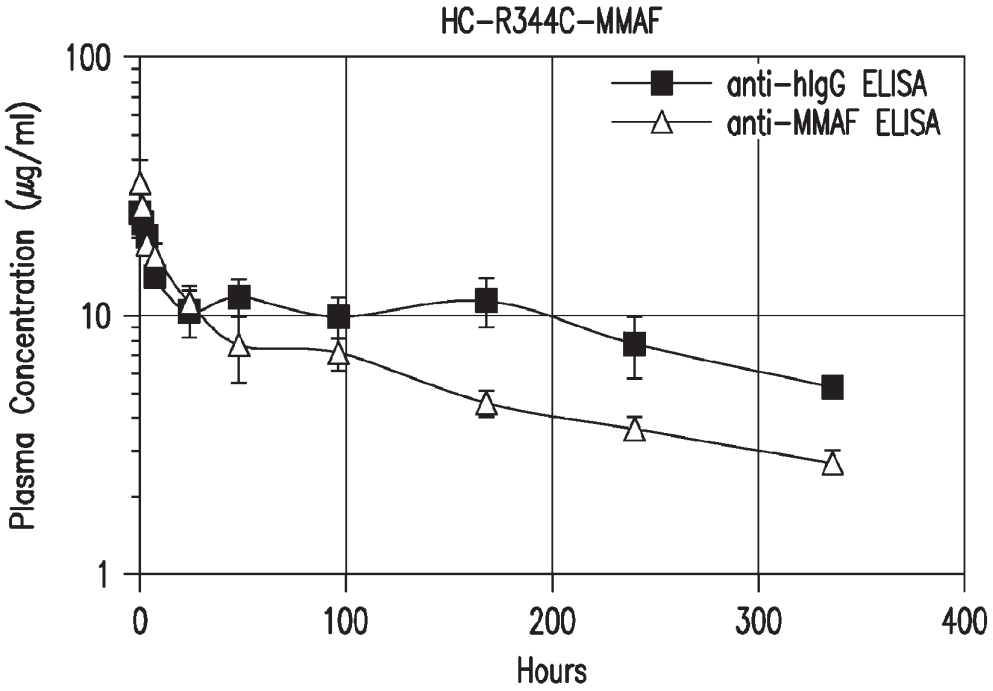


FIG.20D

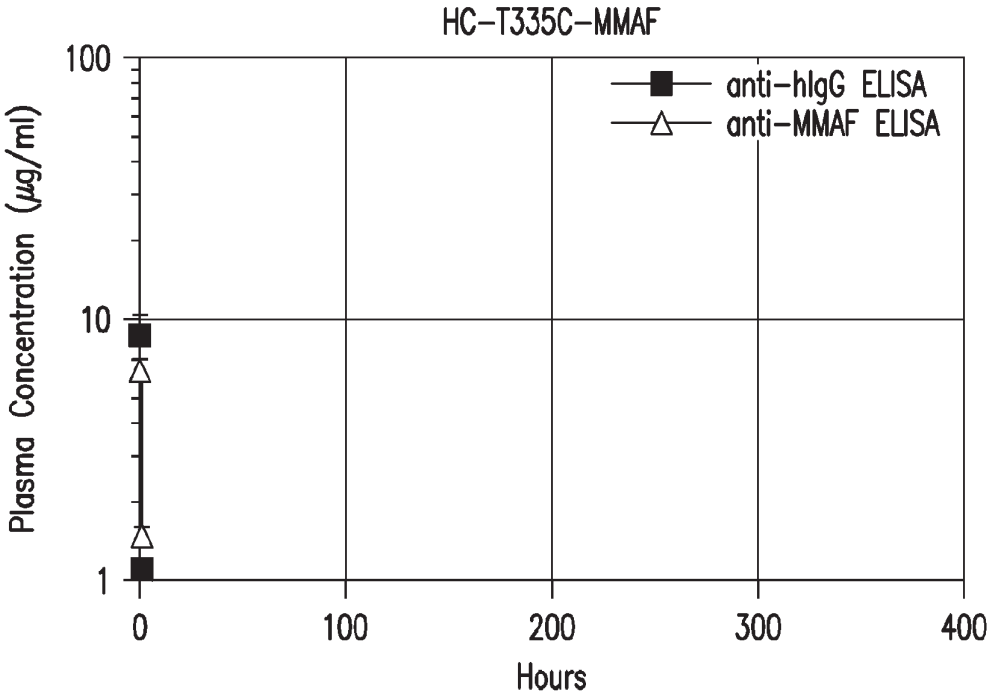


FIG.21A

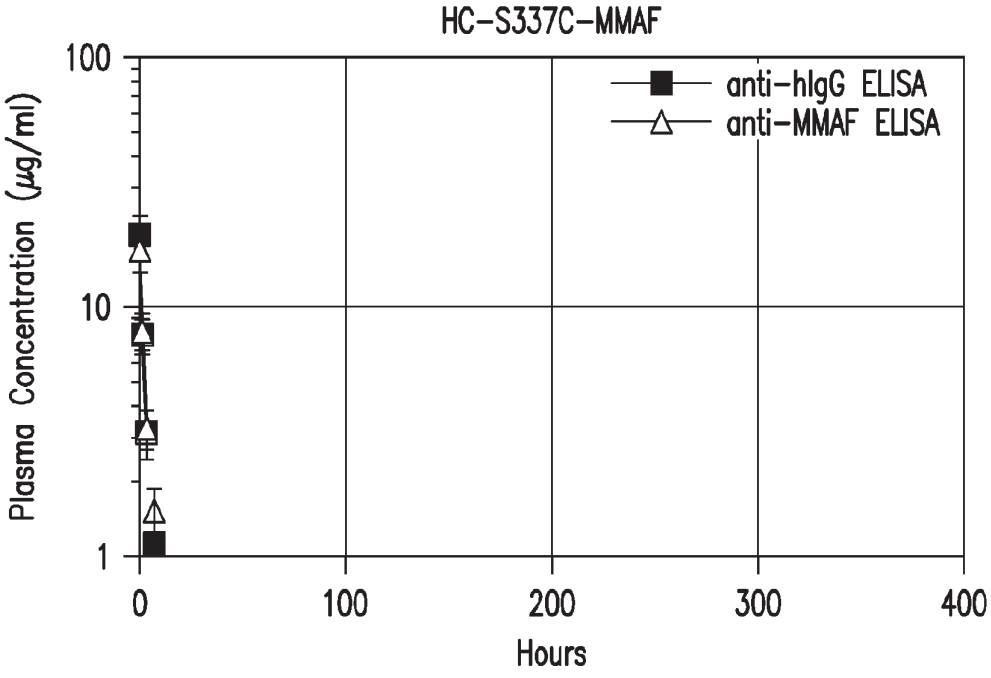


FIG.21B

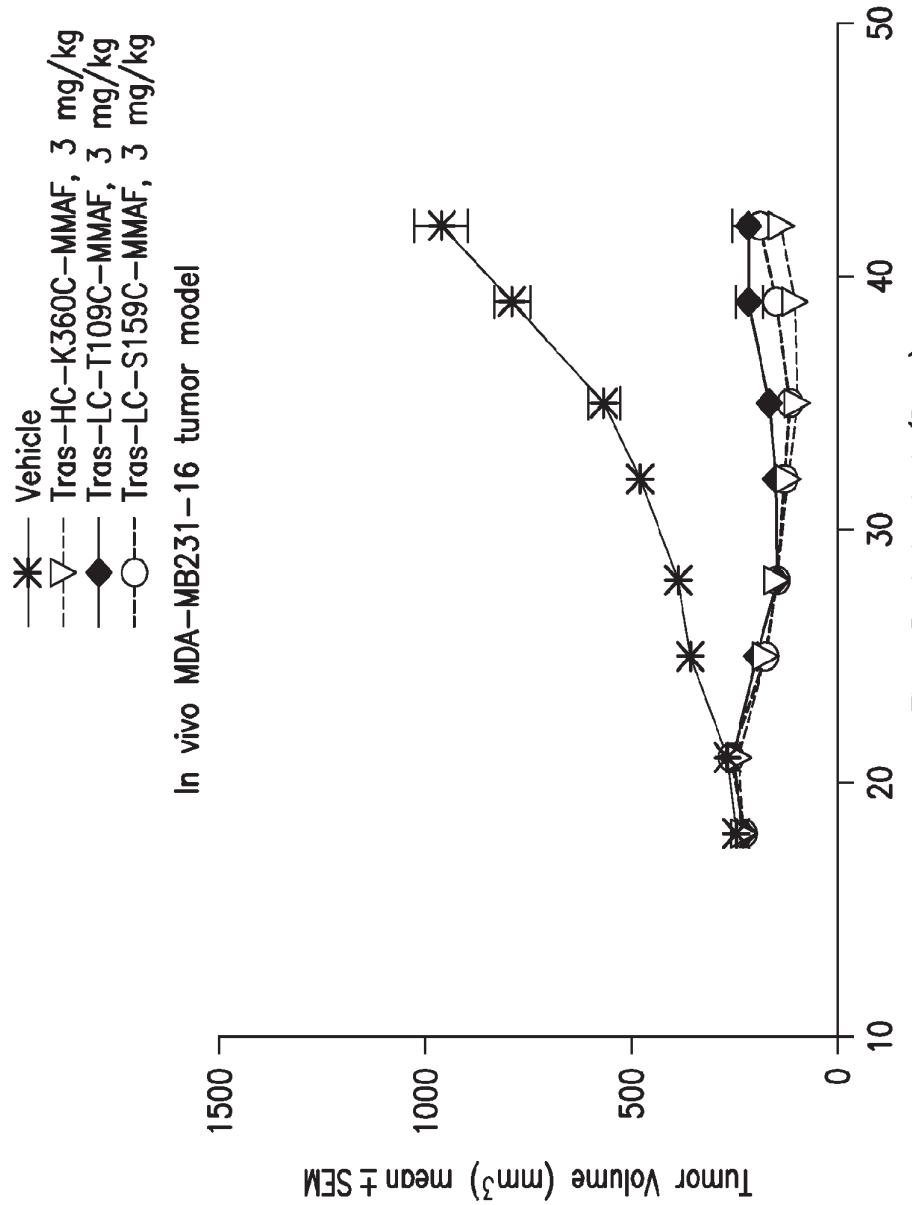

In vivo MDA-MB231-16 tumor model
Vehicle
Tras-HC-K360C-MMAF, 3 mg/kg
Tras-LC-T109C-MMAF, 3 mg/kg
Tras-LC-S159C-MMAF, 3 mg/kg
Tumor Volume (mm3) mean ± SEM
0
500
1000
1500
10
20
30
40
50
Time Post-Implant (Days)

FIG.22

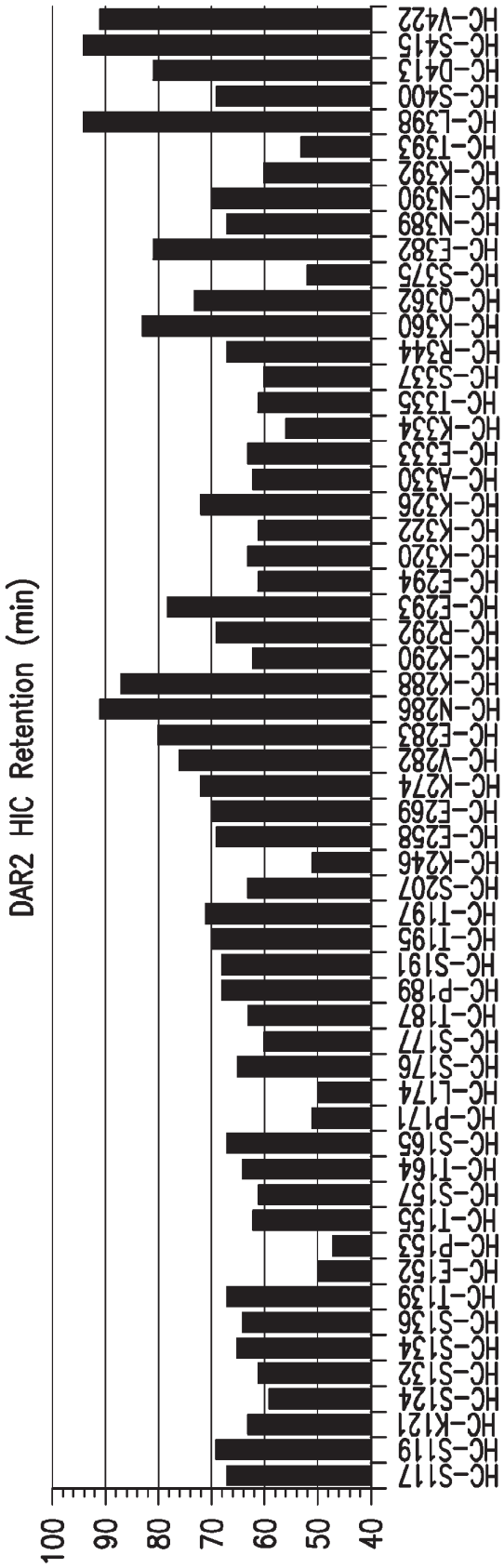

DAR2 HIC Retention (min)
100
90
80
70
60
50
40
HC-S117
HC-S119
HC-K121
HC-S124
HC-S132
HC-S134
HC-S136
HC-T139
HC-E152
HC-P153
HC-T155
HC-S157
HC-T164
HC-S165
HC-P171
HC-L174
HC-S176
HC-S177
HC-T187
HC-P189
HC-S191
HC-T195
HC-T197
HC-S207
HC-K246
HC-E258
HC-E269
HC-K274
HC-V282
HC-E283
HC-N286
HC-K288
HC-K290
HC-R292
HC-E293
HC-E294
HC-K320
HC-K322
HC-K326
HC-A330
HC-E333
HC-K334
HC-T335
HC-S337
HC-R344
HC-K360
HC-Q362
HC-S375
HC-E382
HC-N389
HC-N390
HC-K392
HC-T393
HC-L398
HC-S400
HC-D413
HC-S415
HC-V422

FIG.23A

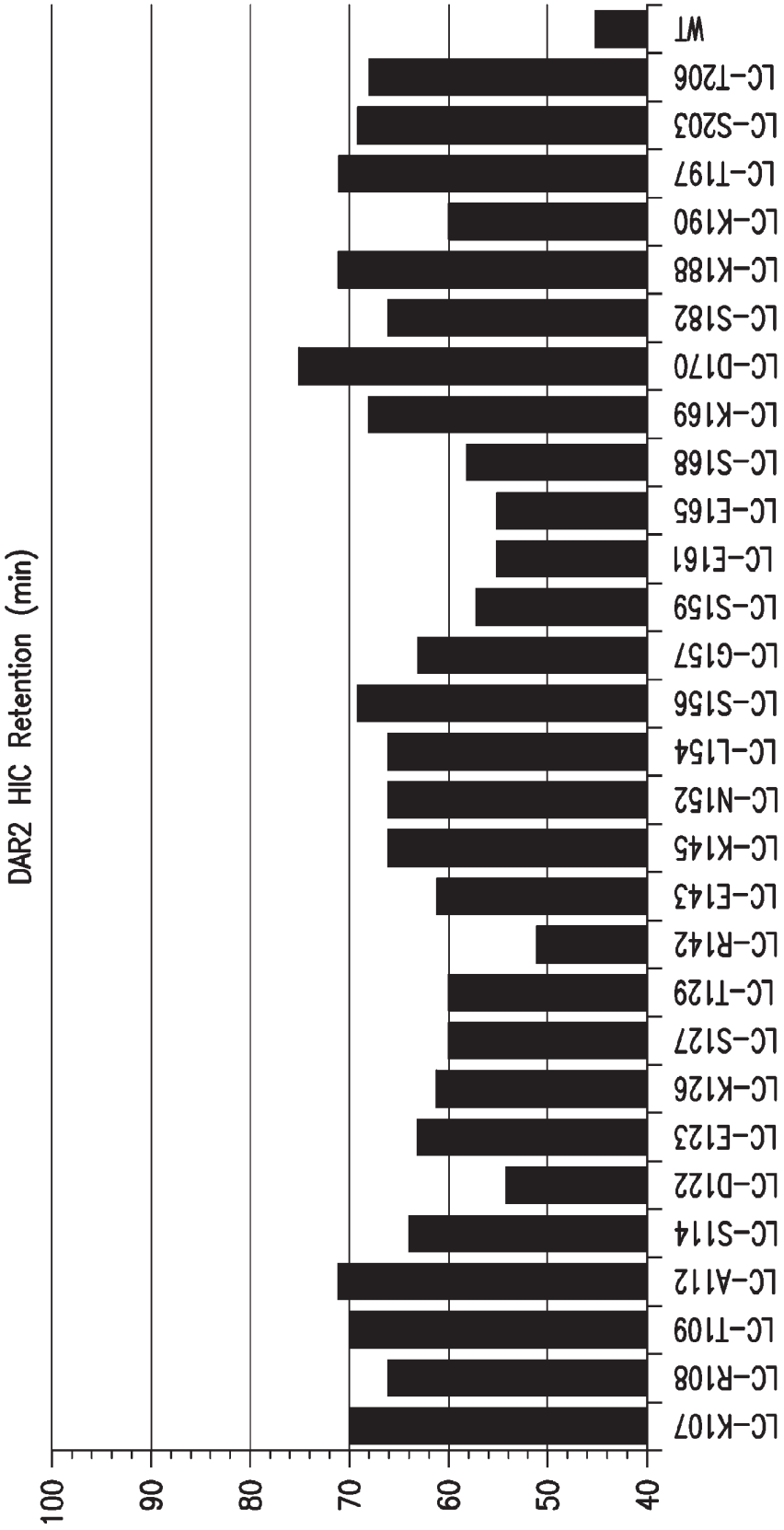

DAR2 HIC Retention (min)
100
90
80
70
60
50
40
WT
LC-T206
LC-S203
LC-T197
LC-K190
LC-K188
LC-S182
LC-D170
LC-K169
LC-S168
LC-E165
LC-E161
LC-S159
LC-G157
LC-S156
LC-L154
LC-N152
LC-K145
LC-E143
LC-R142
LC-T129
LC-S127
LC-K126
LC-E123
LC-D122
LC-S114
LC-A112
LC-T109
LC-R108
LC-K107

FIG.23B

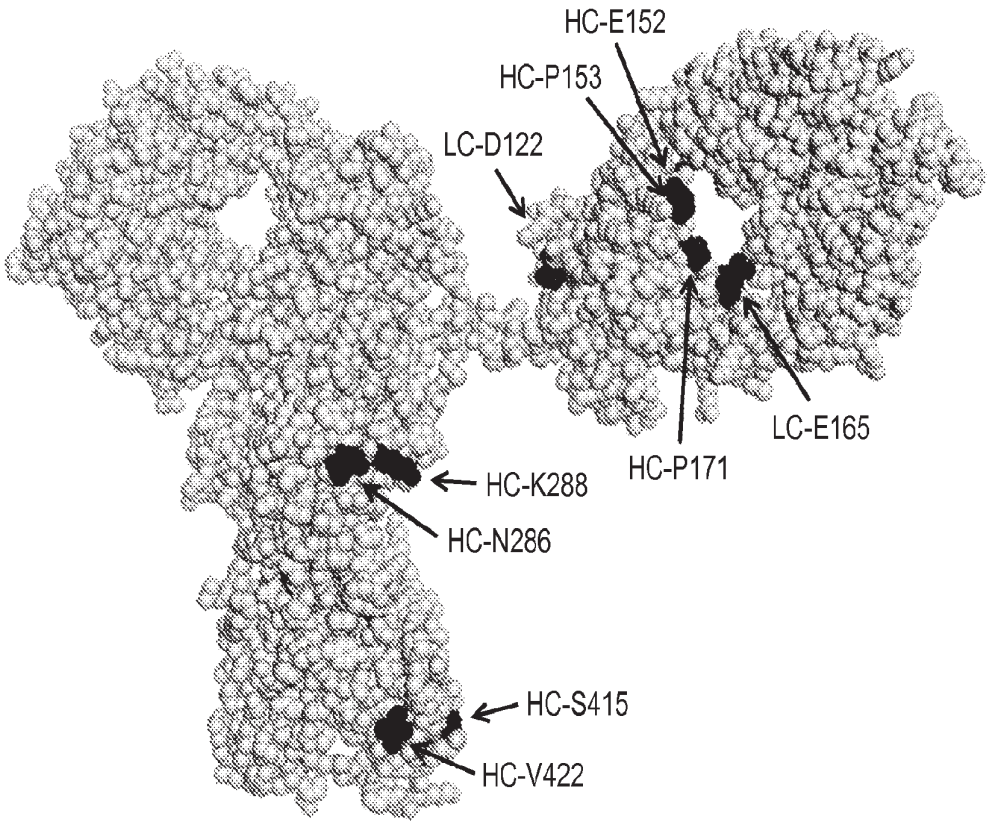

HC-E152
HC-P153
LC-D122
LC-E165
HC-P171
HC-K288
HC-N286
HC-S415
HC-V422

FIG.24A

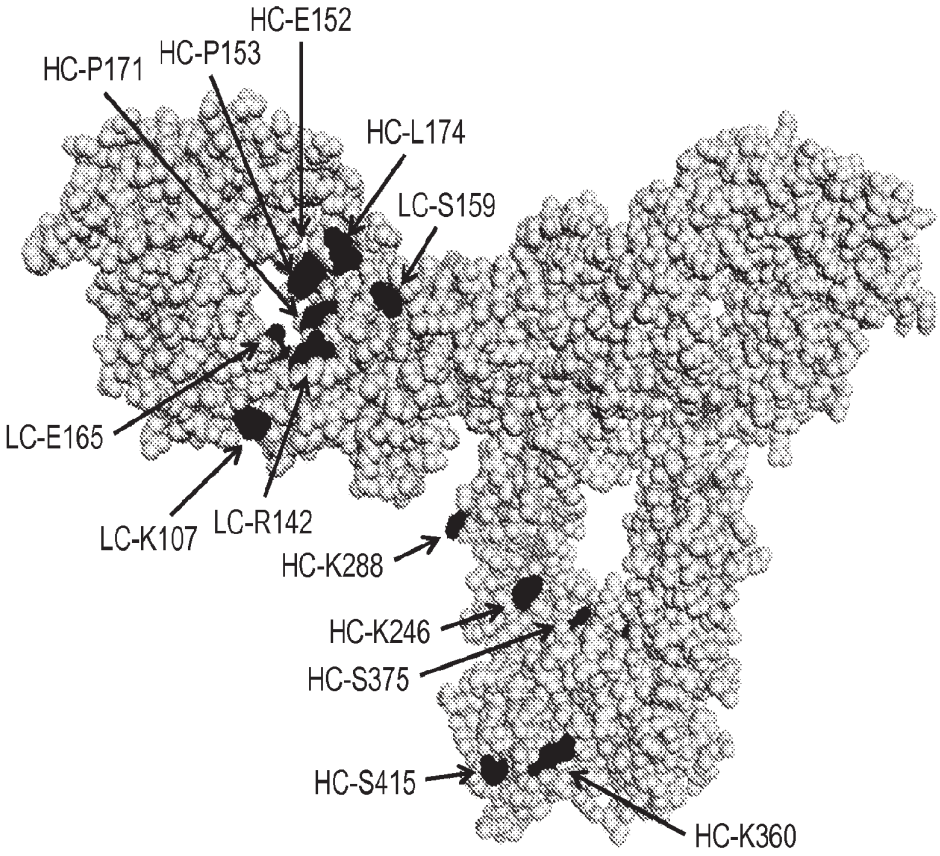

HC-E152
HC-P153
HC-P171
HC-L174
LC-S159
LC-E165
LC-K107
LC-R142
HC-K288
HC-K246
HC-S375
HC-S415
HC-K360

FIG.24B

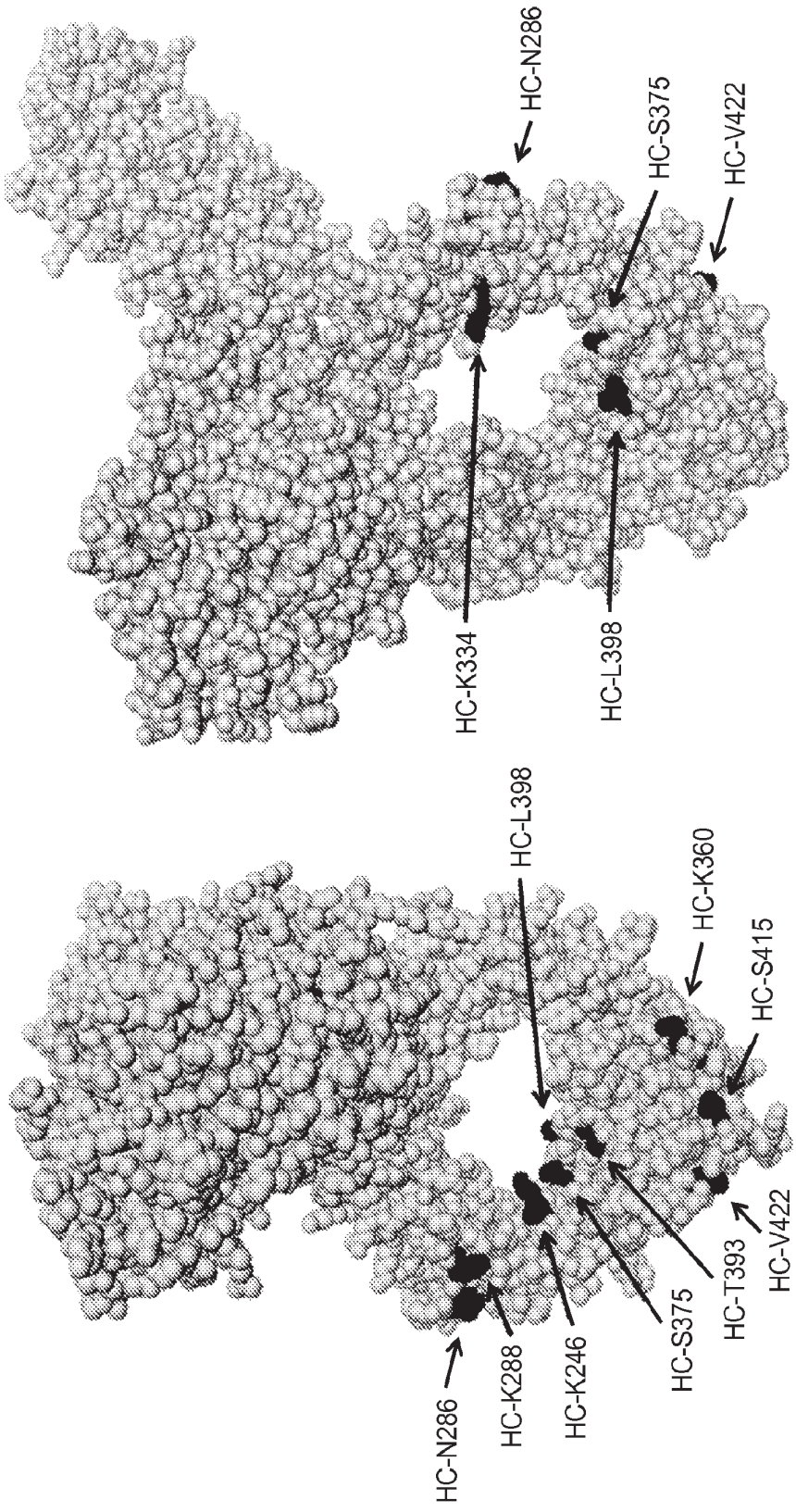

HC-N286
HC-S375
HC-V422
HC-K334
HC-L398
HC-L398
HC-K360
HC-S415
HC-T393
HC-V422
HC-S375
HC-K246
HC-K288
HC-N286

FIG. 24C

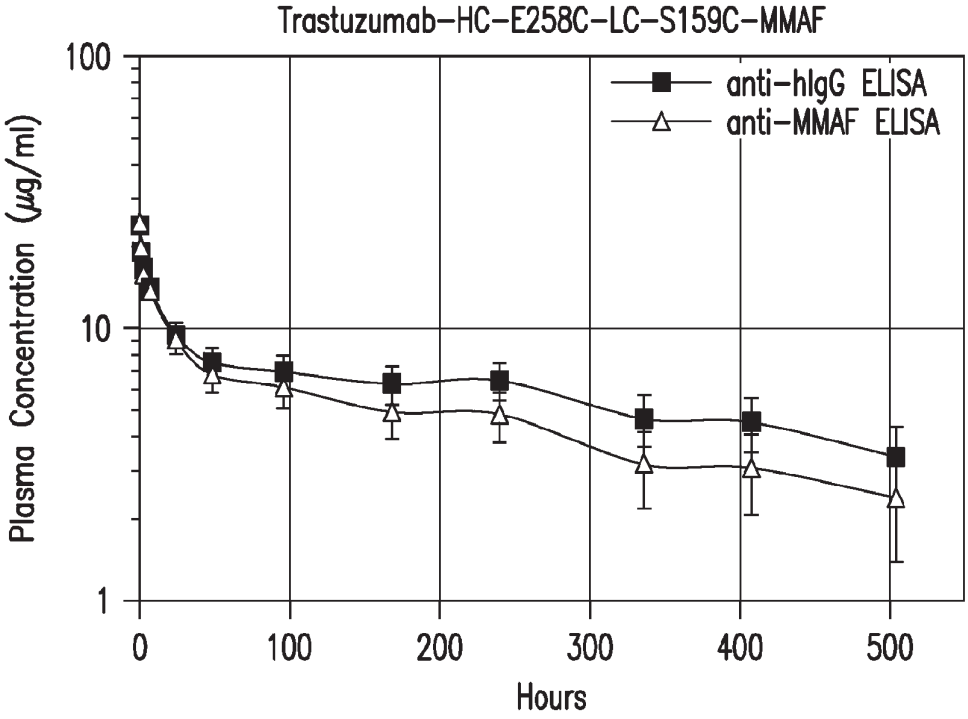

Trastuzumab-HC-E258C-LC-S159C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25A

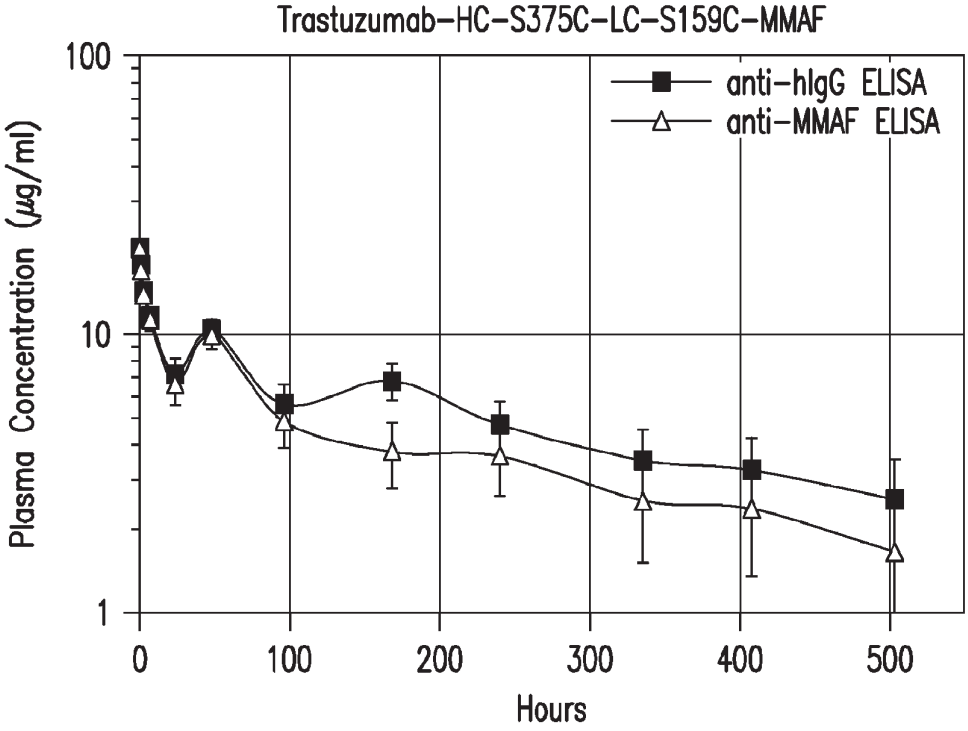

Trastuzumab-HC-S375C-LC-S159C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25B

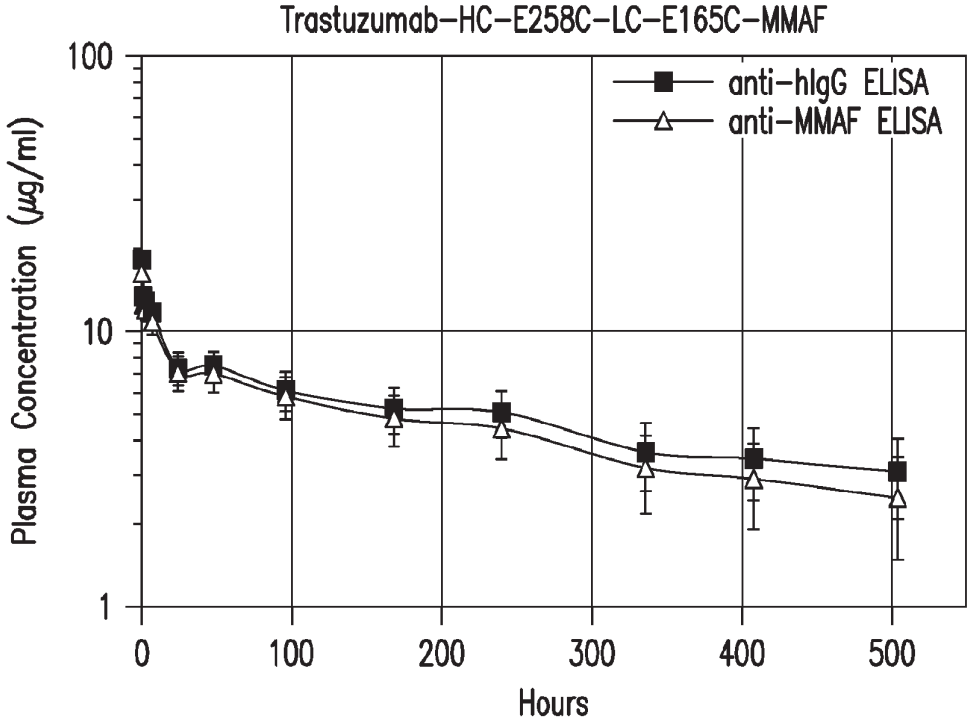

Trastuzumab-HC-E258C-LC-E165C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25C

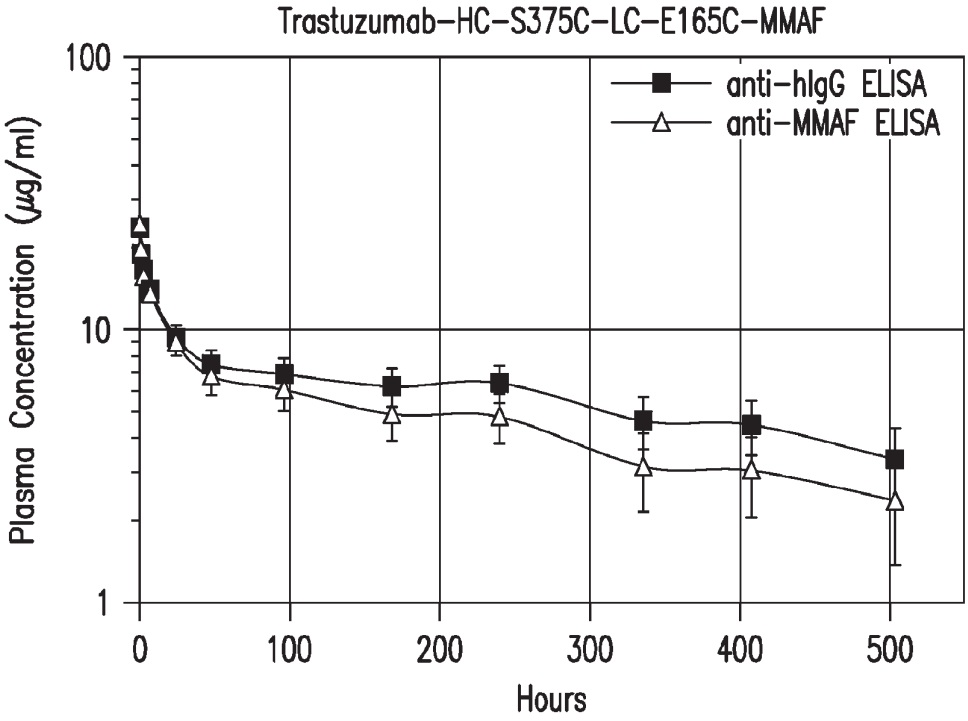

Trastuzumab-HC-S375C-LC-E165C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25D

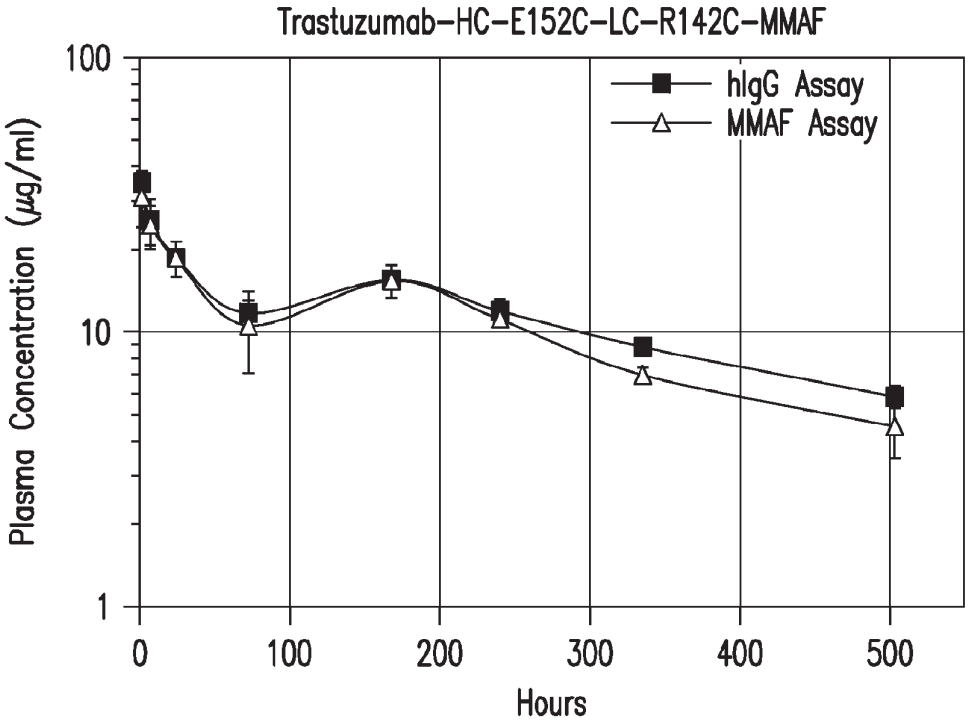

Trastuzumab-HC-E152C-LC-R142C-MMAF
hIgG Assay
MMAF Assay
Plasma Concentration (μg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25E

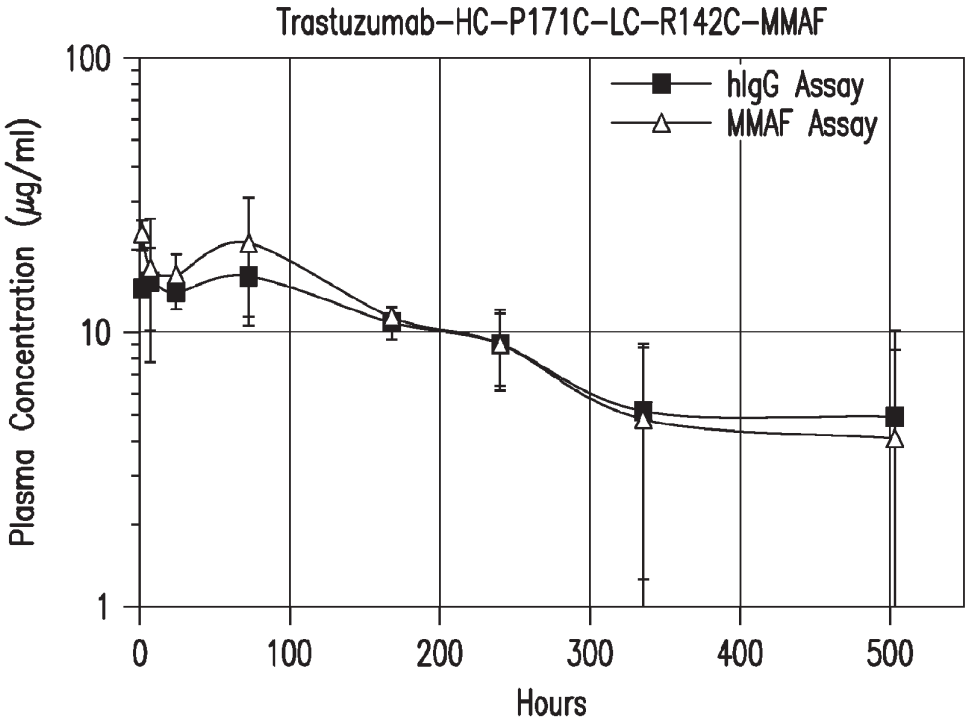

Trastuzumab-HC-P171C-LC-R142C-MMAF
hIgG Assay
MMAF Assay
Plasma Concentration (µg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25F

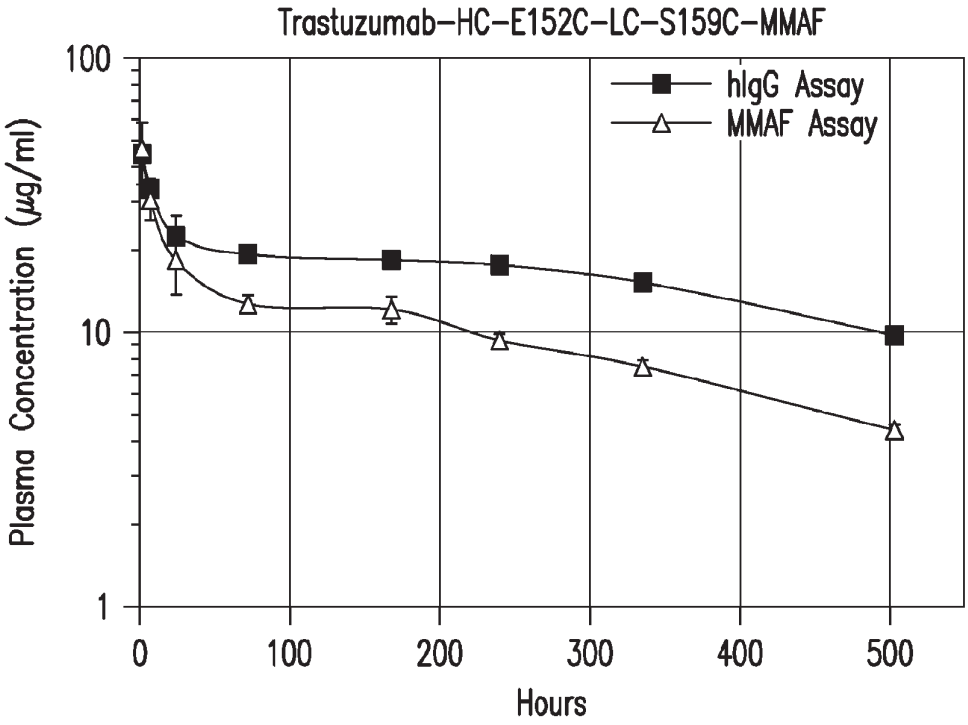

Trastuzumab-HC-E152C-LC-S159C-MMAF
hIgG Assay
MMAF Assay
Plasma Concentration (µg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25G

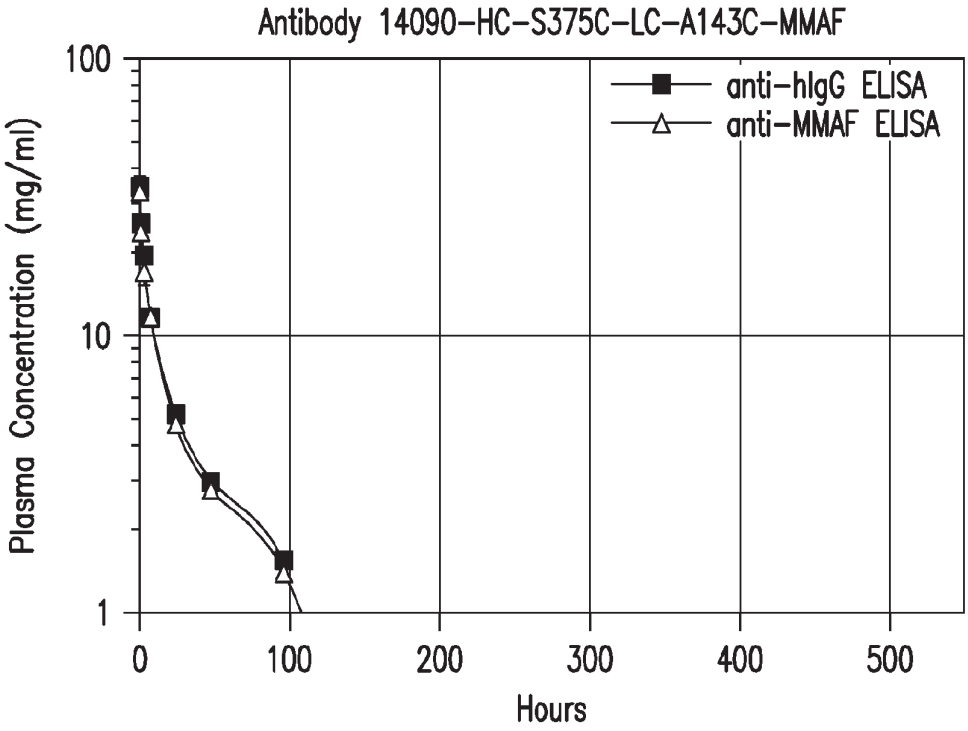

Antibody 14090-HC-S375C-LC-A143C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (mg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25H

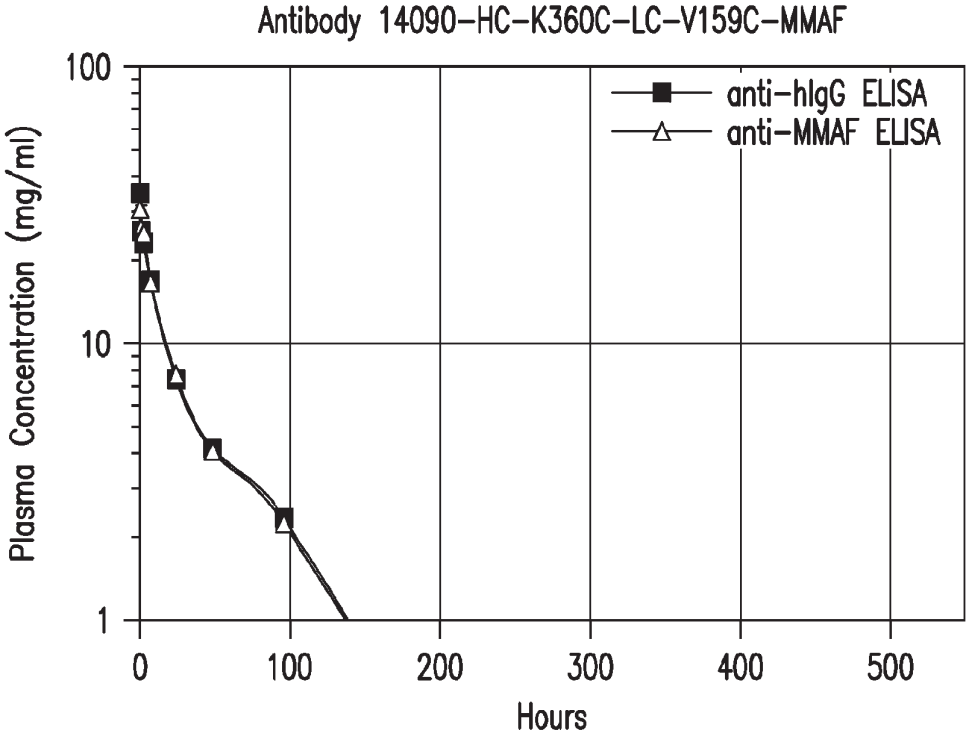

Antibody 14090-HC-K360C-LC-V159C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (mg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25I

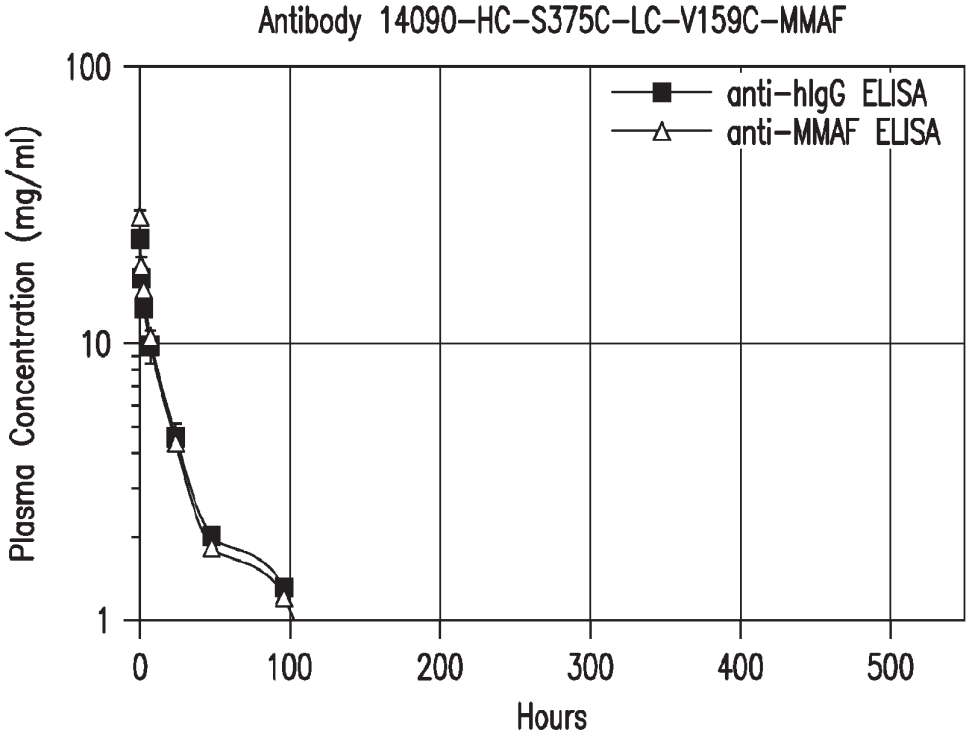

Antibody 14090-HC-S375C-LC-V159C-MMAF
anti-hIgG ELISA
anti-MMAF ELISA
Plasma Concentration (mg/ml)
100
10
1
0
100
200
300
400
500
Hours

FIG.25J

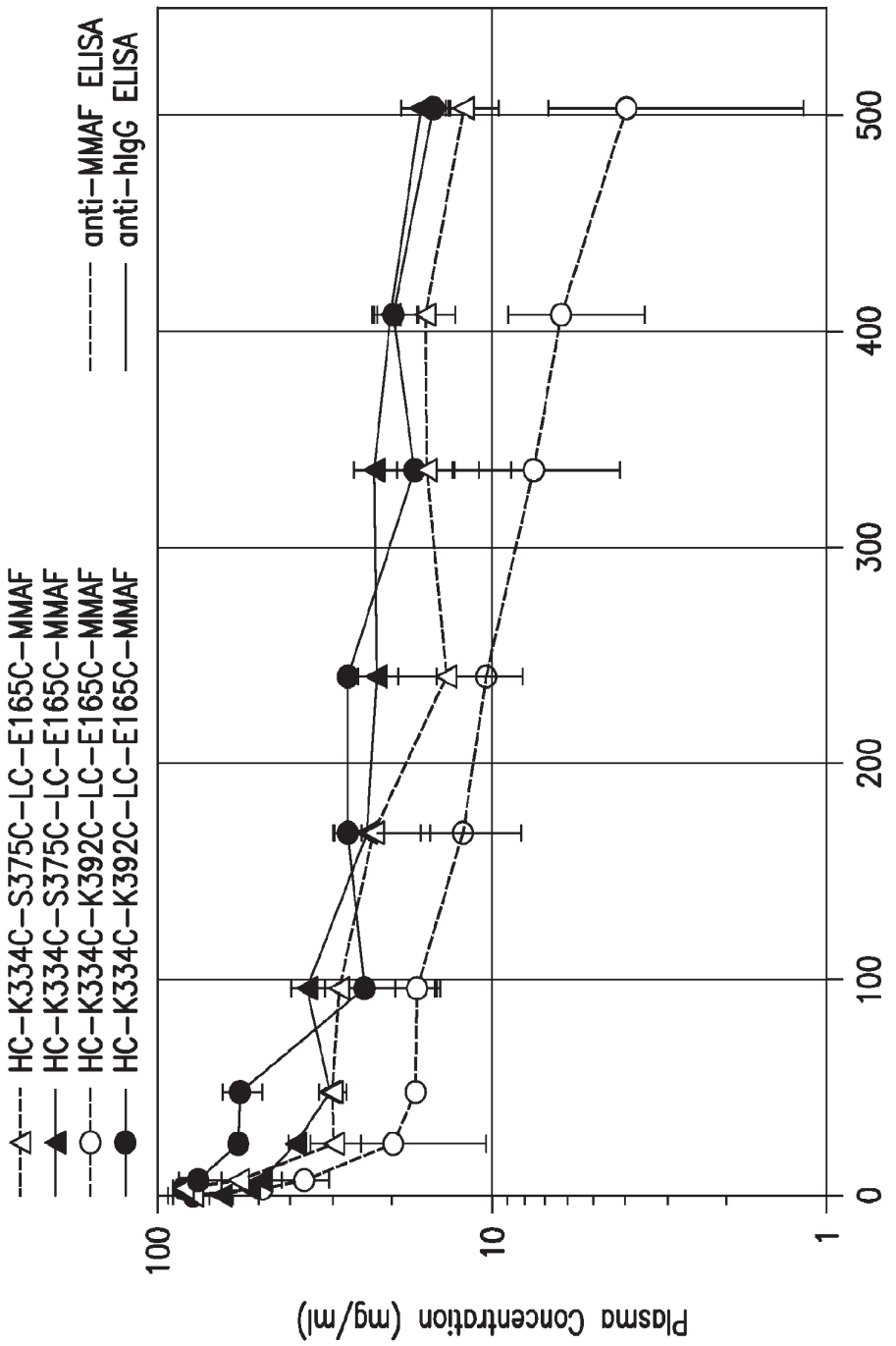

HC-K334C-S375C-LC-E165C-MMAF
HC-K334C-S375C-LC-E165C-MMAF
HC-K334C-K392C-LC-E165C-MMAF
HC-K334C-K392C-LC-E165C-MMAF
anti-MMAF ELISA
anti-hIgG ELISA
Plasma Concentration (mg/ml)
100
10
1
0
100
200
300
400
500
Time (hours)

FIG.25K

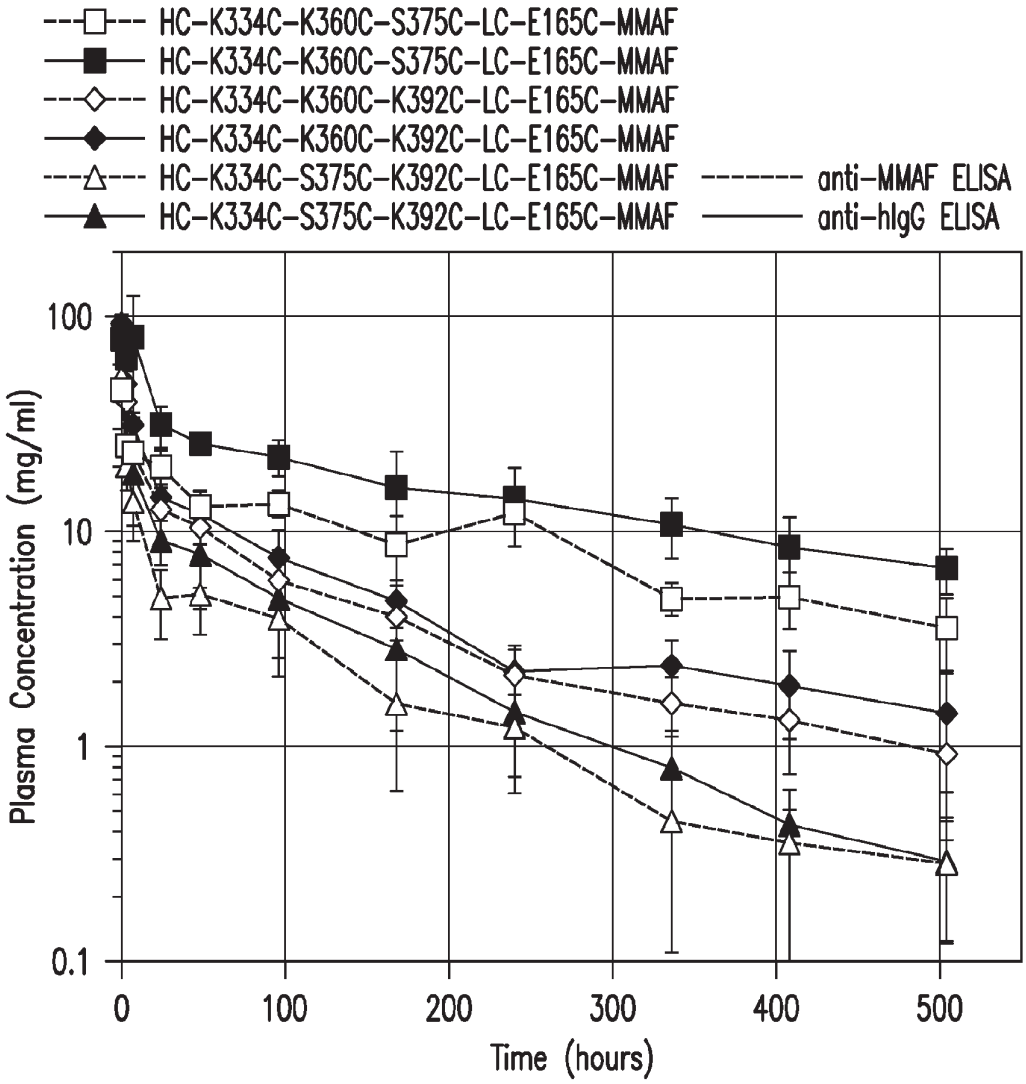

HC-K334C-K360C-S375C-LC-E165C-MMAF
HC-K334C-K360C-S375C-LC-E165C-MMAF
HC-K334C-K360C-K392C-LC-E165C-MMAF
HC-K334C-K360C-K392C-LC-E165C-MMAF
HC-K334C-S375C-K392C-LC-E165C-MMAF
HC-K334C-S375C-K392C-LC-E165C-MMAF
anti-MMAF ELISA
anti-hIgG ELISA
Plasma Concentration (mg/ml)
100
10
1
0.1
0
100
200
300
400
500
Time (hours)

FIG.25L