FIG. 1A

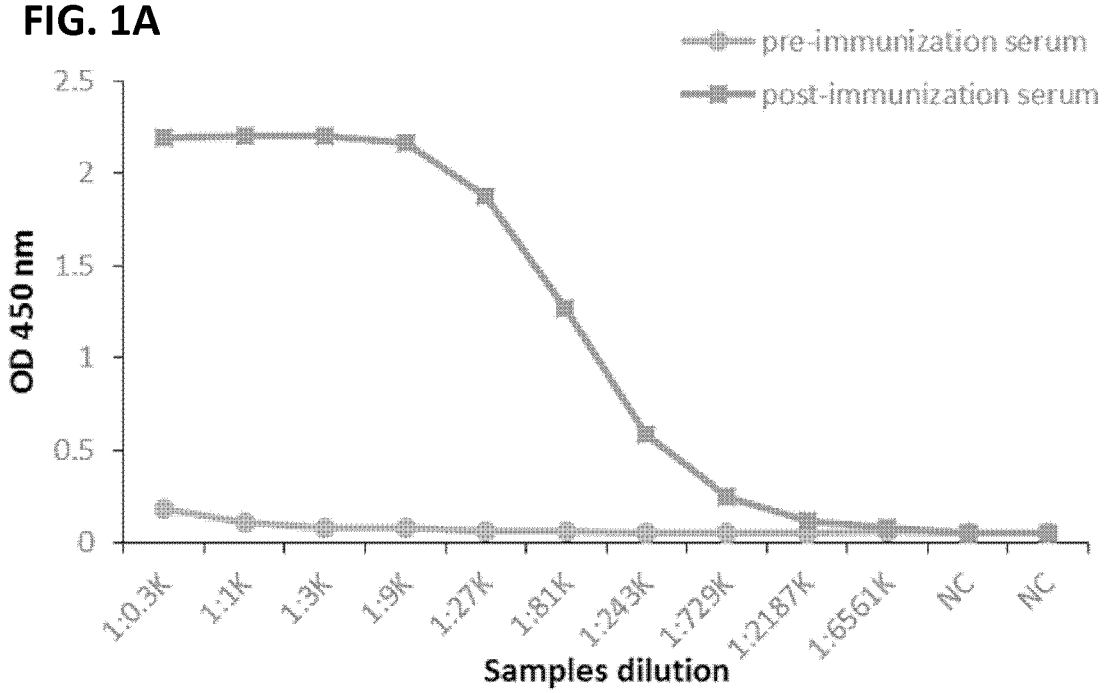

pre-immunization serum
post-immunization serum
OD 450 nm
2.5
2
1.5
1
0.5
0
1:0.3K
1:1K
1:3K
1:9K
1:27K
1:81K
1:243K
1:729K
1:2187K
1:6561K
NC
NC
Samples dilution

FIG. 1B

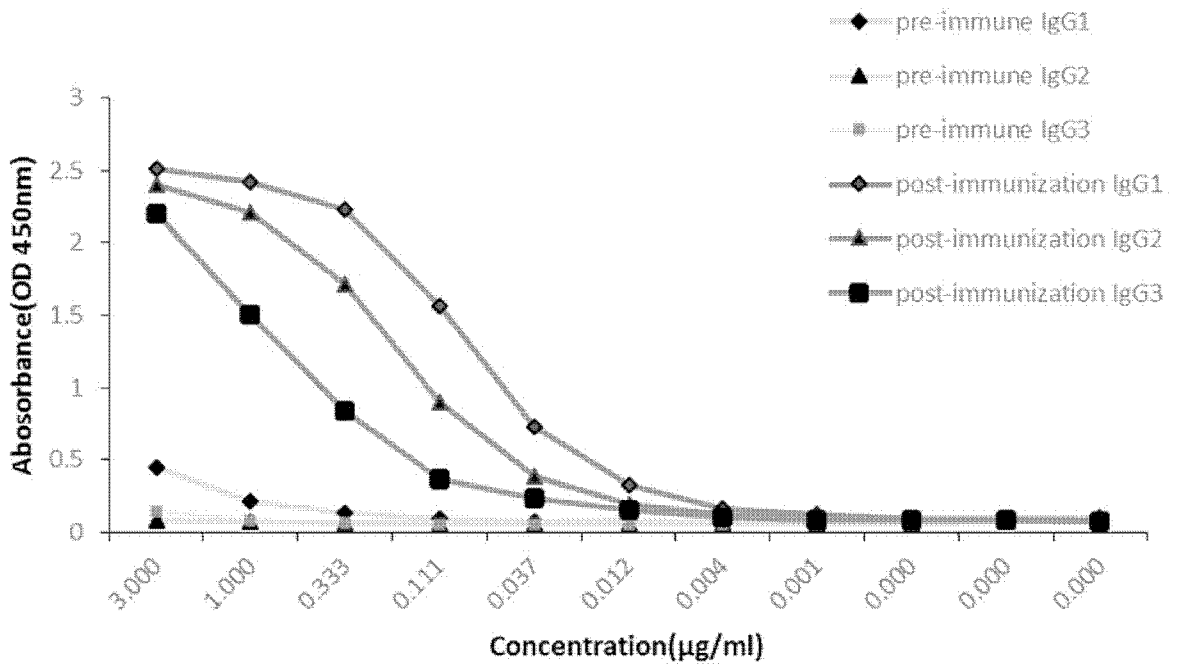

pre-immune IgG1
pre-immune IgG2
pre-immune IgG3
post-immunization IgG1
post-immunization IgG2
post-immunization IgG3
Abosorbance(OD 450nm)
3
2.5
2
1.5
1
0.5
0
3.000
1.000
0.333
0.111
0.037
0.012
0.004
0.001
0.000
0.000
0.000
Concentration(μg/ml)

FIG. 2A

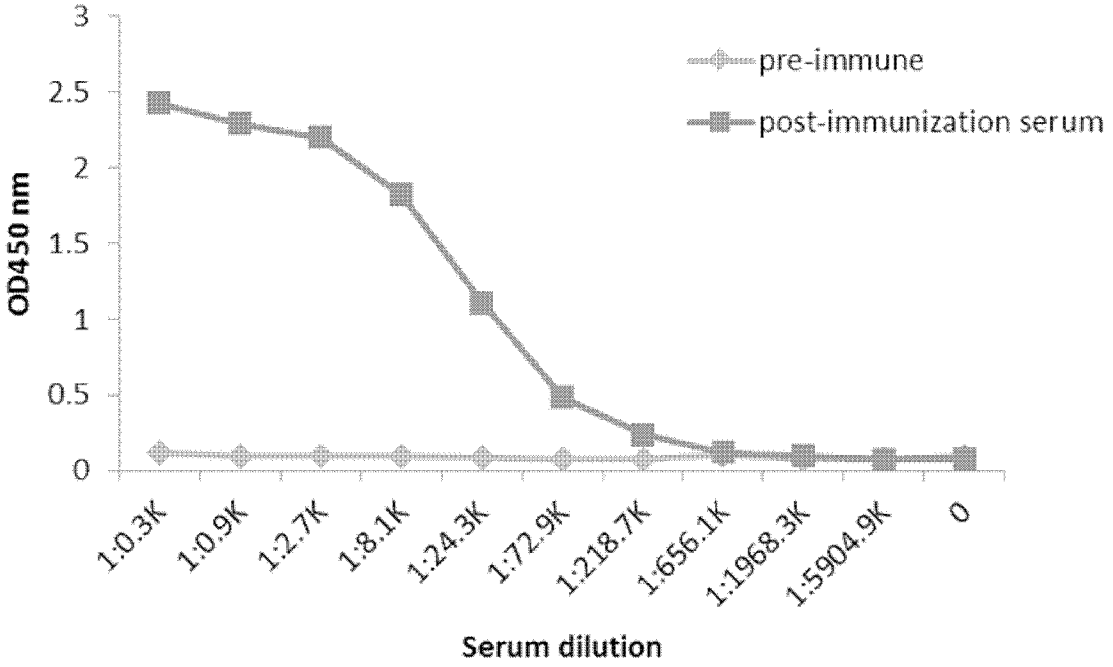

3
2.5
2
1.5
1
0.5
0
OD450 nm
pre-immune
post-immunization serum
1:0.3K
1:0.9K
1:2.7K
1:8.1K
1:24.3K
1:72.9K
1:218.7K
1:656.1K
1:1968.3K
1:5904.9K
0
Serum dilution

FIG. 2B

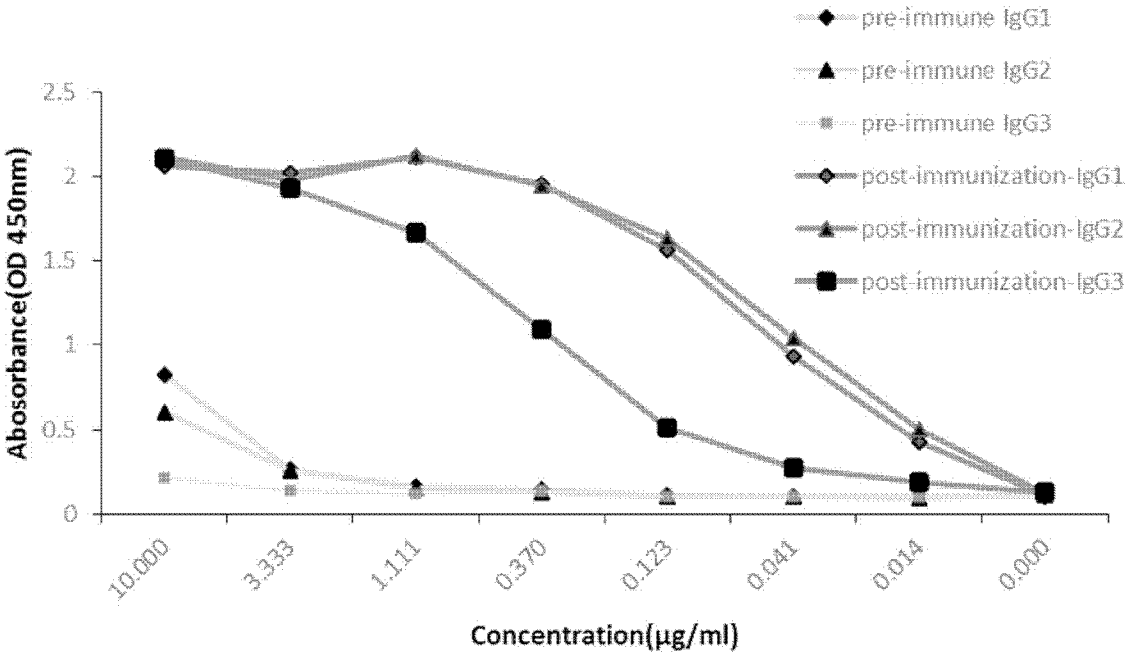

pre-immune IgG1
pre-immune IgG2
pre-immune IgG3
post-immunization-IgG1
post-immunization-IgG2
post-immunization-IgG3
2.5
2
1.5
1
0.5
0
Abosorbance(OD 450nm)
10.000
3.333
1.111
0.370
0.123
0.041
0.014
0.000
Concentration(μg/ml)

FIG. 3A

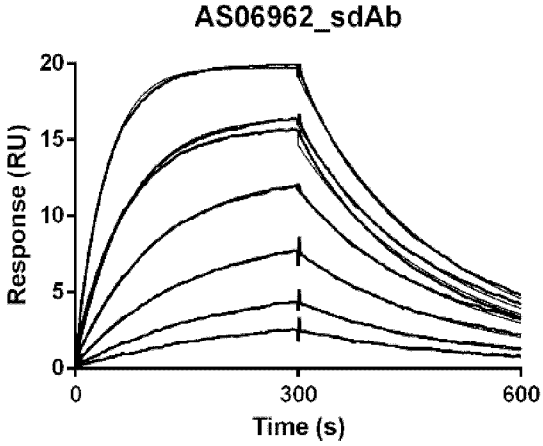


FIG. 3B

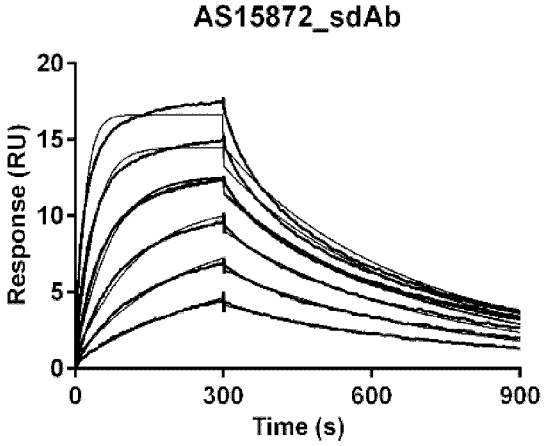


FIG. 3C

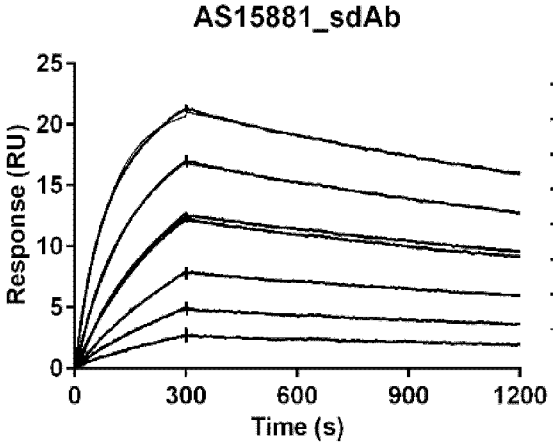


FIG. 3D

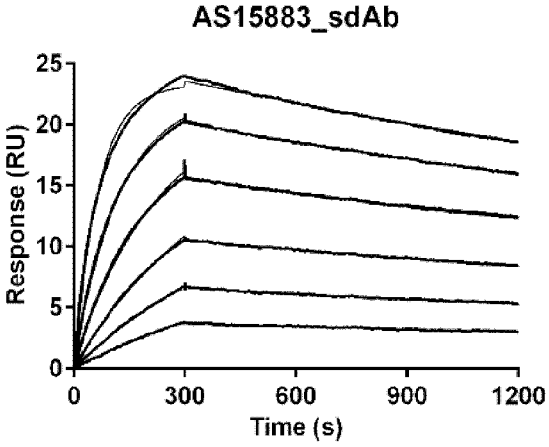


FIG. 3E

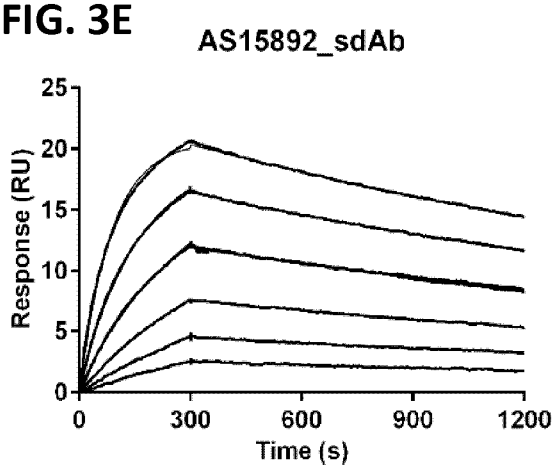


FIG. 3F

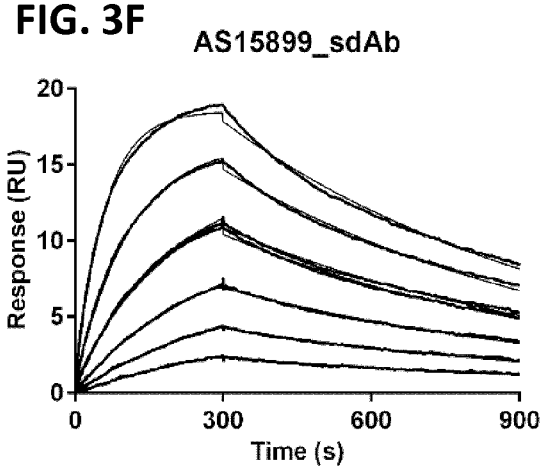


FIG. 3G

Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
AS06962_sdAb	1.26E+07	5.74E-03	4.54E-10
AS15872_sdAb	1.98E+05	2.86E-03	1.45E-08
AS15881_sdAb	1.36E+05	3.13E-04	2.31E-09
AS15883_sdAb	9.49E+04	2.64E-04	2.78E-09
AS15892_sdAb	1.45E+05	3.86E-04	2.67E-09
AS15899_sdAb	4.25E+05	1.37E-03	3.22E-09

FIG. 4A

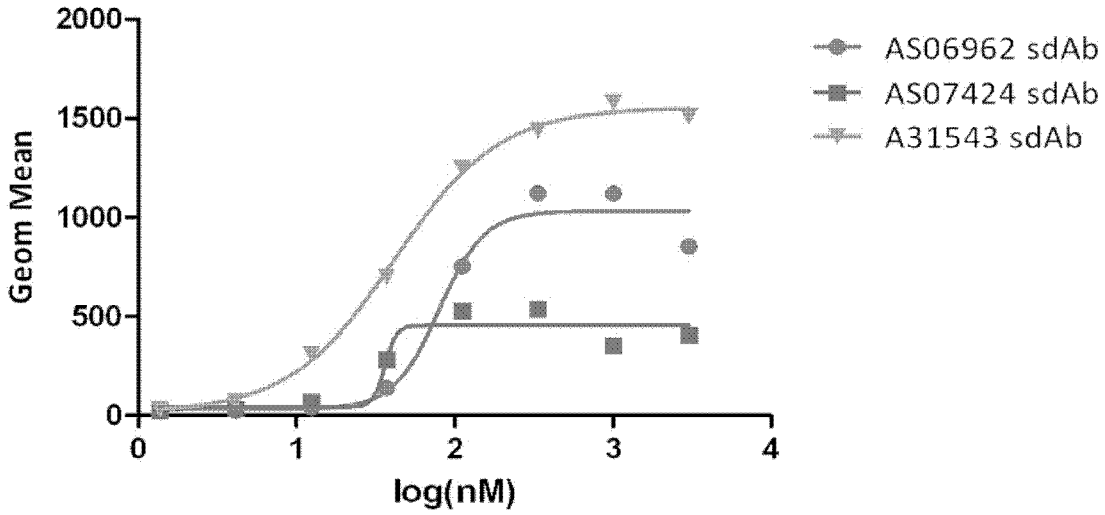


FIG. 4B

	AS06962 sdAb	AS07424 sdAb	A31543 sdAb
EC_{50} (nM)	79.24	36.7	41.17

FIG. 5A

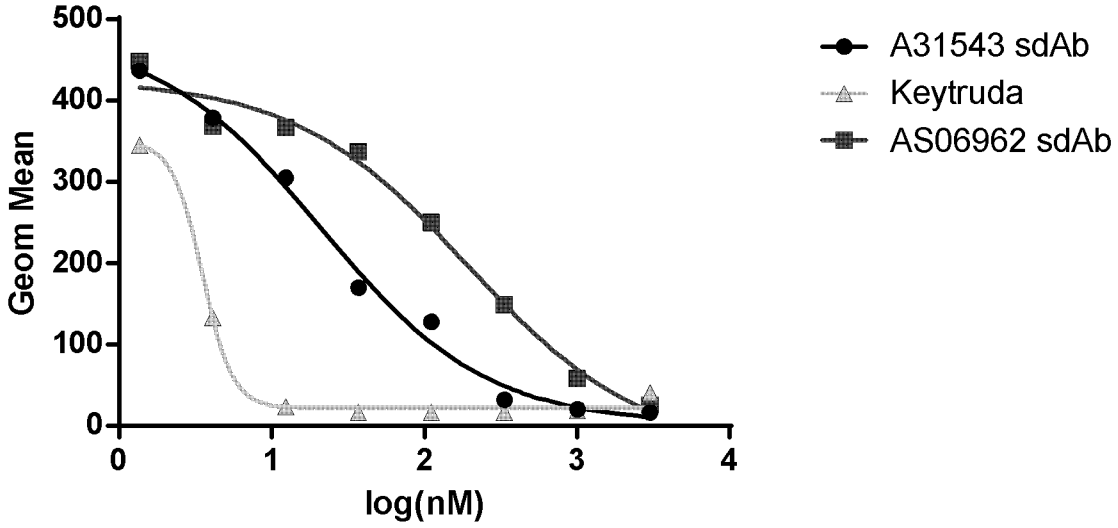


FIG. 5B

	AS06962 sdAb	A31543 sdAb	Keytruda
IC_{50}	193.2	21.1	3.5

FIG. 6A

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
AS15140_HCAb	PD-1 His	1.79E+05	1.88E-03	1.05E-08
AS15152_HCAb		1.94E+05	3.55E-03	1.83E-08
AS15156_HCAb		6.66E+04	1.78E-04	2.67E-09
AS15193_HCAb		7.90E+04	7.51E-05	9.51E-10
AS06962_HCAb		1.80E+05	7.10E-03	3.90E-08
AS15881_HCAb		1.00E+05	3.50E-04	3.30E-09
AS15883_HCAb		8.20E+04	3.20E-04	3.90E-09
AS15892_HCAb		1.10E+05	4.30E-04	4.00E-09
AS15899_HCAb		2.20E+05	8.40E-04	3.80E-09
AS25170_HCAb		5.60E+04	5.50E-04	9.80E-09
Keytruda		2.50E+05	2.60E-03	1.00E-08

FIG. 6B

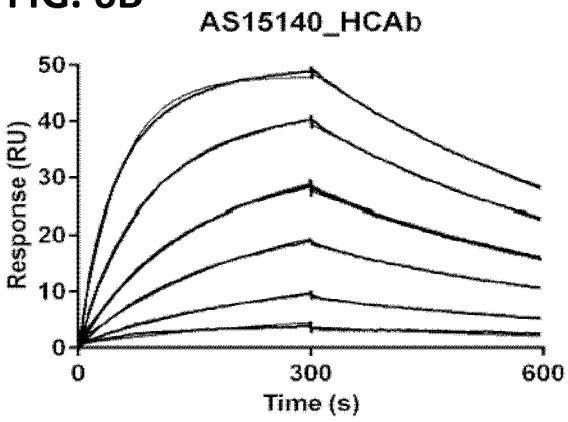


FIG. 6C

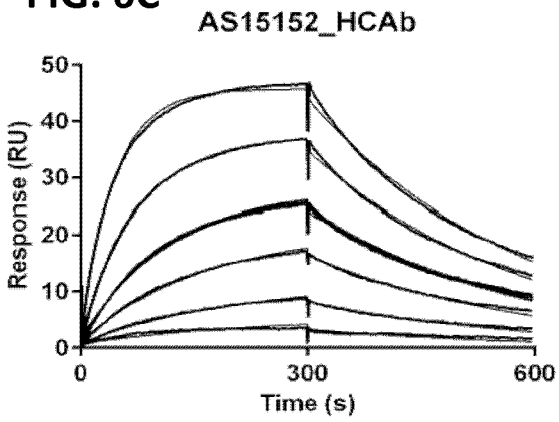


FIG. 6D

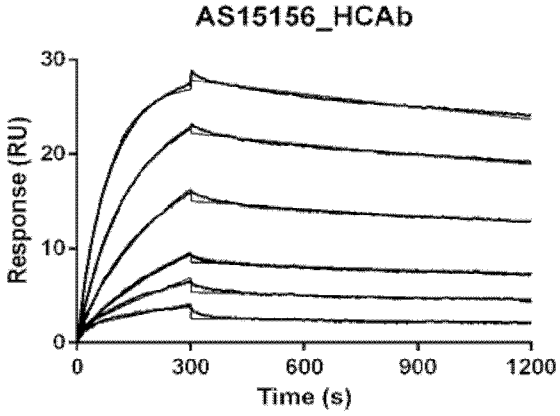


FIG. 6E

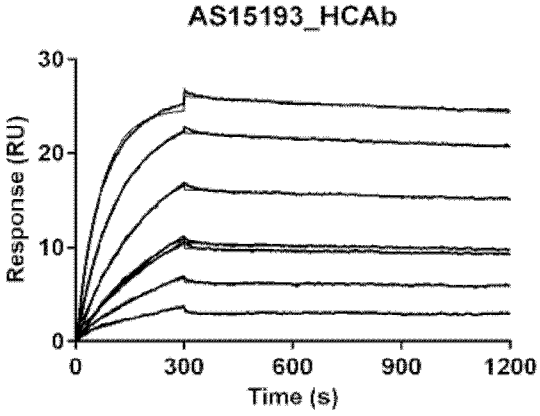


FIG. 6F

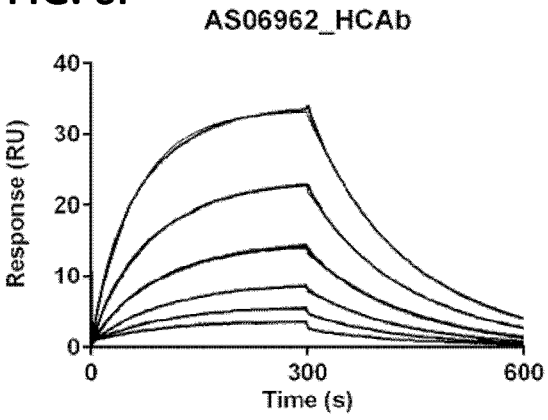


FIG. 6G

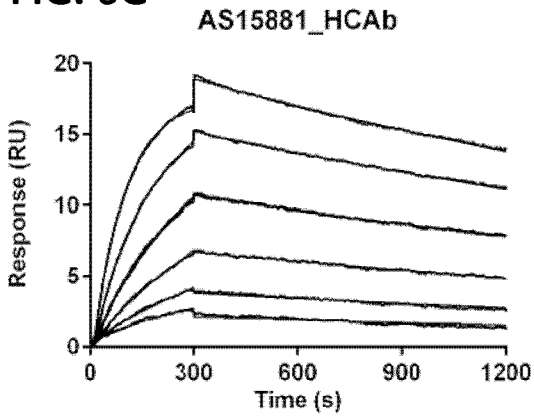


FIG. 6H

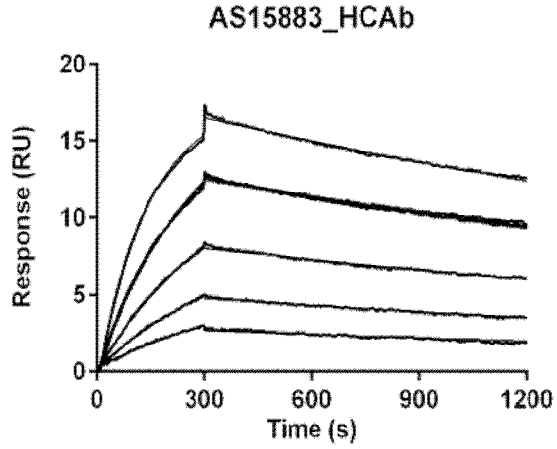


FIG. 6I

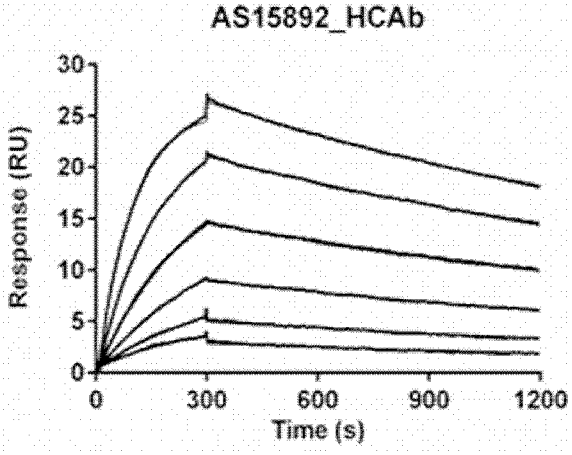


FIG. 6J

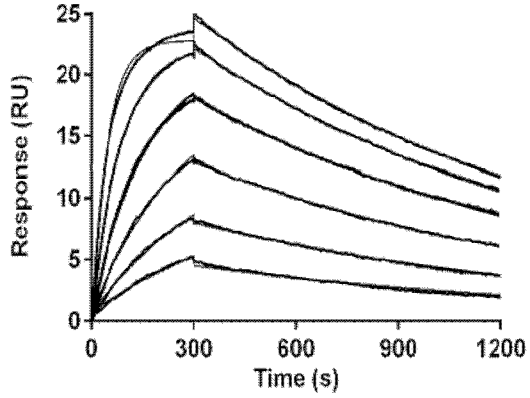


FIG. 6K

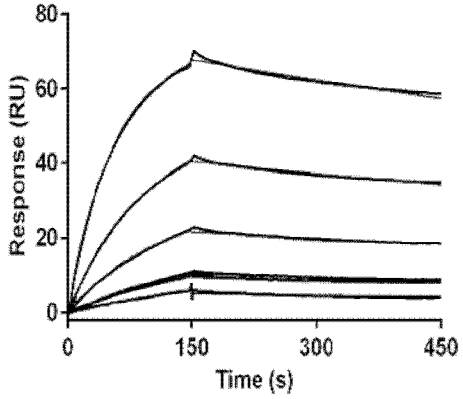


FIG. 6L

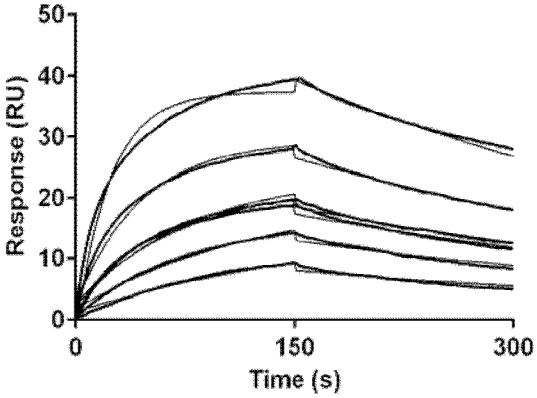


FIG. 7A

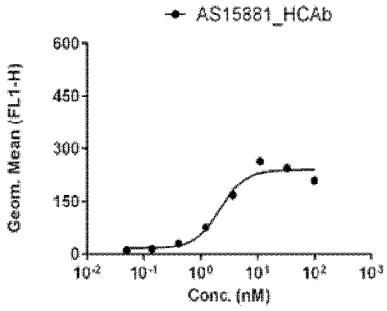


FIG. 7B

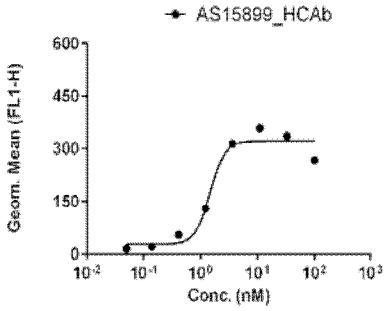


FIG. 7C

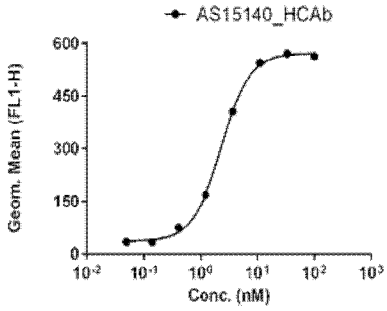


FIG. 7D

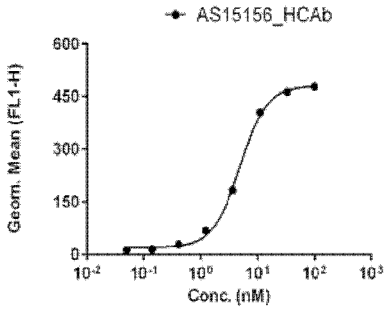


FIG. 7E

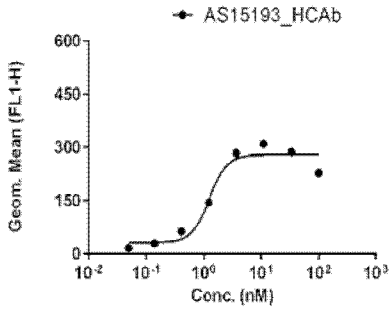


FIG. 7F

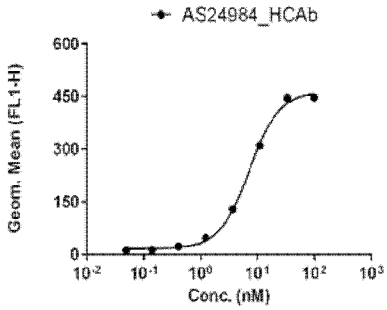


FIG. 7G

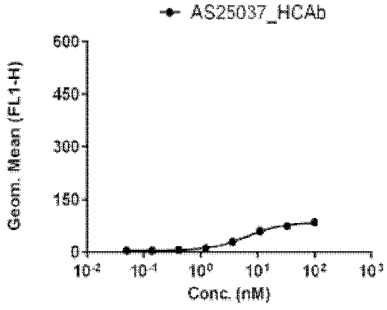


FIG. 7H

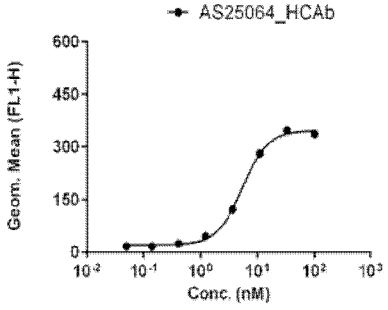


FIG. 7I

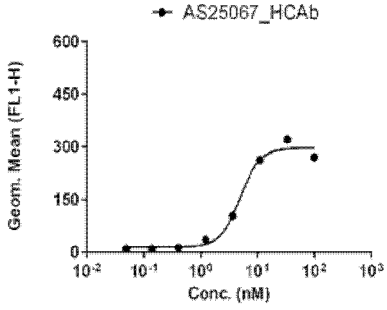


FIG. 7J

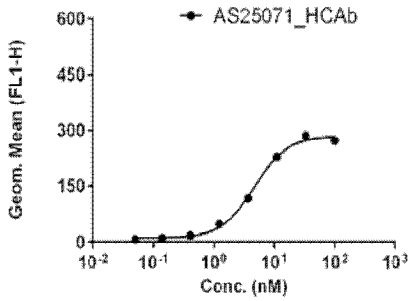


FIG. 7K

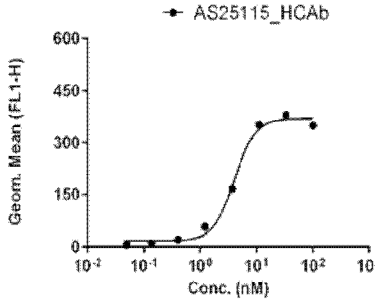


FIG. 7L

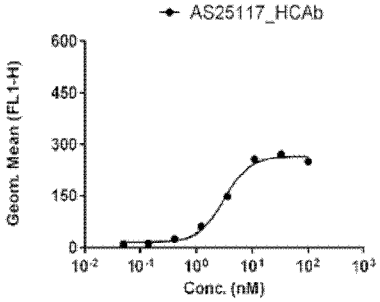


FIG. 7M

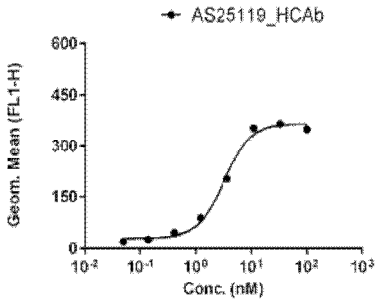


FIG. 7N

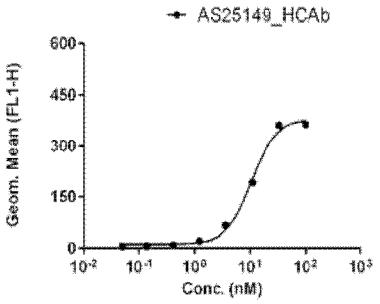
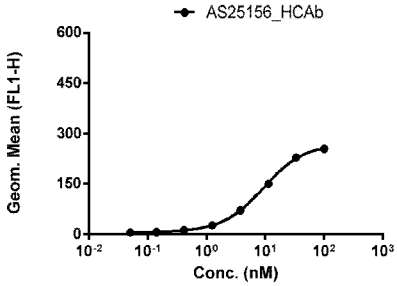


FIG. 7O



EC50	9.180

FIG. 7P

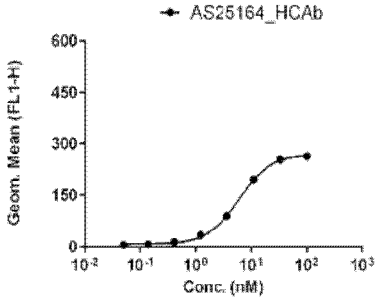


FIG. 7Q

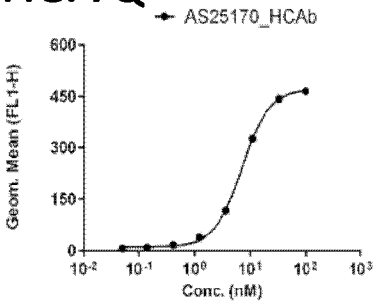


FIG. 7R

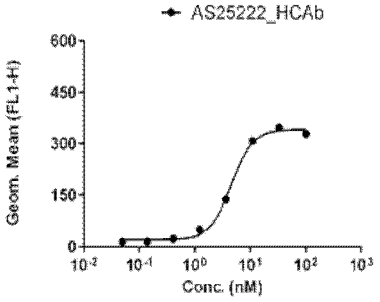


FIG. 7S

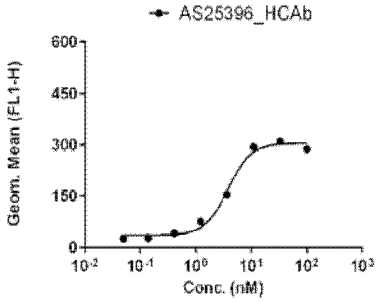


FIG. 7T

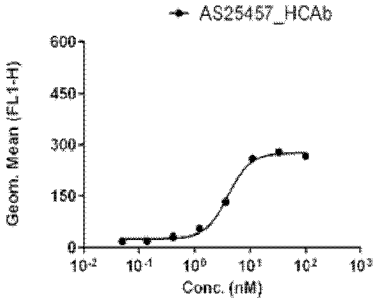


FIG. 7U

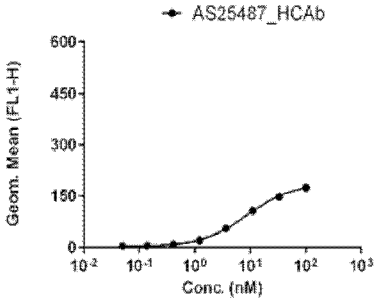


FIG. 7V

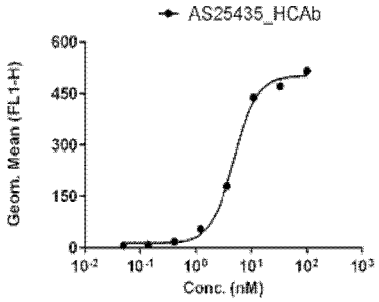


FIG. 7W

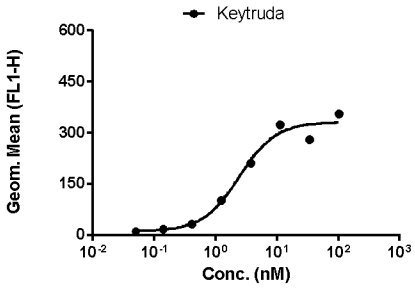


FIG. 7X

Sample	AS15881_ HCAb	AS15899_ HCAb	AS15140_ HCAb	AS15156_ HCAb	AS15193_ HCAb
EC_{50} (nM)	2.18	1.48	2.32	4.91	1.26
Sample	AS25071_ HCAb	AS25115_ HCAb	AS25117_ HCAb	AS25119_ HCAb	AS25149_ HCAb
EC_{50} (nM)	4.74	4.06	3.22	3.29	10.65
Sample	AS25396_ HCAb	AS25457_ HCAb	AS25487_ HCAb	AS25435_ HCAb	Keytruda
EC_{50} (nM)	3.84	4.04	8.94	4.91	2.44
Sample	AS24984_ HCAb	AS25037_ HCAb	AS25064_ HCAb	AS25067_ HCAb	AS25156_ HCAb
EC_{50} (nM)	7.22	6.62	5.46	4.96	9.18
Sample	AS25164_ HCAb	AS25170_ HCAb	AS25222_ HCAb		
EC_{50} (nM)	6.15	7.20	4.59		

FIG. 8A

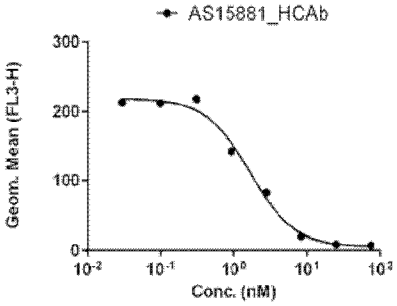


FIG. 8B

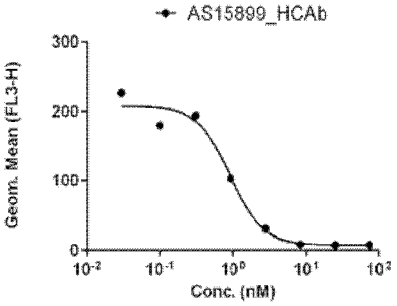


FIG. 8C

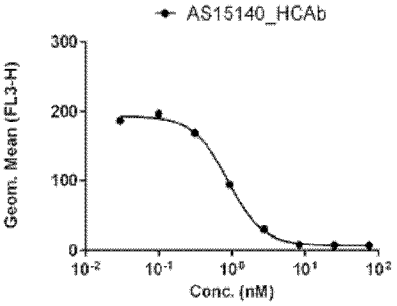


FIG. 8D

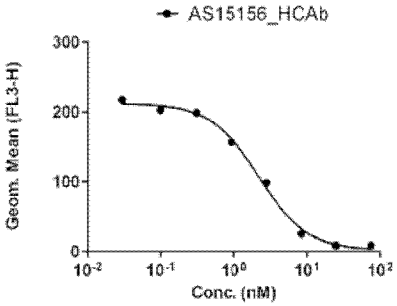


FIG. 8E

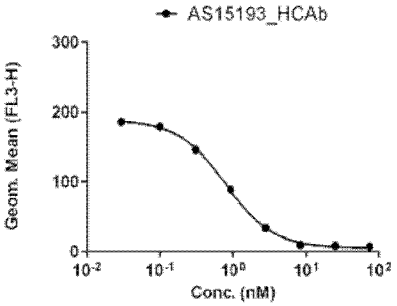


FIG. 8F

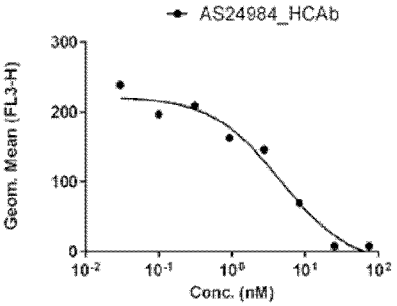


FIG. 8G

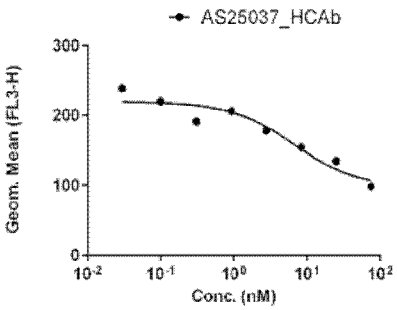


FIG. 8H

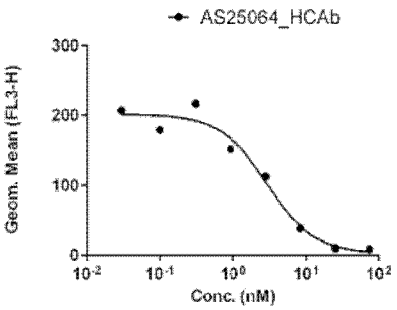


FIG. 8I

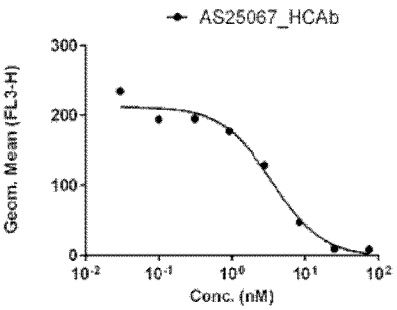


FIG. 8J

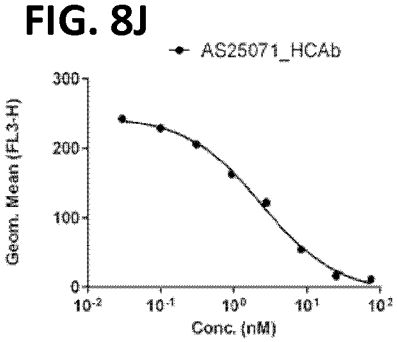


FIG. 8K

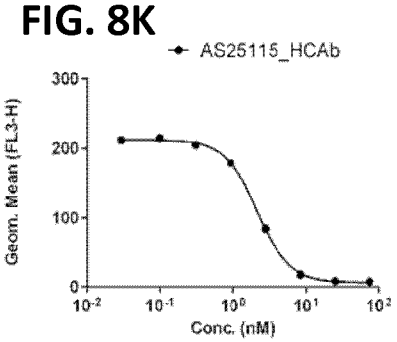


FIG. 8L

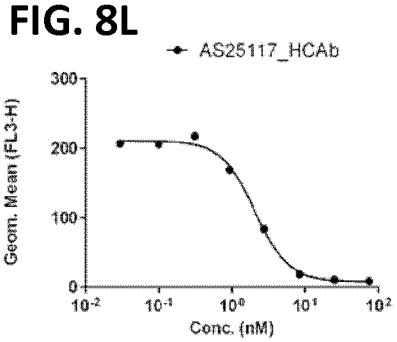


FIG. 8M

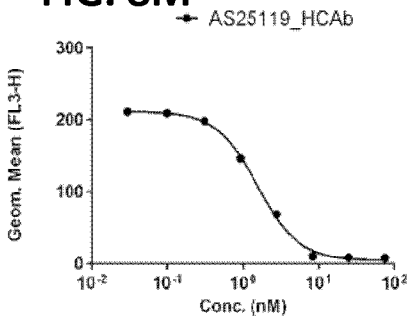


FIG. 8N

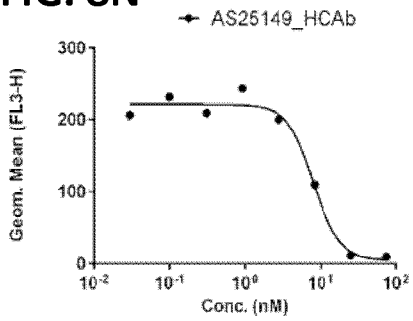


FIG. 8O

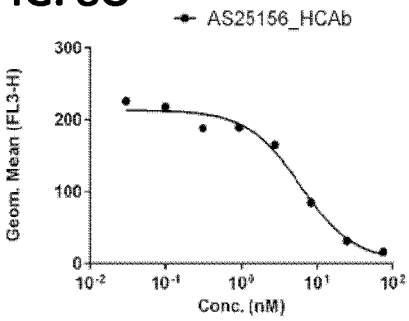


FIG. 8P

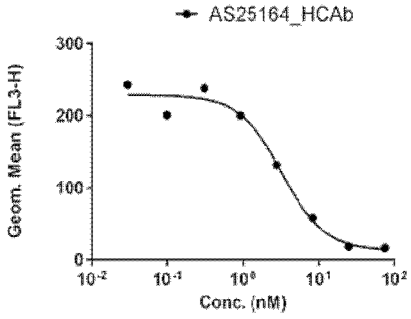


FIG. 8Q

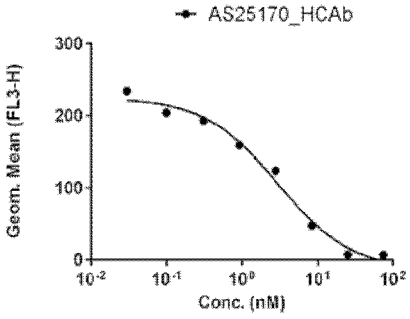


FIG. 8R

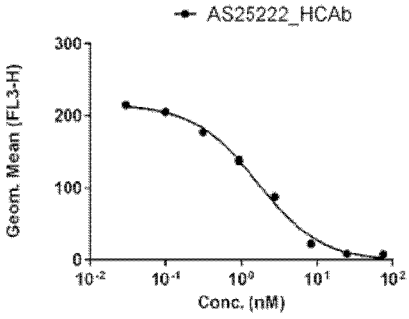


FIG. 8S

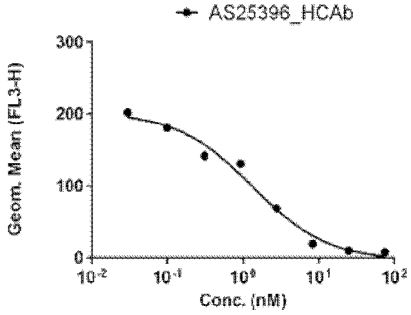


FIG. 8T

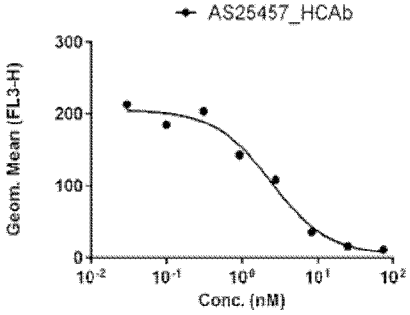


FIG. 8U

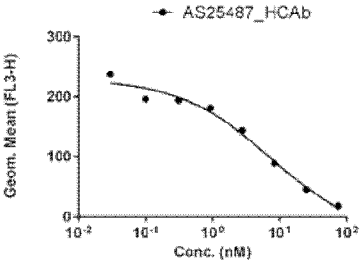


FIG. 8V

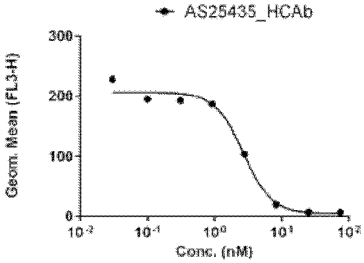


FIG. 8W

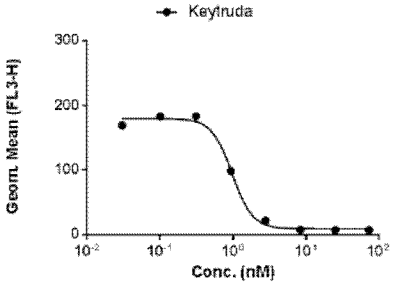


FIG. 8X

Sample	AS15881_ HCAb	AS15899_ HCAb	AS15140_ HCAb	AS15156_ HCAb	AS15193_ HCAb
IC_{50} (nM)	1.66	0.91	0.88	2.20	0.79
Sample	AS25071_ HCAb	AS25115_ HCAb	AS25117_ HCAb	AS25119_ HCAb	AS25149_ HCAb
IC_{50} (nM)	2.41	2.15	2.06	1.57	8.00
Sample	AS25396_ HCAb	AS25457_ HCAb	AS25487_ HCAb	AS25435_ HCAb	Keytruda
IC_{50} (nM)	1.34	2.44	7.24	2.70	0.97
Sample	AS24984_ HCAb	AS25037_ HCAb	AS25064_ HCAb	AS25067_ HCAb	AS25156_ HCAb
IC_{50} (nM)	4.59	6.58	2.91	3.51	6.04
Sample	AS25164_ HCAb	AS25170_ HCAb	AS25222_ HCAb		
IC_{50} (nM)	3.25	3.01	1.66		

FIG. 9A

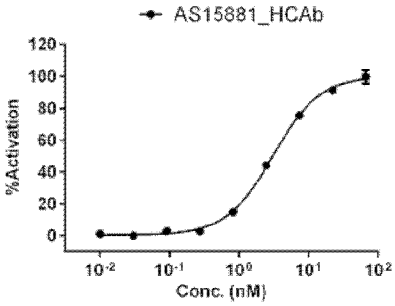


FIG. 9B

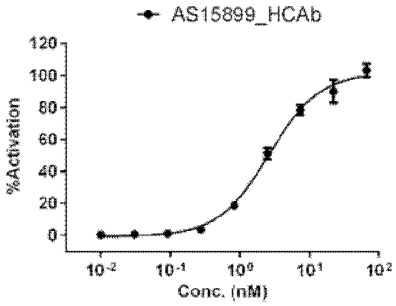


FIG. 9C

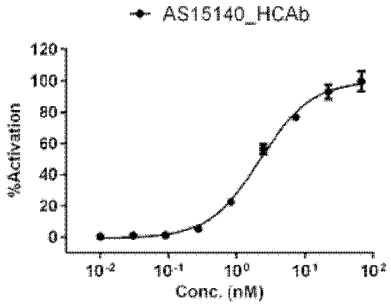


FIG. 9D

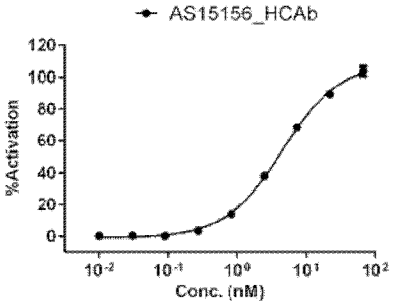


FIG. 9E

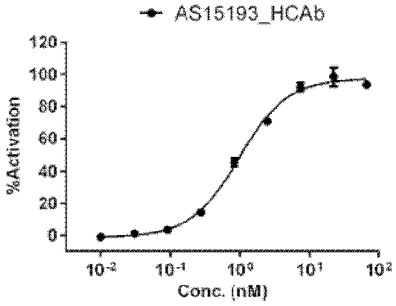


FIG. 9F

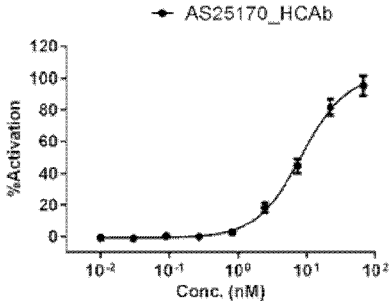


FIG. 9G

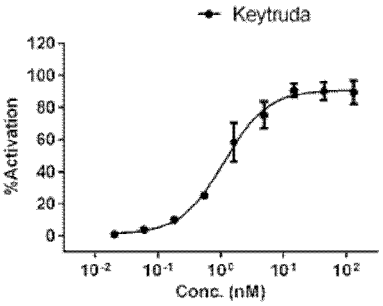


FIG. 9H

Sample	AS15881_HCAb	AS15899_HCAb	AS15140_HCAb	AS15156_HCAb
EC_{50} (nM)	3.09	2.60	2.21	4.51
Sample	AS15193_HCAb	AS25170_HCAb	Keytruda	
EC_{50} (nM)	0.97	8.36	1.59	

FIG. 10A

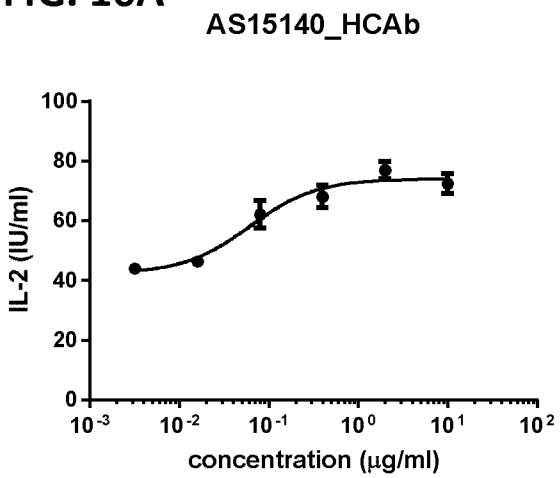


FIG. 10B

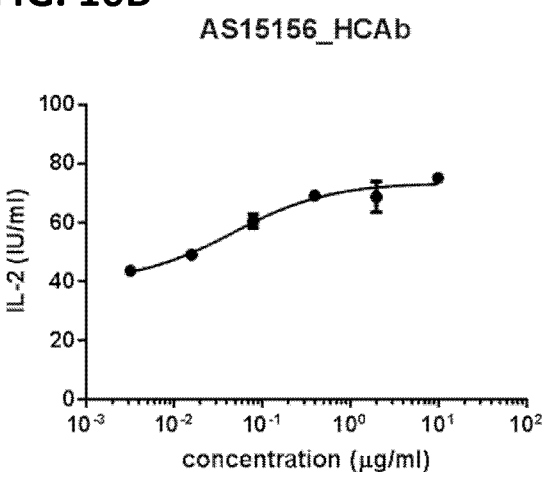


FIG. 10C

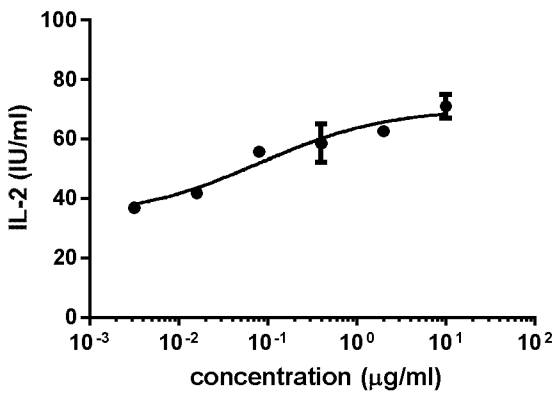


FIG. 10D

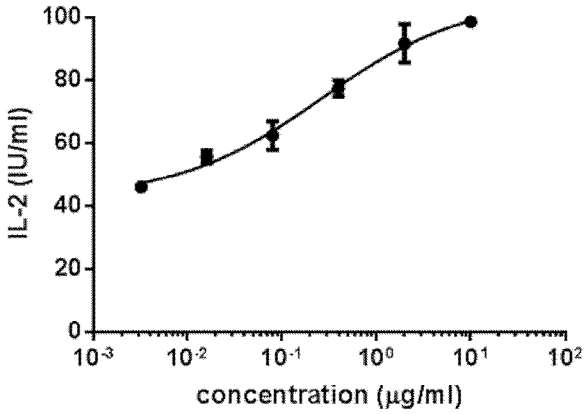


FIG. 10E

Sample ID	Best-fit values		
	Bottom	Top	EC_{50} (μg/ml)
AS15140_HCAb	42.26	74.11	0.062
AS15156_HCAb	40.08	73.57	0.048
AS15193_HCAb	33.56	85.39	0.082
Keytruda	46.59	98	0.330

FIG. 11

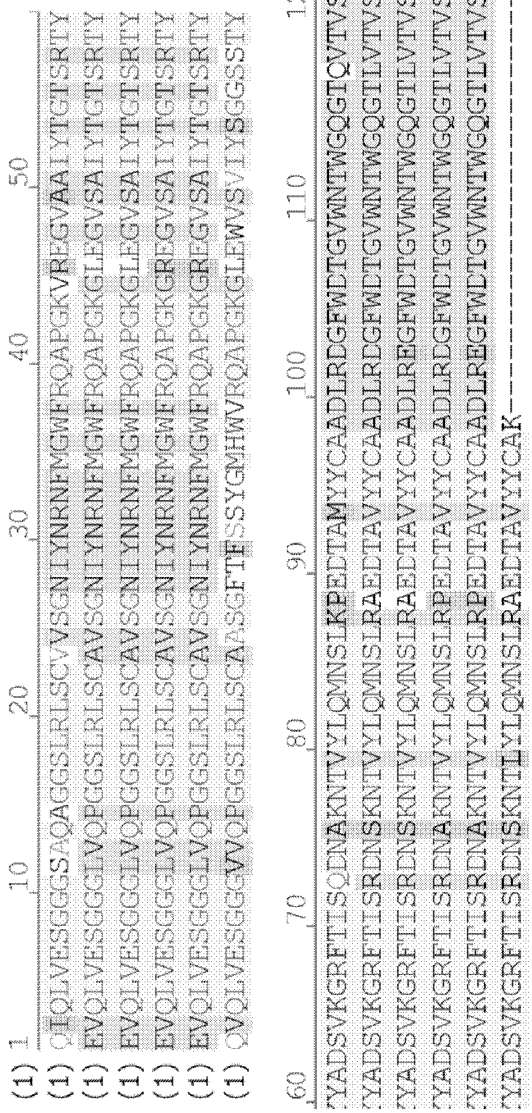

(1) 1 10 20 30 40 50
Camelid AS15193 SEQ ID.No 295 (1) QTQLVESGGGSAQAGGSLRLSCVVSGNIYNRNFMGWFRQAPGKVREGVAAIYTGTSRTY
Var AS15193VH8 SEQ ID.No 321 (1) EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTY
Var AS15193VH8M1 SEQ ID.No 322 (1) EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTY
Var AS15193VH18 SEQ ID.No 323 (1) EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTY
Var AS15193VH18M1 SEQ ID.No 324 (1) EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTY
human acceptor SEQ ID.No 366 (1) QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVSVIYSGGSSTY
60 70 80 90 100 110 123
Camelid AS15193 SEQ ID.No 295 YADSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAADLRDGFWDTGVWNTWGQGTQVTVSS
Var AS15193VH8 SEQ ID.No 321 YADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS
Var AS15193VH8M1 SEQ ID.No 322 YADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS
Var AS15193VH18 SEQ ID.No 323 YADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS
Var AS15193VH18M1 SEQ ID.No 324 YADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS
human acceptor SEQ ID.No 366 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK----------

FIG. 12A

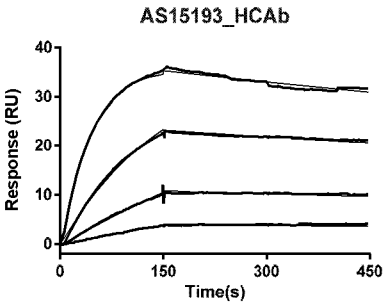


FIG. 12B

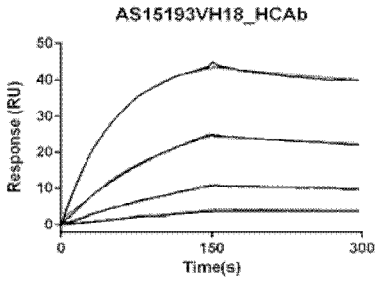


FIG. 12C

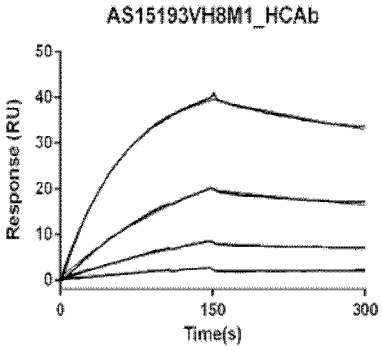


FIG. 12D

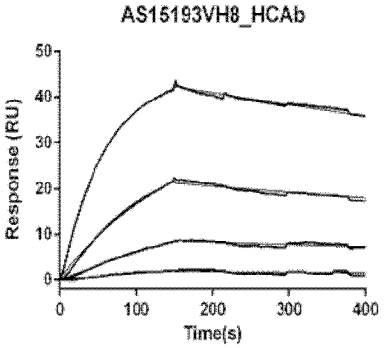


FIG. 12E

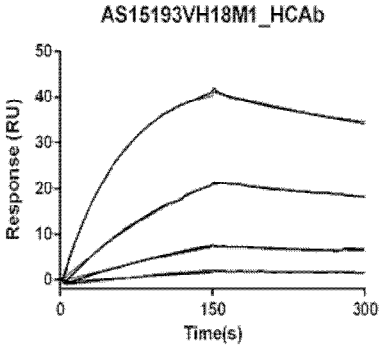


FIG. 12F

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
AS15193_HCAb	PD-1 His	1.2E+05	4.7E-04	4.0E-09
AS15193VH8_HCAb		6.80E+04	6.60E-04	9.70E-09
AS15193VH8M1_HCAb		6.10E+04	1.20E-03	2.00E-08
AS15193VH18_HCAb		8.60E+04	6.00E-04	7.00E-09
AS15193VH18M1_HCAb		9.20E+04	1.40E-03	1.50E-08

FIG. 13A

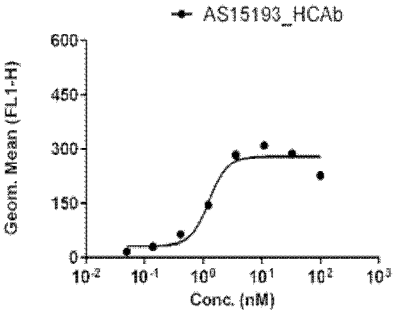


FIG. 13B

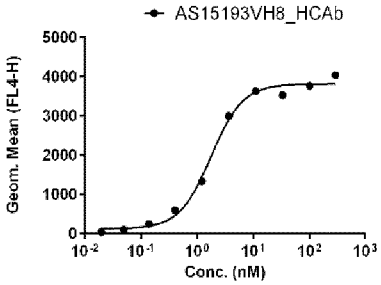


FIG. 13C

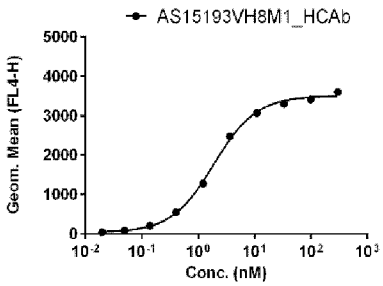


FIG. 13D

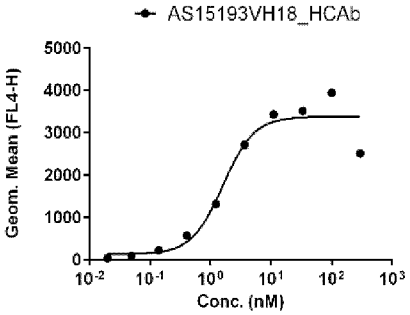


FIG. 13E

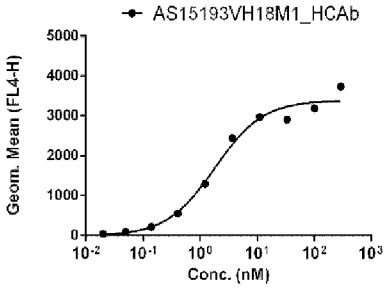


FIG. 13F

	AS15193_HCAb	AS15193VH8_HCAb	AS15193VH8M1_HCAb
EC_{50} (nM)	1.26	1.78	1.92
	AS15193VH18_HCAb	AS15193VH18M1_HCAb	
EC_{50} (nM)	1.61	1.80	

FIG. 14A

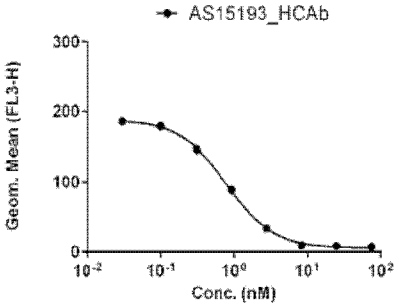


FIG. 14B

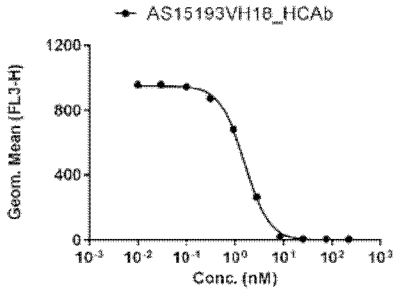


FIG. 14C

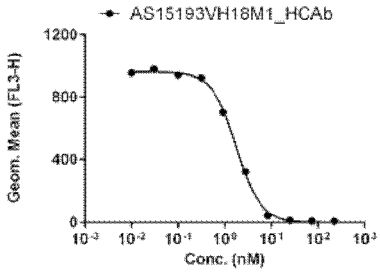


FIG. 14D

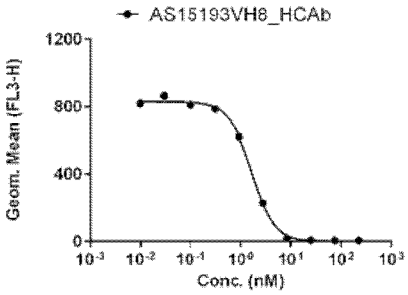


FIG. 14E

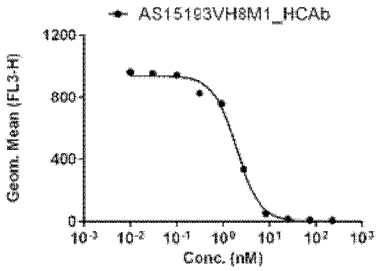


FIG. 14F

	AS15193_HCAb	AS15193 VH8_HCAb	AS15193 VH8M1_HCAb
IC_{50} (nM)	0.79	1.64	1.96
	AS15193 VH18_HCAb	AS15193 VH18M1_HCAb	
IC_{50} (nM)	1.57	1.75	

FIG. 15A

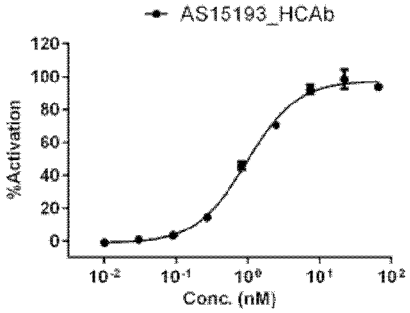


FIG. 15B

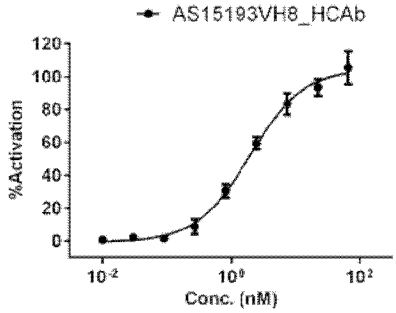


FIG. 15C

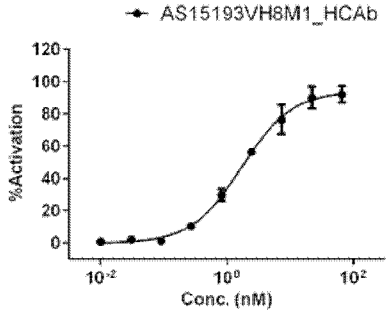


FIG. 15D

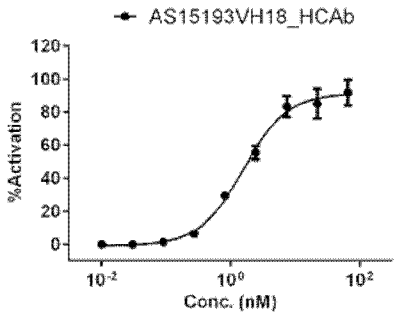


FIG. 15E

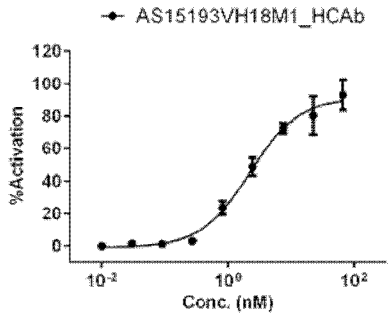


FIG. 15F

	AS15193_HCAb	AS15193VH8_HCAb	AS15193VH8M1_HCAb
EC_{50} (nM)	0.97	1.97	1.72
	AS15193VH18_HCAb	AS15193VH18M1_HCAb	
EC_{50} (nM)	1.58	2.21	

FIG. 16

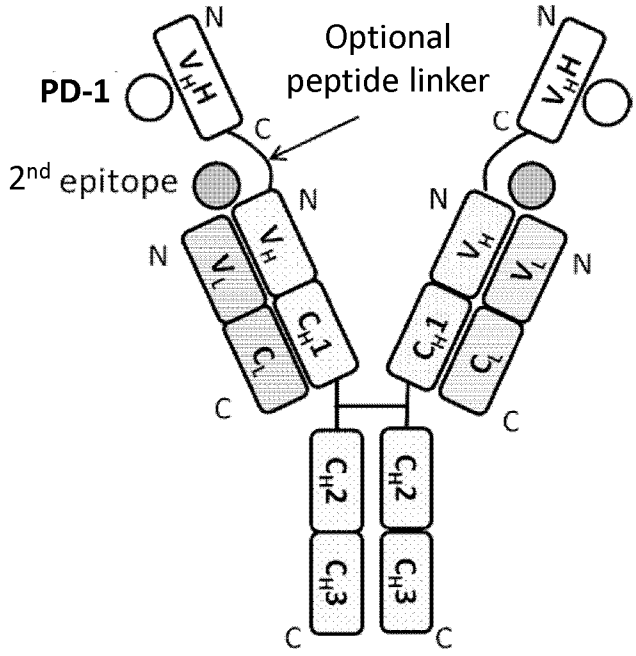

PD-1
Optional
peptide linker
2nd epitope
N
C
VHH
VH
VL
CH1
CL
CH2
CH3

FIG. 17

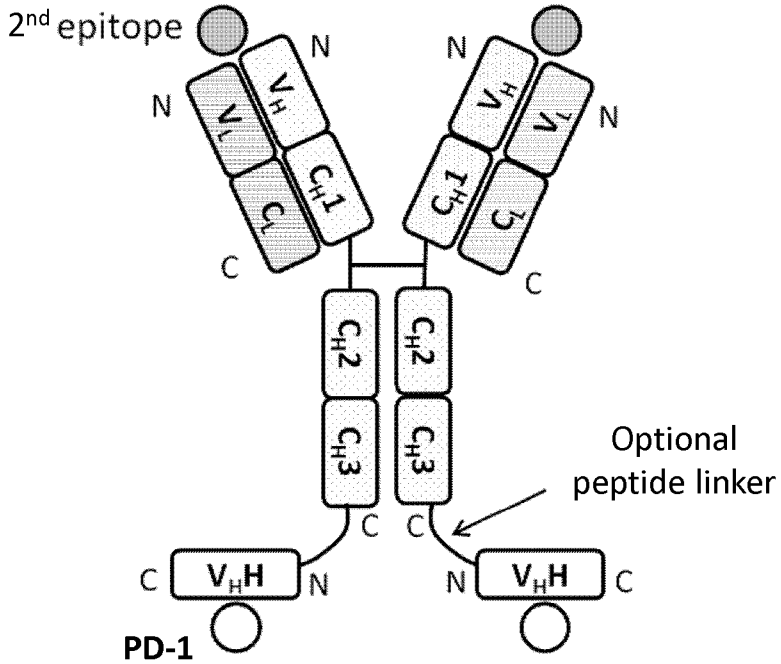

2nd epitope
Optional
peptide linker
PD-1
N
C
VH
VL
CH1
CL
CH2
CH3
VHH

FIG. 18

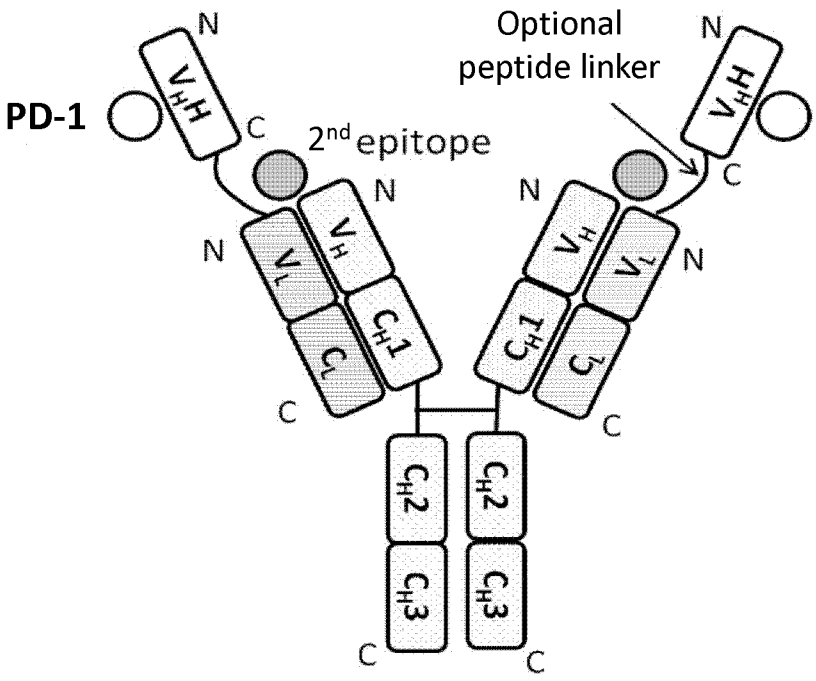

PD-1
N
C
V_HH
2nd epitope
Optional
peptide linker
V_H
V_L
C_H1
C_L
C_H2
C_H3

FIG. 19

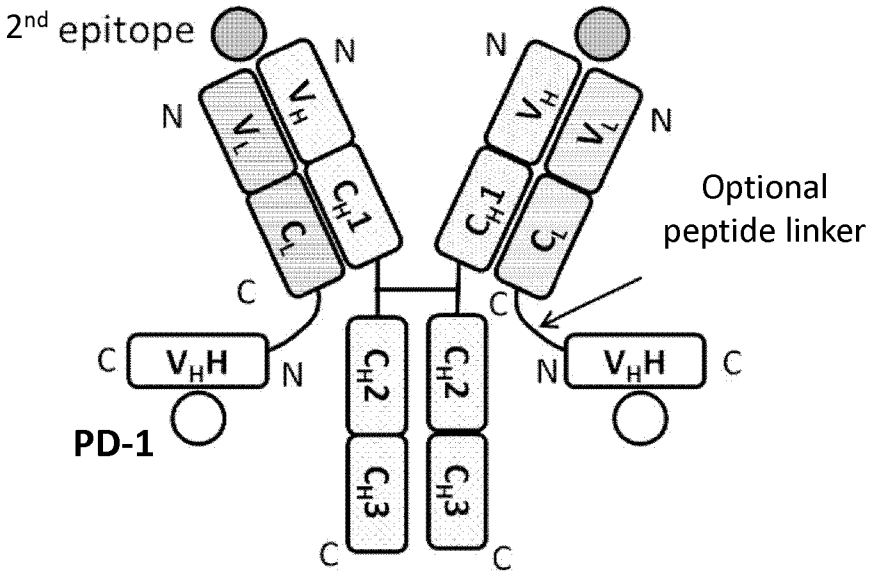

2nd epitope
PD-1
N
C
V_HH
Optional
peptide linker
V_H
V_L
C_H1
C_L
C_H2
C_H3

FIG. 20

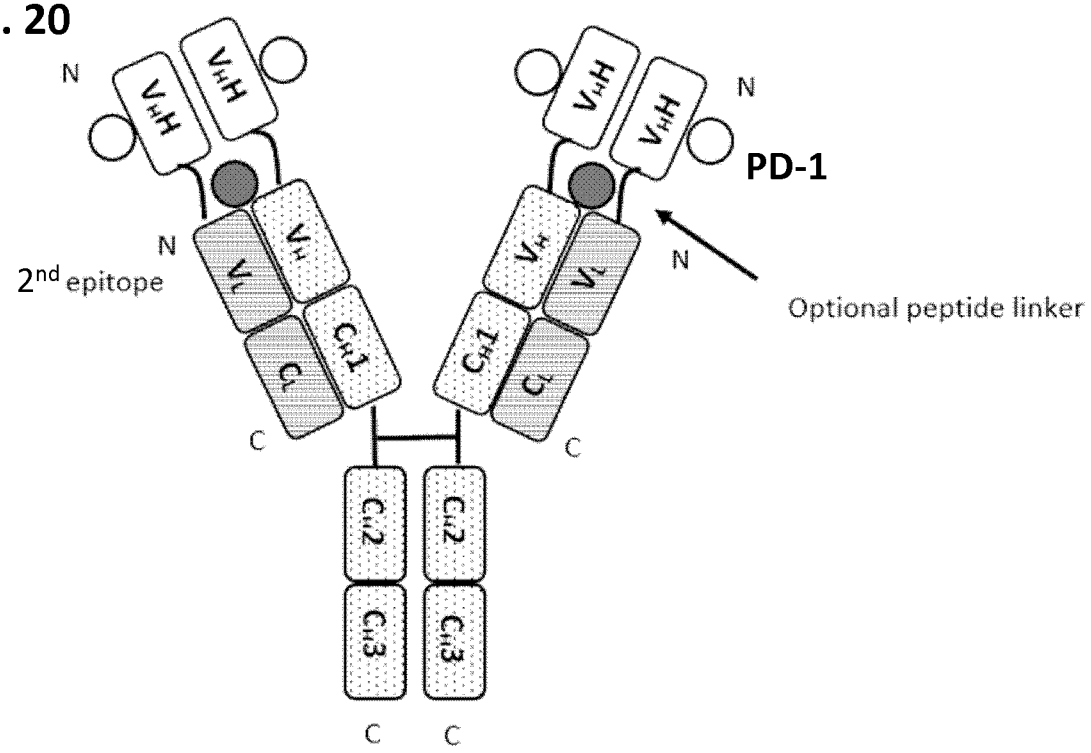

N
VHH
VHH
N
PD-1
N
2nd epitope
VH
VL
CH1
CL
C
Optional peptide linker
CH2
CH3
C

FIG. 21

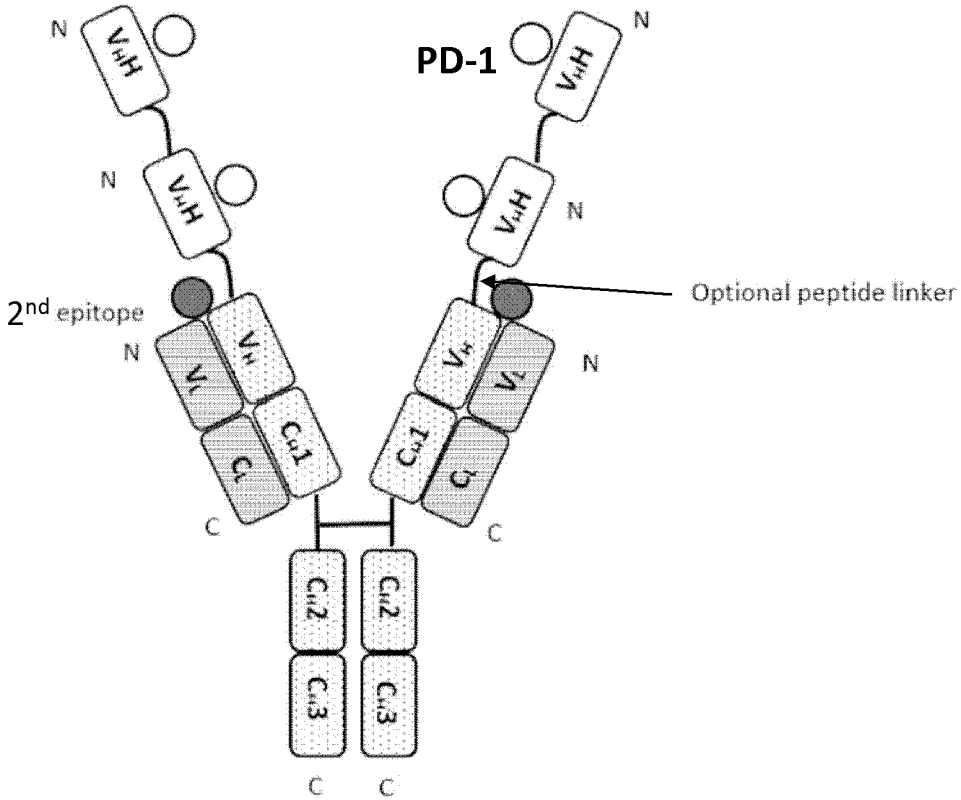

N
VHH
PD-1
N
N
VHH
N
2nd epitope
Optional peptide linker
N
VH
VL
CH1
CL
C
CH2
CH3
C

FIG. 22

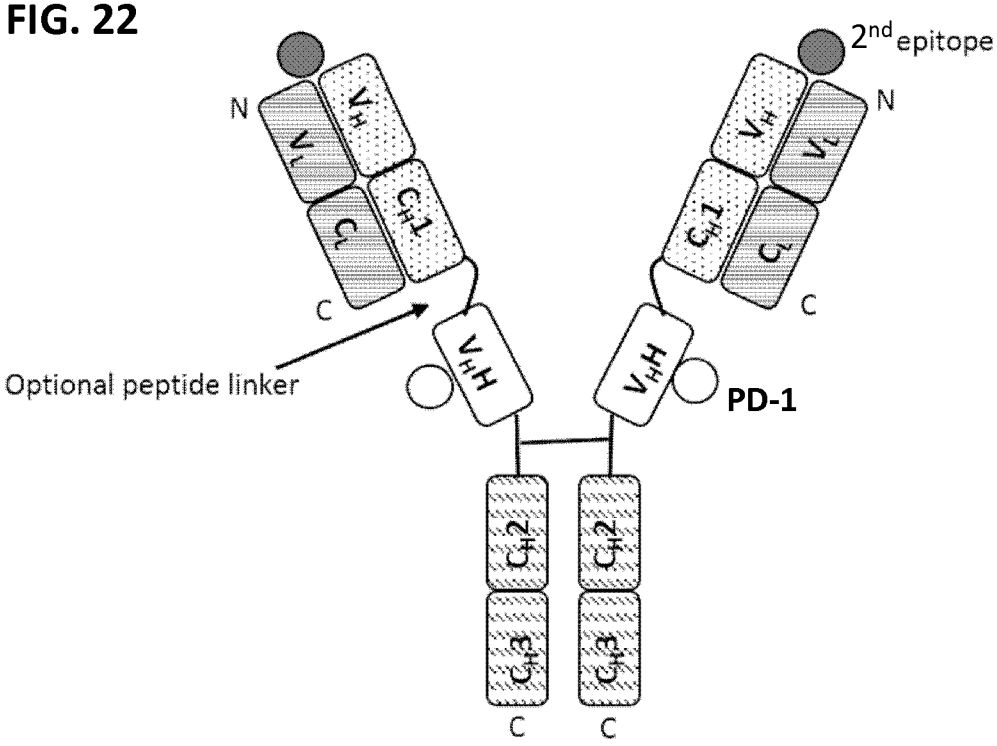

2nd epitope
N
N
C
C
Optional peptide linker
PD-1
C
C

FIG. 23

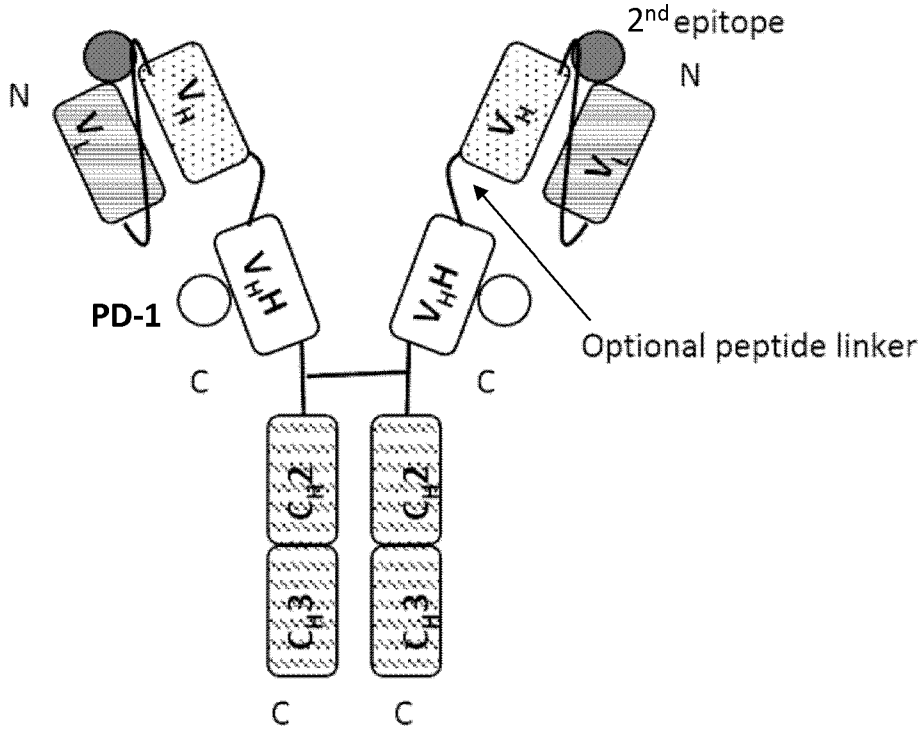

2nd epitope
N
N
PD-1
Optional peptide linker
C
C
C
C

FIG. 24

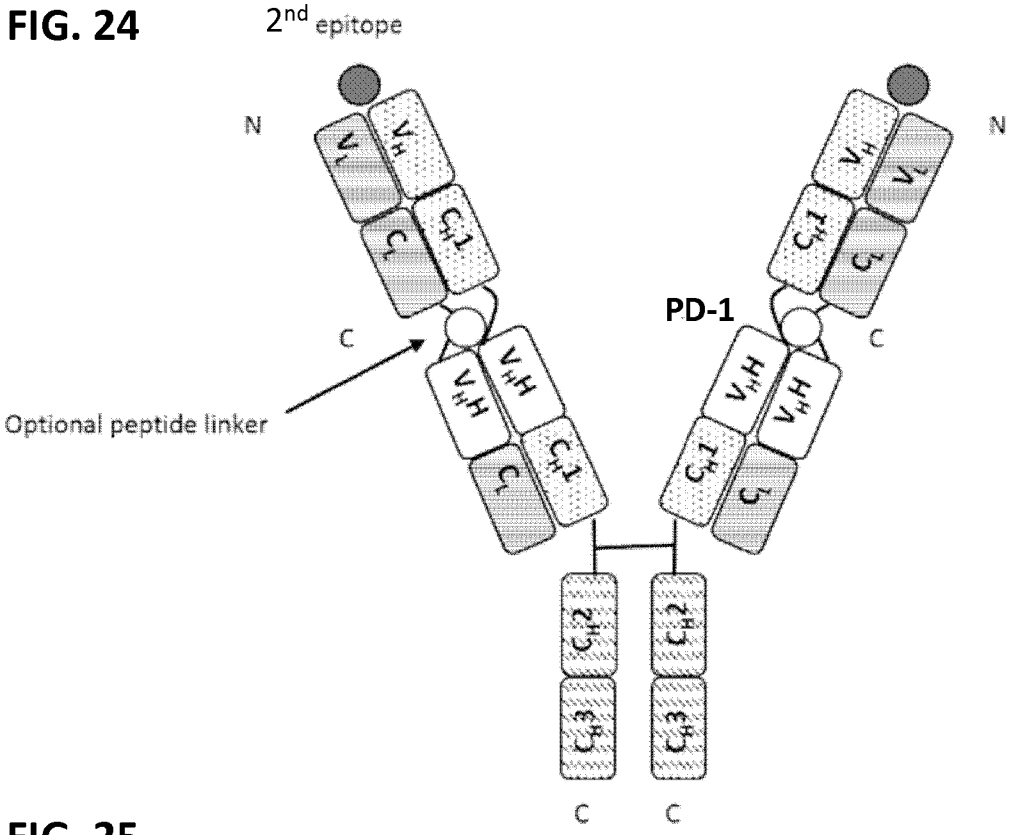

2nd epitope
N
N
PD-1
C
C
Optional peptide linker
C
C

FIG. 25

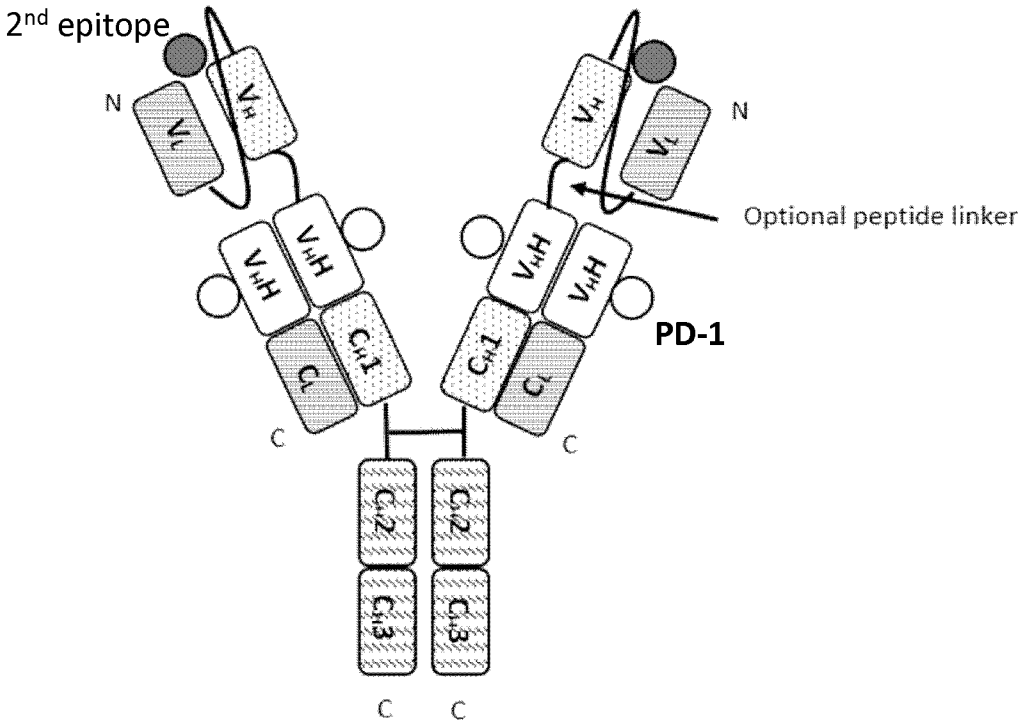

2nd epitope
N
N
Optional peptide linker
PD-1
C
C
C
C

FIG. 26A

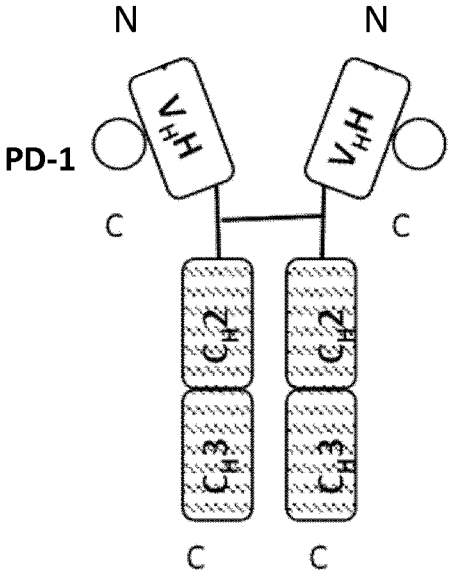


FIG. 26B

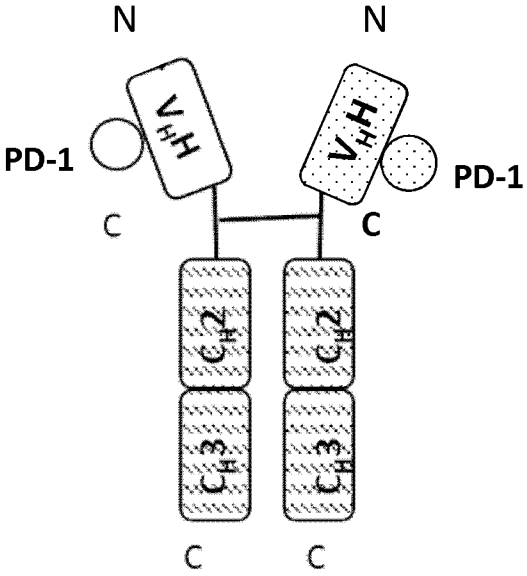


FIG. 27

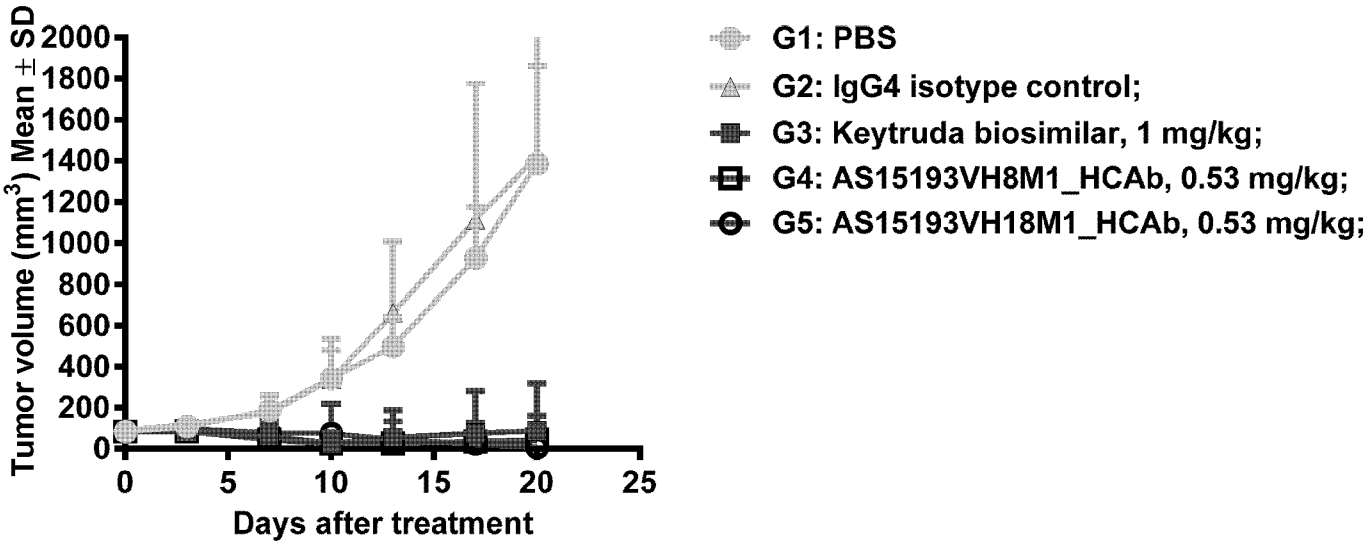

Tumor volume (mm3) Mean ± SD
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Days after treatment
G1: PBS
G2: IgG4 isotype control;
G3: Keytruda biosimilar, 1 mg/kg;
G4: AS15193VH8M1_HCAb, 0.53 mg/kg;
G5: AS15193VH18M1_HCAb, 0.53 mg/kg;

FIG. 28

Sample	AS06962_sdAb	AS15872_sdAb	AS15881_sdAb	AS15883_sdAb	AS15892_sdAb	AS15899_sdAb	AS07424_sdAb	AS31543_sdAb
Amount(mg)	4.39	17.79	8.29	2.22	2.78	0.74	2.34	4.09
Purity	>85%	>90%	>90%	>80%	>90%	80%	>90%	>85%
Endotoxin level(EU/μg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

FIG. 29

Sample	AS15881_HCAb	AS15899_HCAb	AS15140-HCAb	AS15156_HCAb	AS15193_HCAb	AS25071_HCAb	AS25115_HCAb	AS25117_HCAb
Conc.(mg/ml)	1.92	1.58	1.73	2.13	0.96	1.48	1.33	1.24
Amount(mg)	6.73	5.54	6.06	11.70	3.77	4.18	5.06	4.72
Purity	>90%	>90%	>90%	>90%	>90%	>85%	>90%	>90%
Endotoxin level(EU/μg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1
Sample	AS25119_HCAb	AS25149_HCAb	AS25396_HCAb	AS25457_HCAb	AS25487_HCAb	AS25435_HCAb	AS24984_HCAb	AS25037_HCAb
Conc.(mg/ml)	1.15	1.23	1.12	1.47	1.64	1.41	0.65	0.62
Amount(mg)	4.39	4.68	4.05	7.20	9.10	8.86	1.35	1.90
Purity	>90%	>90%	>90%	>90%	>90%	>85%	>85%	>85%
Endotoxin level(EU/μg)	<0.1	<0.1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Sample	AS25064_HCAb	AS25067_HCAb	AS25156_HCAb	AS25164_HCAb	AS25170_HCAb	AS2522_HCAb		
Conc.(mg/ml)	1.35	1.37	1.18	1.18	1.26	1.00		
Amount(mg)	5.57	4.68	4.60	6.10	5.93	5.22		
Purity	>90%	>85%	>90%	>90%	>90%	>90%		
Endotoxin level(EU/μg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		