FIG. 1A

	CDR-L1										
Library	28	29	30	30a	30b	30c	30d	30e	30f	31	32
L1/L3	Ss		NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	-	NNK	Ys
L1/L4	Ss		NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	NNK	Ys
L1/L2/L3-A_1	Ss	V/L	NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	NNK	Ys
L1/L2/L3-A_2	Ss		NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	NNK	Ys
L1/L2/L3-B_1	NNK	NNK	NNK	-	-	-	-	-	-	NNK	NNK
L1/L2/L3-B_2	NNK	NNK	NNK	(NNK)	(NNK)	-	-	-	-	NNK	NNK
L1/L2/L3-C	Ds	I/V	NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	NNK	Y
L1/L2/L3-D	Ds	I/V	NNK	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	(NNK)	NNK	WRG YSPHNTDA Y WRG YSPHNTDA

FIG. 1B

CDR-L2				Framework3						CDR-L3								
50	51	52	53	66	67	68	69	70	91	92	93	a	b	c	d	e	f	94
				NNK	Ss	-	T/Ts	D/Ds		XYZ	XYZ	(XYZ)	(XYZ)					
				NNK	NNK	Ss	NNK	NNK		XYZ	XYZ	-	-					
NNK W	GAS	S	NNK							XYZ	XYZ	(XYZ)	(XYZ)					
NNK W	GAS	S	NNK							XYZ	XYZ	(XYZ)	(XYZ)					
NNK W	GAS	S	NNK							NNK	NNK	-	-					
NNK W	GAS	S	NNK							NNK	NNK	-	-					
NNK W WG	GA	S	YS NNK						YADS H WRG	XYZ	XYZ	(XYZ)	(XYZ)	-	-	-	-	ATSG
NNK W WG	GA	S	YS NNK	NNK	Ss		T Ts	D Ds		XYZ	XYZ	-	-	-	-	-	-	ATSG

FIG. 2

	Library	L1/L3	L1/L4	L1/L2/L3-A	L1/L2/L3-B	L1/L2/L3-C, +L4-D
	template Format Stop	2C4 Fab-C L1	2C4 Fab-C L1	2C4 Fab-C L1	_1 4D5 ScFv L1	_2 G6-Av Fab-C	4D5 ScFv L1
L1	28 29 30 30a-e 31 32 33	Ss *NNK* *(NNK)* 0-4 *NNK* Ys	 *NNK* *(NNK)* 0-5 *NNK* Ys	Ss VL *NNK* *(NNK)* 5 P Y	*NNK* *NNK* *NNK* *NNK* *NNK* *NNK*	*NNK* *NNK* *NNK* *(NNK)* 2 *NNK* *NNK*	Ds I/V *NNK* *(NNK)* 0-5 *NNK* Y/WRG/YSPHSTDA
L2	50 51 52 53			*NNK*/W GAS S *NNK*	*NNK*/W GAS S *NNK*		*NNK*/W/WRG GA S YS/*NNK*
L4	66 67 68 69 70	*NNK* Ss T/Ts D/Ds	*NNK* *NNK* Ss *NNK* *NNK*				*NNK* Ss T/Ts D/Ds
L3	91 92 93 93a 94	*XYZ* *(XYZ)* *(XYZ)* 0-2	*XYZ* *(XYZ)*	*XYZ* *XYZ* *(XYZ)* 0-2	*NNK* *NNK*	*NNK* *NNK*	YADS/H/WRG *XYZ* *XYZ* *(XYZ)* 0-2 ATSG	*XYZ* *XYZ* ATSG
	Display	5-7%	5-7%	5-7%	~15-25%		~17%
	Target	VEGF DR5 Albumin		VEGF DR5	VEGF		VEGF DR5 IgG-Fc-fusion protein

FIG. 3

	CDR-L1							CDR-L2				FR3(CDR-L4)					CDR-L3						
	28	29	30	30a-f	31	32	33	50	51	52	53	66	67	68	69	70	91	92	93	93a-e	94	95	96
hu4D5	D	V	S		T	A	V	S	A	S	F	G	S	G	T	D	H	Y	T		T	P	P
2C4	D	V	S		I	G	V	S	A	S	Y	G	S	G	T	D	Y	Y	I		Y	P	Y
Fab12-G	D	V	S		T	A	V	S	A	S	F	G	S	G	T	D	Y	S	T		V	P	W

	CDR-H1						CDR-H2											CDR-H3							
	29	30	31	32	33	34	49	50	51	52	52a	53	54	55	56	57	58	59	94	95	96	97	98	99	100	100a
hu4D5	I	K	D	T	Y	I	A	R	I	Y	P	T	N	G	Y	T	R	Y	R	W	G	G	D	G	F	Y
2C4	F	T	D	Y	T	M	A	D	V	N	P	N	S	G	G	S	I	Y	R	N	L	G	P	S	F	
Fab12-G	I	S	D	S	G	I	A	A	I	A	P	G	A	G	S	T	Y	Y	R	F	V	S	A	P	P	S

FIG. 4

LC CDR	Position	Natural Diversity									Design Diversity										
		Prevalence (%)									Prevalence (%)										
CDR1	28(D)	S	G	D	N	T	R				D	E	G	A	V	N	Y	H	X		
		71	12	7	4	3	2				39	10	7	7	7	6	6	6	<2		
	29(V)	I	V	L							I	V									
		43	42	12							50	50									
	30(N)	S	L	R	G	N	V	T	D		S	L	R	G	A	V	T	P	X		
		55	17	6	5	4	4	2	2		9	9	9	6	6	6	6	6	3		
	30a-e (*)	S	N	Y	G	K	H	D			S	L	R	G	A	V	T	P	X		
		34	23	10	9	8	6	5			9	9	9	6	6	6	6	6	3		
	31(T)	S	N	T	R	D	I	K			S	L	R	G	A	V	T	P	X		
		44	32	11	3	2	2	2			9	9	9	6	6	6	6	6	3		
	32(A)	Y	N	W	F	S	D	R			Y	W	G	R	N	S	D	T	A	H	P
		68	8	6	5	4	3	2			31	8	8	8	6	6	6	6	6	6	6
	33(V)	L	V	F	X						V	L									
		94	3	1	2						50	50									
CDR2	50(S)	G	A	D	W	K	L	E	S		W	G	S	L	R	A	V	T	P	X	
		25	22	19	10	8	6	3	2		16	12	7	7	7	5	5	5	5	2	
	51(A)	A	V	G	T						A	G									
		79	7	6	5						50	50									
	52(S)	S									S										
		95									100										
	53(F)	S	N	T	K	R	I				S	Y	X								
		36	28	26	3	2	2				44	43	<2								
CDR3	91(H)	Y	S	R	A	G	H	F	L	D	H	Y	S	A	D	R	G	W			
		52	12	10	7	4	3	2	2	2	20	15	15	15	15	7	7	7			
	92(Y)	G	Y	N	S	D	L	T	H	I	G	S	D	R	A	V	Y	L	C	N	X
		22	22	15	12	7	6	4	3	2	11	9	8	7	7	6	6	5	5	5	<5
	93(T)	S	N	Q	T	H	G	D	R		G	S	D	R	A	V	Y	L	C	N	X
		46	21	7	6	4	3	3	2		11	9	8	7	7	6	6	5	5	5	<5
	93a-b(*)	W	S	T	P	L	Y	V	A	N	G	D	S	Y	R	A	V	C	N	X	
		28	25	17	11	6	4	2	2	2	12	10	9	7	7	7	6	6	5	<5	
	94(T)	T	P	S	W	Y	L	F	A	V	G	T	S	A	G							
		19	19	18	12	10	7	5	3	2	1	25	25	25	25							

FIG. 5A

	CDR-L1								CDR-L2							CDR-L3						Mutations/ Insertions
	28	29	30	30a	30b	30c	30d	30e	31	32	33	50	51	52	53	91	92	93	93a	93b	94	
hu4D5	D	V	N						T	A	V	S	A	S	F	H	Y	T			T	-
anti-VEGF	D	I	G	G					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	H	S					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	V	F						T	S	V	D	A	S	Y	R	Y	I	W		A	8
	D	I	W						R	A	V	P	A	S	N	G	Y	Y	I		A	9
	D	I	W						R	W	V	A	A	S	S	H	A	G			A	9
	Y	V	W						Q	Y	V	H	A	S	S	G	Y	W	V		A	10
	D	I	P	S					I	Y	V	G	A	S	Y	R	Y	W	V		A	11
	D	I	W						R	W	L	A	G	S	S	H	D	Q			A	11
	E	V	Y						S	Y	V	P	A	S	S	G	F	W	I		G	11
	N	V	W						D	W	V	P	A	S	S	G	W	Y	I		A	11
	N	V	W						S	H	V	G	G	S	S	R	L	W	F		T	11
	W	V	P	S					H	T	V	L	G	S	Y	S	D	Y			T	11
	Y	V	Y	S					T	T	V	N	G	S	S	A	S	S			A	11
	E	I	F	P					Y	Y	V	L	G	S	S	G	W	D			G	13
	N	I	F						S	H	V	P	G	S	Y	A	F	W	V		S	13
	Q	I	W						R	H	L	T	G	S	S	S	Y	W	V		A	13
	Y	I	W						N	Y	V	G	G	S	S	S	W	W	A		G	13
anti-DR5	S	V	S						S	A	V	S	A	S	S	Y	S	S			S	8
	V	V	S						M	T	V	G	A	S	S	Y	G	S	Y		S	11
	N	V	G						R	P	V	G	G	S	S	Y	G	S	F		G	12
	R	I	N	S					H	T	V	W	G	S	H	Y	S	N	R		T	12
	D	I	W	N	R				R	A	L	E	G	S	S	G	G	S	Y		S	14
	D	I	W	N	R				R	A	L	K	G	S	S	G	G	S	Y		S	14
anti-Fc	E	V	L						T	S	V	S	A	S	F	H	Y	T			T	3
	K	I	Q						A	Y	V	S	A	S	F	H	Y	T			T	5
	N	I	V	V	R				P	Y	V	S	A	S	F	H	Y	T			T	7
	D	V	G	G	G				S	G	V	G	G	S	S	H	Y	T			T	8
	D	I	G						A	G	L	S	A	S	F	S	E	S	R		S	10
	D	I	S						R	Y	L	S	A	S	F	Y	G	W	R	R	T	10
	D	V	G	G					L	G	L	S	A	S	S	G	G	A	D		T	10
	D	V	N						R	Y	V	A	G	S	Y	G	I	D	L		A	10
	D	V	S	R					Y	D	L	F	A	S	S	S	G	Y	H		T	11
	V	V	R						Q	H	V	R	A	S	S	S	D	A	S		A	11
	D	V	H	R					D	S	V	W	G	S	Q	W	T	W	A	D	T	12
	D	V	H	P	S				P	R	V	L	G	S	S	A	N	V	D		T	12
	E	I	S	R					Y	A	V	W	A	S	S	G	V	Y	N	E	A	12
	N	V	P	R					W	S	L	W	A	S	S	W	V	T	H	E	T	12
	P	V	F	R					R	G	L	S	A	S	S	G	L	R	H		G	12
	A	V	P	R					Y	G	V	W	G	S	D	S	G	W	S		A	13
	A	V	S	R					H	A	L	W	G	S	S	W	D	G	K		A	13
	D	I	G	L					L	N	L	W	A	S	S	W	A	D	I	S	T	13
	D	I	R	G	Q				R	G	V	S	G	S	S	G	S	G	S	S	T	13
	D	V	S	G	R				R	G	L	D	A	S	S	G	A	A	Y		A	13
	E	I	V						F	S	V	G	G	S	S	G	D	S	K		S	13
	N	V	P	L					N	D	V	H	G	S	S	W	A	H	Y		G	13
	V	I	A	R					Y	D	L	W	A	S	S	A	G	A	R		A	14
	D	I	P	E	H				F	R	L	G	G	S	S	G	G	W	S	E	T	15
	Y	I	P	R					D	A	L	W	G	S	Y	W	A	S	W	D	A	15
	R	V	S	D	S	L	Q		N	S	V	L	G	S	S	G	L	D	L		A	16
	N	V	S	R	V	S	W	F	E	T	L	G	G	S	Y	W	F	T	W		G	17

FIG. 5B-1

	CDR-L1									CDR-L2				CDR-L3							Mutations/ Insertions
	28	29	30	30a	30b	30c	30d	30e	31	32	33	50	51	52	53	91	92	93	93a	93b	94	
hu4D5	D	V	N						T	A	V	S	A	S	F	H	Y	T			T	-
anti-	D	I	W						K	W	V	A	A	S	S	H	Y	T			T	5
HER2/VEGF	D	I	K	N					G	S	V	W	A	S	F	H	Y	T			T	6
	D	I	L	G					G	S	V	W	A	S	F	H	Y	T			T	6
	D	I	M	S					G	S	V	W	A	S	F	H	Y	T			T	6
	D	I	R	A					G	S	V	W	A	S	F	H	Y	T			T	6
	D	I	R	G					G	S	V	W	A	S	F	H	Y	T			T	6
	D	V	R	Q					G	S	V	W	G	S	F	H	Y	T			T	6
	D	I	A	A					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	A	G					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	A	H					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	A	K					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	G	A					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	G	A					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	G	G					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	G	L					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	G	M					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	K	H					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	L	A					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	L	G					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	L	I					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	L	T					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	M	L					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	Q	S					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	R	I					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	R	M					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	R	Q					G	S	V	W	A	S	Y	H	Y	T			T	7
	D	I	R	T					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	R	V					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	S	M					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	S	R					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	S	V					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	V	S					G	S	V	W	G	S	F	H	Y	T			T	7
	D	I	W						H	W	V	A	G	S	S	H	Y	T			T	7
	N	I	A	Q					G	S	V	W	A	S	F	H	Y	T			T	7
	D	I	A	F					G	S	L	W	G	S	F	H	Y	T			T	8
	D	I	A	M					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	A	R					G	S	V	W	G	S	M	H	Y	T			T	8
	D	I	A	S					G	S	V	W	G	S	L	H	Y	T			T	8
	D	I	A	S					G	S	V	W	G	S	S	H	Y	T			T	8
	D	I	G	S					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	I	G					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	K	A					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	K	F					G	S	V	W	G	S	S	H	Y	T			T	8
	D	I	K	L					G	S	V	W	G	S	L	H	Y	T			T	8
	D	I	K	L					G	S	V	W	G	S	M	H	Y	T			T	8
	D	I	K	S					G	S	V	W	G	S	T	H	Y	T			T	8

FIG. 5B-2

	CDR-L1								CDR-L2				CDR-L3						Mutations/ Insertions
	28	29	30	30a	30b	30c	30d	30e	31	32	33	50	51	52	53	91	92	93	93a	93b	94	
hu4D5	D	V	N						T	A	V	S	A	S	F	H	Y	T			T	-
	D	I	K	V					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	K	W					G	S	V	W	G	S	T	H	Y	T			T	8
	D	I	L	K					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	L	S					G	S	V	W	G	S	W	H	Y	T			T	8
	D	I	Q	R					G	S	V	W	G	S	C	H	Y	T			T	8
	D	I	Q	S					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	Q	T					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	E					G	S	V	W	G	S	S	H	Y	T			T	8
	D	I	R	F					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	G					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	L					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	M					G	S	V	W	G	S	S	H	Y	T			T	8
	D	I	R	R					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	R					G	S	V	W	G	S	A	H	Y	T			T	8
	D	I	R	S					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	R	S					G	S	V	W	G	S	T	H	Y	T			T	8
	D	I	R	S					G	S	V	W	G	S	N	H	Y	T			T	8
	D	I	R	S					G	S	V	W	G	S	E	H	Y	T			T	8
	D	I	R	S					G	S	V	W	G	S	S	H	Y	T			T	8
	D	I	R	V					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	S	S					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	T	M					G	S	V	W	G	S	L	H	Y	T			T	8
	D	I	Y	M					G	S	V	W	G	S	Y	H	Y	T			T	8
	D	I	A	T					G	S	L	W	G	S	Y	H	Y	T			T	9
	D	I	K	S					G	S	L	W	G	S	Y	H	Y	T			T	9
	D	I	R	G					G	S	V	G	G	S	Y	Y	Y	T			T	9
	G	I	R	T					G	S	V	W	G	S	Y	H	Y	T			T	9
	N	I	A	M					G	S	V	W	G	S	Y	H	Y	T			T	9
	N	I	R	S					G	S	V	W	G	S	V	H	Y	T			T	9
	N	I	R	T					G	S	V	W	G	S	Y	H	Y	T			T	9
	D	I	R	A					G	S	V	W	G	S	Y	H	F	N			A	11
	N	I	Y	A					G	S	L	W	G	S	Y	H	Y	T			T	10
	N	I	Y	S					G	S	L	W	G	S	Y	H	Y	T			T	10
	D	I	P	R	S	I	S		G	Y	V	W	G	S	Y	H	Y	T			T	11
anti-HER2/DR5	N	I	R	N	G				G	G	L	S	A	S	F	H	Y	T			T	8
	N	V	S						K	H	V	W	G	S	Y	S	Y	S			G	10
anti-HER2/Fc	Q	V	S	K					Y	D	V	W	G	S	S	S	G	F	R		S	13

FIG. 6

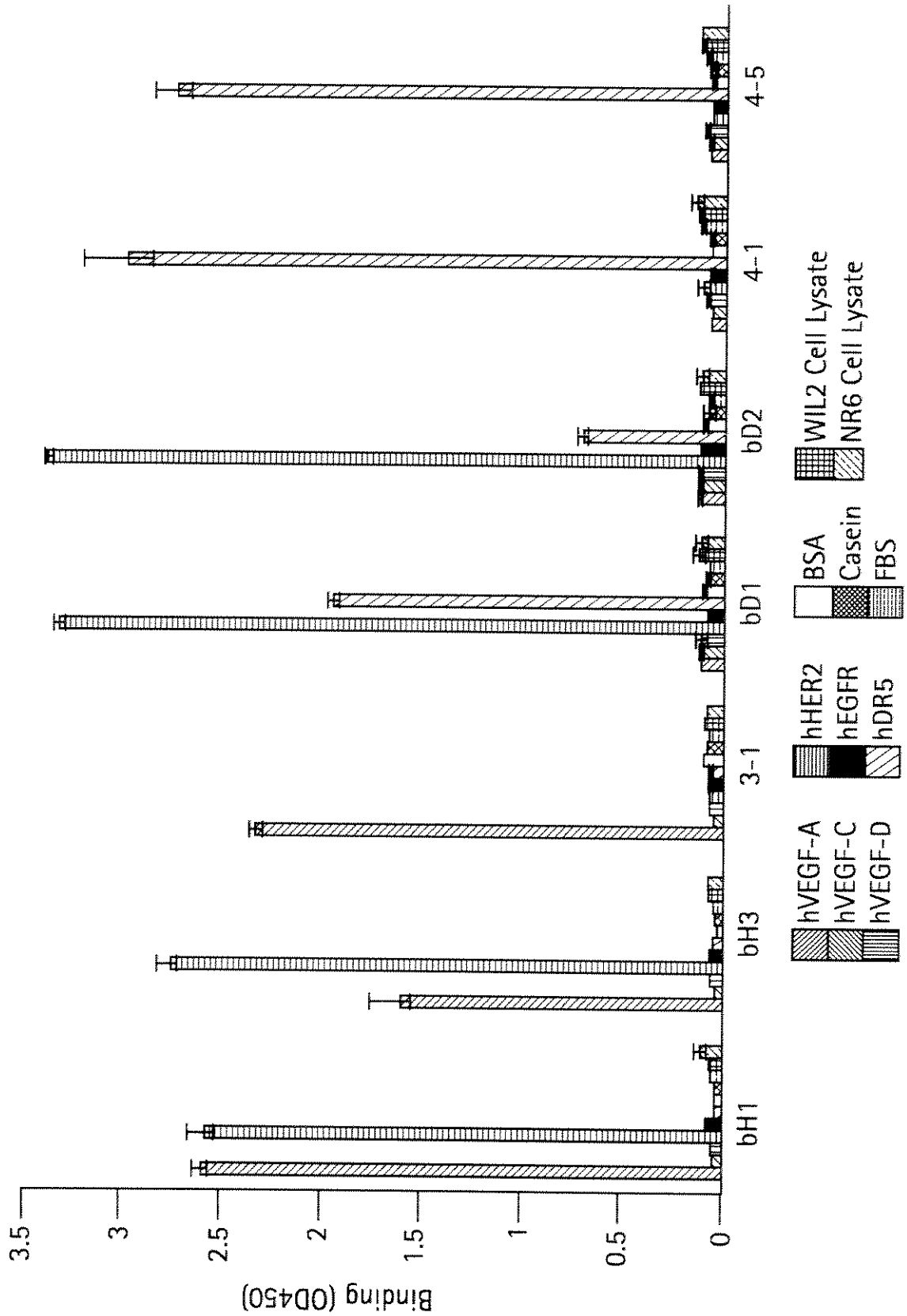

Binding (OD450)
3.5
3
2.5
2
1.5
1
0.5
0
bH1
bH3
3-1
bD1
bD2
4-1
4-5
hVEGF-A
hVEGF-C
hVEGF-D
hHER2
hEGFR
hDR5
BSA
Casein
FBS
WIL2 Cell Lysate
NR6 Cell Lysate

FIG. 7

Sorting Round	Binding condition	Protein	Titer	Enrichment	OD/mL
1	bind 4 hrs. wash 4X BSA	Human Fc fusion hVEGF hDR5 – long L – 3 (short L1's) L – 4 (long L1's)	1.8×10^6 1.5×10^6 8×10^5 4.8×10^6 1×10^6		21 18.3 20.9
2	bind 3hrs. wash 5X BSA/ovalbumin	Human Fc fusion hVEGF hDR5 – long	5.2×10^5 3.6×10^6 4.2×10^5	1.86 1.33 1.05	26.6 18.2 15.4
3	bind 3hrs. wash 10X BSA/milk	Human Fc fusion hVEGF hDR5 – long	9×10^3 8×10^3 1.4×10^4	1.5 0.75 2.8	16.2 8.17 17.3
4	bind 2 hrs. wash 12X casein	Human Fc fusion hVEGF hDR5 – long	5.5×10^5 7.4×10^6 4.5×10^7	22.9 96.1 818.2	25.3 14.8 9.7
5	bind 1.75 hrs. wash 12X BSA	Human Fc fusion hVEGF hDR5 – long	1.4×10^6 2.2×10^7 6.5×10^7	14 110 325	

FIG. 8

28	29	30	30a					31	32	33	50	51	52	53	91	92	93
Dsoft	I/V	*NNK*						*NNK*		L/V		G/A					*XYZ*
D	I	R	S					G	S	V	W	G	S	Y	H	Y	T
D	I	R	T					G	S	V	W	G	S	F	H	Y	T
D	I	R	V					G	S	V	W	G	S	F	H	Y	T
D	I	A	G					G	S	V	W	A	S	Y	H	Y	T
D	I	R	F					G	S	V	W	G	S	F	H	Y	T
D	I	K	S					G	S	V	W	G	S	Y	H	Y	T
D	I	A	R					G	S	V	W	G	S	Y	H	Y	T
W	V	P	S					H	T	V	L	G	S	Y	S	D	Y
D	I	N	G					G	S	V	W	A	S	F	H	Y	T
D	I	L	Q					G	S	V	W	G	S	Y	H	Y	T
D	V	R	M					G	S	V	W	G	S	Y	H	Y	T
D	I	R	T					G	S	V	W	G	S	S	H	Y	T
D	I	K	H					G	S	V	W	G	S	F	H	Y	T
D	I	A	S					G	S	V	W	A	S	F	H	Y	T
D	I	K	S					G	S	L	W	G	S	Y	H	Y	T
N	V	W						D	W	V	P	A	S	S	G	W	Y
D	I	K	H					G	S	V	W	G	S	S	H	Y	T
D	I	R	M					G	S	V	W	G	S	V	H	Y	T
Q	I	W						R	H	L	T	G	S	S	S	Y	W
D	I	S	H					G	S	V	W	G	S	F	H	Y	T
N	I	A	M					G	S	V	W	G	S	Y	H	Y	T
E	V	Y						S	Y	V	P	A	S	S	G	F	W
V	I	R	K	A	F	G		Q	P	L	G	A	S	Y	Y	R	A
D	I	K	K					G	S	V	W	G	S	S	H	Y	T
N	I	F						S	H	V	P	G	S	Y	A	F	W
D	I	R	T					G	S	V	W	G	S	Y	H	Y	T
D	I	L	F					G	S	V	W	G	S	F	H	Y	T
D	I	W						R	W	L	A	G	S	S	H	D	Q
D	I	Y	M					G	S	V	W	G	S	Y	H	Y	T
D	I	S	M					G	S	V	W	G	S	Y	H	Y	T
N	I	A	Q					G	S	V	W	A	S	F	H	Y	T

FIG. 9

hVEGF Binders-Combined plate and solution selection

	L1										L2				L3						
	28	29	30	30a	30b	30c	30d	31	32	33	50	51	52	53	91	92	93	93a	94	Phage IC50(nM)	
4D5	D	V	S					T	A	V	S	A	S	F	H	Y	T		T	hVEGF	HER2
H6	Y	I	W					N	Y	V	G	G	S	S	S	W	W	A	G	87	ND
H7	N	V	W					D	W	V	P	A	S	S	G	W	Y	I	A	60	ND
H8	E	I	F	P				Y	Y	V	L	G	S	S	G	W	D		G	226	ND
H9	Y	V	W					Q	Y	V	H	A	S	S	G	Y	W	V	A	41	ND
H10	D	V	F					T	S	V	D	A	S	Y	R	Y	I	W	A	170	ND

FIG. 10A-1

		Affinity screening assays						L1							L2				L3			
	clone	SSC+/- 50nM Vegf			SSC+/- 20nM HER2			28	29	30			32	33	50	51	52	53	91			
H11	59	2.28	2.06	90.5	2.79	0.12	4.4	D	I	M	L	G	S	V	W	G	S	F	H	Y	T	T
H3	92	2.57	1.72	67.0	1.96	0.11	5.8	D	I	G	L	G	S	V	W	A	S	Y	H	Y	T	T
	1	2.38	2.90	96.9	2.69	0.10	6.6	D	I	R	G	G	S	V	W	A	S	F	H	Y	T	T
	64	2.91	2.92	100.3	3.29	0.23	7.1	D	I	A	K	G	S	V	W	G	S	F	H	Y	T	T
	38	2.15	2.00	96.7	2.77	0.20	7.2	D	I	L	A	G	S	V	W	G	S	F	H	Y	T	T
	67	2.36	2.35	99.5	2.50	0.19	7.6	D	I	A	K	G	S	V	W	G	S	F	H	Y	T	T
	56	2.67	2.61	97.7	2.95	0.24	8.0	D	I	S	R	G	S	V	W	G	S	F	H	Y	T	T
	60	2.02	2.00	99.0	2.78	0.23	8.2	N	I	Y	A	G	S	L	W	G	S	Y	H	Y	T	T
	71	2.44	2.34	95.9	2.82	0.24	8.4	D	I	A	F	G	S	L	W	G	S	F	H	Y	T	T
	56	2.43	2.46	101.3	2.38	0.22	9.3	D	I	K	A	G	S	V	W	G	S	Y	H	Y	T	T
	25	2.98	2.88	96.7	3.31	0.31	9.4	N	I	A	M	G	S	V	W	G	S	Y	H	Y	T	T
	14	2.54	2.40	94.4	2.83	0.27	9.5	D	I	L	K	G	S	V	W	G	S	Y	H	Y	T	T
	90	2.71	2.57	94.7	2.93	0.28	9.6	D	I	L	I	G	S	V	W	G	S	F	H	Y	T	T
	72	2.25	2.15	95.7	2.78	0.27	9.7	D	I	V	S	G	S	V	W	G	S	F	H	Y	T	T
	21	2.59	2.44	94.1	2.80	0.30	10.6	D	I	K	H	G	S	V	W	G	S	F	H	Y	T	T
	76	3.35	3.25	97.1	3.34	0.36	10.6															
	53	2.45	2.37	96.8	2.41	0.26	10.8	D	I	R	G	G	S	V	W	G	S	Y	H	Y	T	T
	37	2.29	2.21	96.4	2.73	0.30	10.9	D	I	R	Q	G	S	V	W	A	S	Y	H	Y	T	T
	76	2.65	2.49	94.0	2.83	0.31	11.1	D	I	S	M	G	S	V	W	G	S	F	H	Y	T	T
	86	2.49	2.41	96.6	2.78	0.31	11.1	D	I	K	V	G	S	V	W	G	S	Y	H	Y	T	T
	36	3.08	3.11	101.2	3.36	0.38	11.2	D	I	R	E	G	S	V	W	G	S	S	H	Y	T	T
	89	2.92	2.68	91.9	2.86	0.32	11.2	D	I	G	S	G	S	V	W	G	S	Y	H	Y	T	T
	17	2.82	2.67	94.7	2.85	0.32	11.3	D	I	L	T	G	S	V	W	G	S	F	H	Y	T	T
	2	3.02	3.09	102.3	3.32	0.39	11.8	D	I	R	V	G	S	V	W	G	S	Y	H	Y	T	T
H4	42	2.61	2.26	86.8	2.87	0.35	12.1	N	I	R	T	G	S	V	W	G	S	Y	H	Y	T	T
	16	2.56	2.57	100.5	3.07	0.38	12.3	D	I	S	R	G	S	V	W	G	S	F	H	Y	T	T
	23	3.11	2.95	94.8	3.31	0.42	12.8	D	I	Y	M	G	S	V	W	G	S	Y	H	Y	T	T
	17	3.07	2.96	96.2	3.29	0.43	13.1	D	I	S	S	G	S	V	W	G	S	Y	H	Y	T	T

FIG. 10A-2

		Affinity screening assays						L1							L2				L3			
	clone	SSC+/- 50nM Vegf			SSC+/- 20nM HER2			28	29	30			32	33	50	51	52	53	91			
	53	2.56	2.71	105.8	2.87	0.38	13.1	D	I	S	V	G	S	V	W	G	S	F	H	Y	T	T
	85	2.13	2.00	94.0	2.67	0.35	13.1	D	V	R	Q	G	S	V	W	G	S	F	H	Y	T	T
	9	3.21	3.14	98.0	3.30	0.43	13.1	D	I	Q	S	G	S	V	W	G	S	Y	H	Y	T	T
	2	2.38	2.33	97.9	2.84	0.37	13.1	D	I	V	S	G	S	V	W	G	S	F	H	Y	T	T
H5	95	2.77	2.46	88.8	2.87	0.38	13.2	D	I	R	G	G	S	V	G	G	S	Y	Y	Y	T	T
	48	2.77	2.70	97.5	2.86	0.39	13.6	D	I	K	S	G	S	L	W	G	S	Y	H	Y	T	T
	75	2.18	2.16	98.7	2.14	0.30	14.0	D	I	M	S	G	S	V	W	A	S	F	H	Y	T	T
	85	2.62	2.47	94.3	2.95	0.42	14.3	D	I	I	G	G	S	V	W	G	S	Y	H	Y	T	T
	40	2.36	2.31	97.8	2.69	0.40	15.0	D	I	L	G	G	S	V	W	G	S	F	H	Y	T	T
	5	3.01	2.97	98.5	2.96	0.46	15.4	D	I	R	L	G	S	V	W	G	S	Y	H	Y	T	T
	47	2.61	2.55	97.6	2.89	0.45	15.6	D	I	R	F	G	S	V	W	G	S	Y	H	Y	T	T
	94	2.77	2.64	95.2	2.87	0.45	15.7															
	32	2.65	2.60	98.1	2.87	0.45	15.7	D	I	G	A	G	S	V	W	G	S	F	H	Y	T	T
H3	95	2.55	2.26	88.8	2.67	0.43	16.1															
	44	2.73	2.79	102.0	3.28	0.54	16.4	D	I	A	T	G	S	L	W	G	S	Y	H	Y	T	T
	74	2.18	2.19	100.6	2.89	0.48	16.6	D	I	A	T	G	S	L	W	G	S	Y	H	Y	T	T
	38	2.66	2.60	100.7	2.84	0.48	16.8	D	I	R	F	G	S	V	W	G	S	Y	H	Y	T	T
	25	2.73	2.59	94.9	3.03	0.51	16.8	D	I	L	S	G	S	V	W	G	S	W	H	Y	T	T
	63	3.27	3.20	97.8	3.32	0.58	17.4															
	69	1.49	1.51	101.5	2.74	0.48	17.6	G	I	R	T	G	S	V	W	G	S	Y	H	Y	T	T
	58	2.90	2.09	92.7	2.93	0.52	17.7	D	I	A	M	G	S	V	W	G	S	Y	H	Y	T	T
	87	2.49	2.40	100.2	2.73	0.49	18.0	D	I	L	G	G	S	V	W	A	S	F	H	Y	T	T
	40	2.52	2.30	91.2	2.86	0.52	18.2	D	I	K	L	G	S	V	W	G	S	L	H	Y	T	T
	87	3.33	3.19	95.7	3.33	0.61	18.3	D	I	A	G	G	S	V	W	A	S	Y	H	Y	T	T
	4	2.87	2.84	99.0	3.10	0.57	18.4	N	I	Y	S	G	S	L	W	G	S	Y	H	Y	T	T
	88	3.06	2.94	95.9	3.05	0.57	18.6															
	94	2.59	2.63	101.7	2.95	0.55	18.8	D	I	R	M	G	S	V	W	G	S	F	H	Y	T	T

FIG. 10B-1

	Affinity screening assays						L1							L2				L3			
clone	SSC+/- 50nM Vegf			SSC+/- 20nM HER2			28	29	30			32	33	50	51	52	53	91			
9	3.12	2.96	94.8	3.01	0.59	19.5	D	I	R	A	G	S	V	W	A	S	F	H	Y	T	T
13	3.02	2.84	94.1	2.94	0.59	20.0	D	I	K	V	G	S	V	W	G	S	Y	H	Y	T	T
78	2.37	2.35	98.8	2.88	0.58	20.2	D	I	A	A	G	S	V	W	A	S	Y	H	Y	T	T
43	2.23	2.20	99.0	2.70	0.56	20.7	D	I	A	R	G	S	V	W	G	S	M	H	Y	T	T
79	2.83	2.81	99.4	2.98	0.62	20.7	D	I	Q	T	G	S	V	W	G	S	Y	H	Y	T	T
36	2.95	2.92	99.0	3.02	0.63	21.0	D	I	Q	S	G	S	V	W	G	S	F	H	Y	T	T
57	2.59	2.53	97.7	2.98	0.63	21.2	D	I	R	V	G	S	V	W	G	S	F	H	Y	T	T
21	2.86	2.74	95.7	2.97	0.64	21.6	D	I	T	M	G	S	V	W	G	S	L	H	Y	T	T
33	2.50	2.45	97.9	2.84	0.61	21.6	D	I	G	M	G	S	V	W	A	S	Y	H	Y	T	T
69	2.66	2.63	99.0	2.98	0.65	21.7	D	I	R	G	G	S	V	W	G	S	Y	H	Y	T	T
54	2.49	2.41	96.7	2.18	0.51	23.4	D	I	K	S	G	S	V	W	G	S	T	H	Y	T	T
82	2.59	2.44	94.4	2.87	0.67	23.4	N	I	A	M	G	S	V	W	G	S	Y	H	Y	T	T
24	2.75	2.51	91.2	2.88	0.67	23.4	D	I	Q	R	G	S	V	W	G	S	C	H	Y	T	T
82	2.87	2.74	95.5	2.84	0.69	24.4	D	I	R	S	G	S	V	W	G	S	Y	H	Y	T	T
57	2.45	2.32	94.5	2.56	0.63	24.6	D	I	R	S	G	S	V	W	G	S	T	H	Y	T	T
7	3.05	2.90	95.1	3.02	0.77	25.5	D	I	G	G	G	S	V	W	G	S	F	H	Y	T	T
17	2.30	2.42	104.9	2.79	0.71	25.6	D	I	K	F	G	S	V	W	G	S	S	H	Y	T	T
57	3.00	2.88	96.1	2.93	0.75	25.6	D	I	A	S	G	S	V	W	G	S	L	H	Y	T	T
66	2.70	2.98	110.4	2.99	0.78	25.9	D	I	R	R	G	S	V	W	G	S	Y	H	Y	T	T
92	2.91	2.93	100.7	2.98	0.78	26.1	D	I	R	I	G	S	V	W	G	S	F	H	Y	T	T
25	2.66	2.57	96.6	2.85	0.75	26.3	D	I	G	A	G	S	V	W	G	S	F	H	Y	T	T
31	2.13	1.98	92.9	2.90	0.82	28.5	D	I	A	S	G	S	V	W	G	S	S	H	Y	T	T
26	2.84	2.97	104.6	3.07	0.90	29.4	D	I	V	X	G	S	V	W	G	S	F	H	Y	T	T

FIG. 10B-2

		Affinity screening assays						L1							L2				L3			
	clone	SSC+/- 50nM Vegf			SSC+/- 20nM HER2			28	29	30			32	33	50	51	52	53	91			
	60	2.40	2.29	95.4	2.76	0.81	29.4	D	I	R	M	G	S	V	W	G	S	S	H	Y	T	T
	18	2.66	2.49	93.7	2.82	0.84	29.6															
	63	2.48	2.41	97.2	2.95	0.88	29.8	D	I	K	L	G	S	V	W	G	S	M	H	Y	T	T
	11	2.73	2.51	91.9	2.98	0.93	31.4	D	I	A	H	G	S	V	W	A	S	Y	H	Y	T	T
H1	48	2.03	1.44	71.0	2.18	0.74	33.8	D	I	P	R	S I S G	Y	V	W	G	S	Y	H	Y	T	T
	39	2.76	2.59	93.9	2.80	0.96	34.1	D	V	R	Q	G	S	V	W	G	S	F	H	Y	T	T
	45	2.73	2.60	95.2	2.84	0.97	34.2	N	I	A	Q	G	S	V	W	A	S	F	H	Y	T	T
	10	2.34	2.15	91.7	2.78	1.01	36.2	D	I	K	N	G	S	V	W	A	S	F	H	Y	T	T
	32	3.15	3.12	99.2	3.31	1.23	37.0	D	I	L	G	G	S	V	W	A	S	F	H	Y	T	T
	19	2.40	2.33	97.0	2.63	1.04	39.7	D	I	R	A	G	S	V	W	G	S	Y	H	F	N	A
	91	3.15	3.05	96.9	3.30	1.34	40.5	D	I	R	S	G	S	V	W	G	S	N	H	Y	T	T
	44	2.45	2.41	98.1	2.75	1.25	45.4	D	I	R	S	G	S	V	W	G	S	E	H	Y	T	T
	29	3.16	3.16	100.0	3.35	1.55	46.4	D	I	K	L	G	S	V	W	G	S	M	H	Y	T	T
	19	2.96	3.01	101.6	2.86	1.35	47.0	D	I	K	W	G	S	V	W	G	S	T	H	Y	T	T
	34	2.85	2.72	95.5	3.01	1.42	47.3	N	I	R	S	G	S	V	W	G	S	V	H	Y	T	T
	49	2.57	2.52	98.2	2.99	1.44	48.1	D	I	R	S	G	S	V	W	G	S	S	H	Y	T	T
	13	2.77	2.74	98.8	3.03	1.82	60.1	D	I	K	S	G	S	V	W	G	S	T	H	Y	T	T
	16	3.26	3.17	97.4	3.35	2.23	66.5	D	I	A	R	G	S	V	W	G	S	M	H	Y	T	T
	35	2.31	2.41	104.4	1.04	0.94	90.2	D	I	R	T	G	S	V	W	G	S	F	H	Y	T	T
	32	2.46	2.26	91.9	1.08	1.00	92.3	D	V	W		K	W	V	A	A	S	S	H	Y	T	T
	10	3.41	3.35	98.2	3.09	3.02	97.7	D	I	R	R	G	S	V	W	G	S	A	H	Y	T	T
	61	2.05	2.09	101.7	0.60	0.62	103.6	D	I	W		H	W	V	A	G	S	S	H	Y	T	T

FIG. 11

		ELISA Affinity screen						L1							L2				L3				
		SSC+/- 50 or 100nM VEGF			SSC+/- 20nM HER2			28	29	30		31	32	33	50			53	91	92	93	94	94a
	6	2.12	1.81	85.6	0.04	0.04	103.3	D	I	W		R	A	V	P	A	S	N	G	Y	Y	I	A
	8	2.48	1.88	76.1	0.05	0.06	126.5	Y	I	W		N	Y	V	G	G	S	S	S	W	W	A	G
	12	2.23	1.81	81.3	0.04	0.04	97.9	D	I	G	G	G	S	V	W	G	S	F	H	Y	T	T	
	18	2.88	2.56	88.8	0.04	0.04	96.7	W	V	P	S	H	T	V	L	G	S	Y	S	D	Y	T	
	20	2.46	2.21	90.9	0.05	0.04	91.5	Q	I	W		R	H	L	T	G	S	S	S	Y	W	V	A
	43	1.96	1.76	89.5	0.04	0.04	96.1	D	I	W		R	W	V	A	A	S	S	H	A	G	A	
	5	2.47	0.41	16.6	0.04	0.04	99.7	N	V	W		D	W	V	P	A	S	S	G	W	Y	I	A
	26	2.68	2.11	78.5	0.04	0.04	110.8	N	I	F		S	H	V	P	G	S	Y	A	F	W	V	S
	27	2.82	2.70	95.7	0.04	0.04	98.5	Y	V	Y	S	T	T	V	N	G	S	S	A	S	S	A	
	28	2.58	1.83	71.2	0.09	0.04	47.3	E	I	F	P	Y	Y	V	L	G	S	S	G	W	D	G	
	38	3.09	0.69	22.2	0.04	0.04	105.5	Y	V	W		Q	Y	V	H	A	S	S	G	Y	W	V	A
	45	3.08	2.72	88.2	0.05	0.04	93.6	D	I	H	S	G	S	V	W	G	S	Y	H	Y	T	T	
	67	2.08	1.02	49.0	0.04	0.04	103.1	D	V	F		T	S	V	D	A	S	Y	R	Y	I	W	A
	75	2.90	2.47	85.1	0.07	0.07	102.3	D	I	P	S	I	Y	V	G	A	S	Y	R	Y	W	V	A
	86	3.25	2.57	79.0	0.06	0.07	104.6	N	V	W		S	H	V	G	G	S	S	R	L	W	F	T
	19	2.13	1.86	87.6	0.05			W	V	P	S	H	T	V	L	G	S	Y	S	D	Y	T	
c3-1	37	1.36	0.26	18.8	0.05			N	V	W		D	W	V	P	A	S	S	G	W	Y	I	A
c3-3	41	1.36	0.72	53.3	0.05			Q	I	W		R	H	L	T	G	S	S	S	Y	W	V	A
c3-4	46	1.22	0.24	20.1	0.05			E	V	Y		S	Y	V	P	A	S	S	G	F	W	I	G
	51	1.05	0.59	58.5	0.05			N	I	F		S	H	V	P	G	S	Y	A	F	W	V	S
	67	1.05	0.90	85.8	0.05			D	I	W		R	W	L	A	G	S	S	H	D	Q	A	
c3-6	70	1.84	0.43	23.3	0.05			Y	V	W		Q	Y	V	H	A	S	S	G	Y	W	V	A
c3-7	84	1.37	0.33	23.9	0.05			W	V	P	S	H	T	V	L	G	S	Y	S	D	Y	T	
	88	2.00	1.56	77.8	0.05			N	V	W		S	H	V	G	G	S	S	R	L	W	F	T
c3-8	90	1.30	0.68	52.3	0.05			D	I	W		R	W	V	A	A	S	S	H	A	G	A	
	92	1.39	1.02	72.3	0.05				I	W		R	Y	V	W	G	S	S	G	L	W	Y	S

FIG. 12

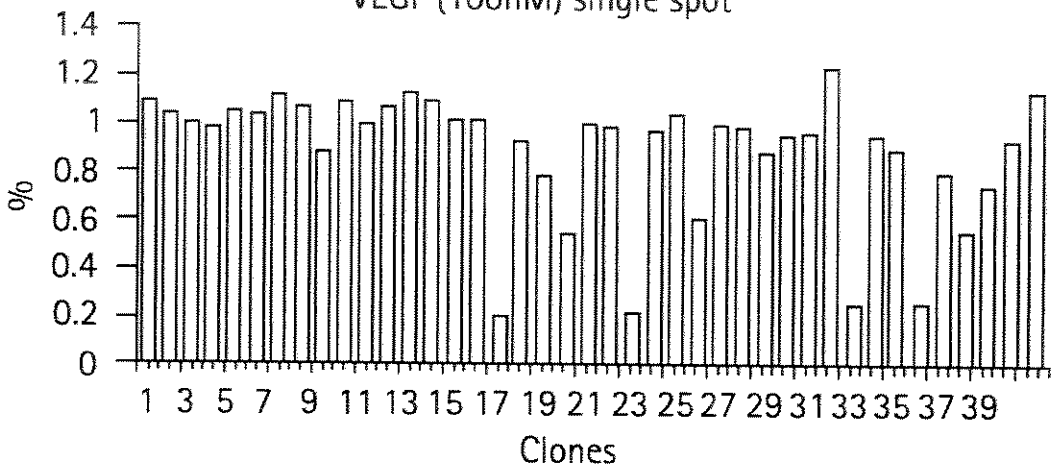


FIG. 13A

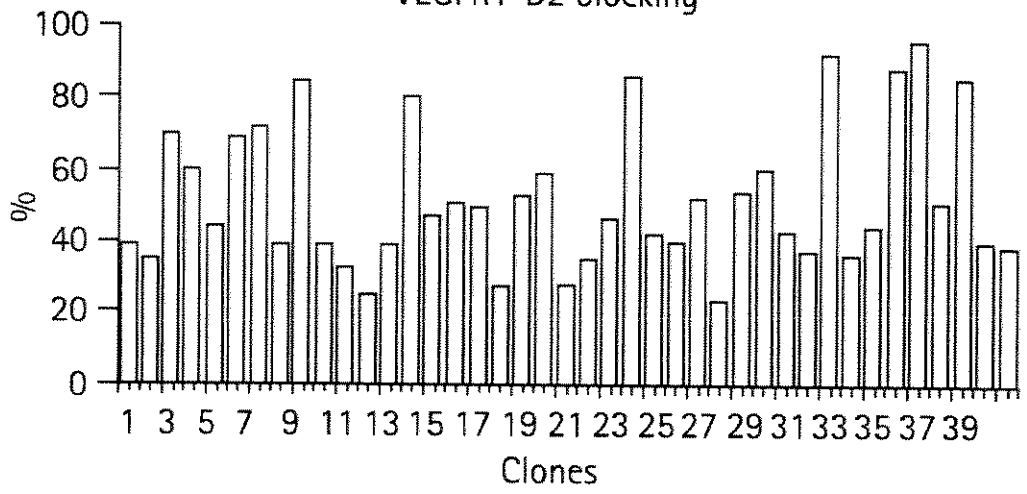


FIG. 13B

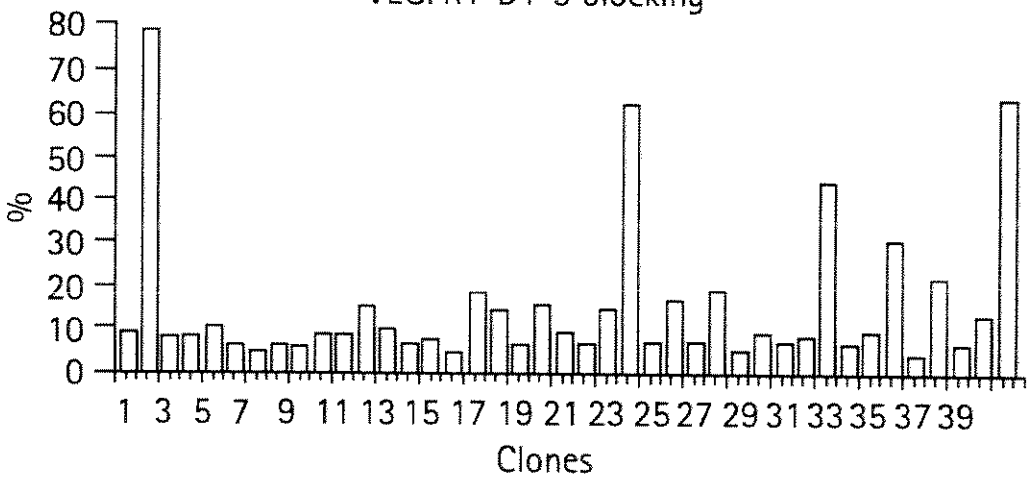


FIG. 14A

hVEGF Binders	L1										L2				L3							
	28	29	30	30a	30b	30c	30d	31	32	33	50	51	52	53	91	92	93	93a	93b	94	Phage IC50 (nM)	
4D5	D	V	S					T	A	V	S	A	S	F	H	Y	T			T	hVEGF	HER2
3-1	N	V	W					D	W	V	P	A	S	S	G	W	Y	I		A	22	ND
3-2	D	I	R	M				G	S	V	W	G	S	V	H	Y	T			T	>1000	1.56
3-3	Q	I	W					R	H	L	T	G	S	S	S	Y	W	V		A	150	ND
3-4	E	V	Y					S	Y	V	P	A	S	S	G	F	W	I		G	32	ND
3-5	N	I	F					S	H	V	P	G	S	Y	A	F	W	V		S	101	ND
3-6	Y	V	W					Q	Y	V	H	A	S	S	G	Y	W	V		A	8	ND
3-7	Y	I	W					R	Y	V	W	G	S	S	G	L	W	Y		S	16	ND
3-8	N	V	W					S	H	V	G	G	S	S	R	L	W	F		T	69	ND

FIG. 14B

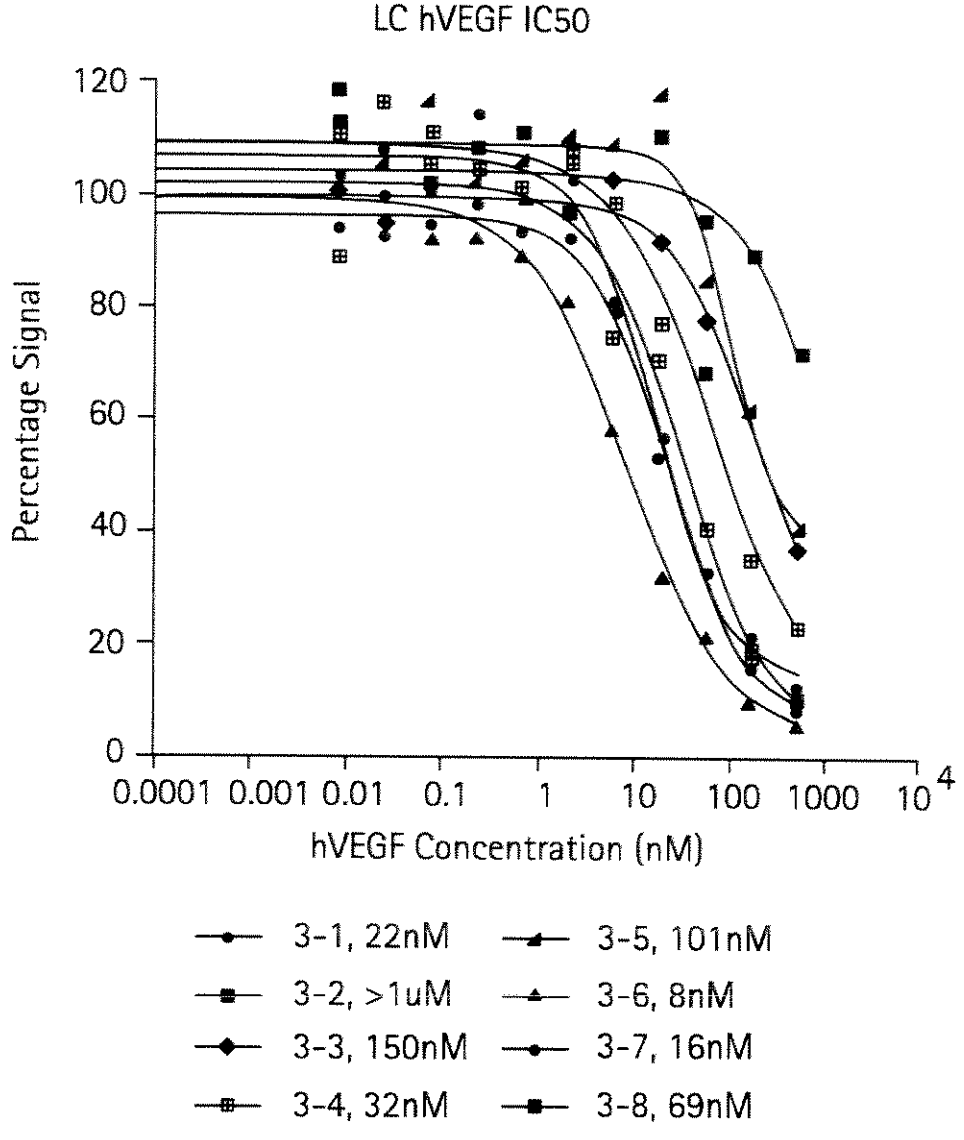


FIG. 15

hVEGF Binders Plate-Sorted Directly on HER2

	L1										L2				L3					Phage IC50(nM)	
	28	29	30	30a	30b	30c	30d	31	32	33	50	51	52	53	91	92	93	93a	94		
4D5	D	V	S					T	A	V	S	A	S	F	H	Y	T		T	hVEGF	HER2
H1	D	I	P	R	S	I	S	G	Y	V	W	G	S	Y	H	Y	T		T	77	10
H3	D	I	G	L				G	S	V	W	A	S	Y	H	Y	T		T	232	2.6
H4	N	I	R	T				G	S	V	W	G	S	Y	H	Y	T		T	115	0.54
H5	D	I	R	M				G	S	V	W	G	S	F	H	Y	T		T	242	721

FIG. 16

	L1										L2				L3						
	28	29	30	30a	30b	30c	30d	31	32	33	50	51	52	53	91	92	93	93a	93b	94	Single/Dual
4D5	D	V	S					T	A	V	S	A	S	F	H	Y	T			T	Specificity
3-1	N	V	W					D	W	V	P	A	S	S	G	W	Y	I		A	
3-6	Y	V	W					Q	Y	V	H	A	S	S	G	Y	W	V		A	hVEGF
3-7	Y	I	W					R	Y	V	W	G	S	S	G	L	W	Y		S	
H1	D	I	P	R	S	I	S	G	Y	V	W	G	S	Y	H	Y	T			T	
H3	D	I	G	L				G	S	V	W	A	S	Y	H	Y	T			T	hVEGF/Her2
H4_N	N	I	R	S				G	S	V	W	A	S	Y	H	Y	T			T	
H4_D	D	I	R	S				G	S	V	W	G	S	Y	H	Y	T			T	
4-1	D	I	W	N	R			R	A	L	E	G	S	S	G	G	S	Y	S	S	
4-5	N	V	G					R	P	V	G	G	S	S	Y	G	S	F	G	T	DR5
4-6	S	V	S					S	A	V	S	A	S	S	Y	S	S			S	
D1	N	V	S					K	H	V	W	G	S	Y	S	Y	S			G	DR5/Her2
D2	N	I	R	N	G			G	G	L	S	A	S	F	H	Y	T			T	

FIG. 17

Clone	Specificity	Yield			Aggregation (%)	
		Fab/L *E. Coli* Small Scale	Fab/L *E. Coli* Fermentor Run	hIgG/L 293	Fab	hIgG
3_1	hVEGF	8.5 mg **	/	60 mg	3	2
H1	hVEGF/Her2	0.8 mg*	66.8 mg	40 mg	4	5
H3	hVEGF/Her2	9.2 mg**	/	40 mg	/	4
H4_N	hVEGF/Her2	0.8 mg*	/	37 mg	/	/
H4_D	hVEGF/Her2	/	/	43 mg	/	/
4_1	DR5	15.2 mg**	/	10 mg	/	/
4_5	DR5	ND	/	6.7 mg	/	/
D1	DR5/Her2	ND	/	10 mg	/	/
D2	DR5/Her2	ND	/	6.7 mg	/	/

*Based on 1 L Culture
**Based on 4 L Culture
/ Not Determined
ND=No Fab detected in soluble fraction

FIG. 18A

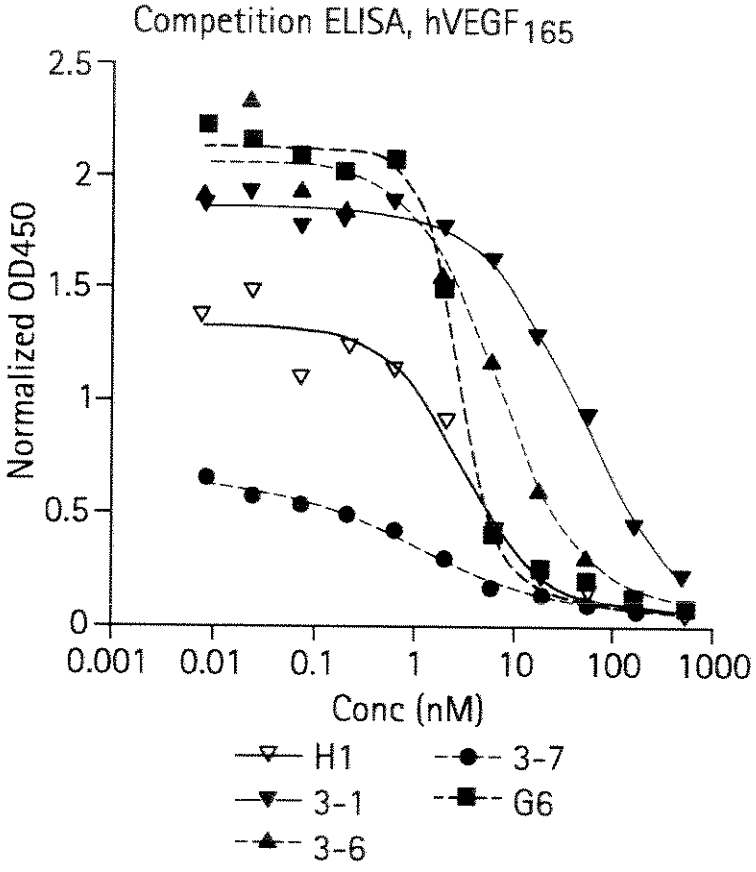


FIG. 18B

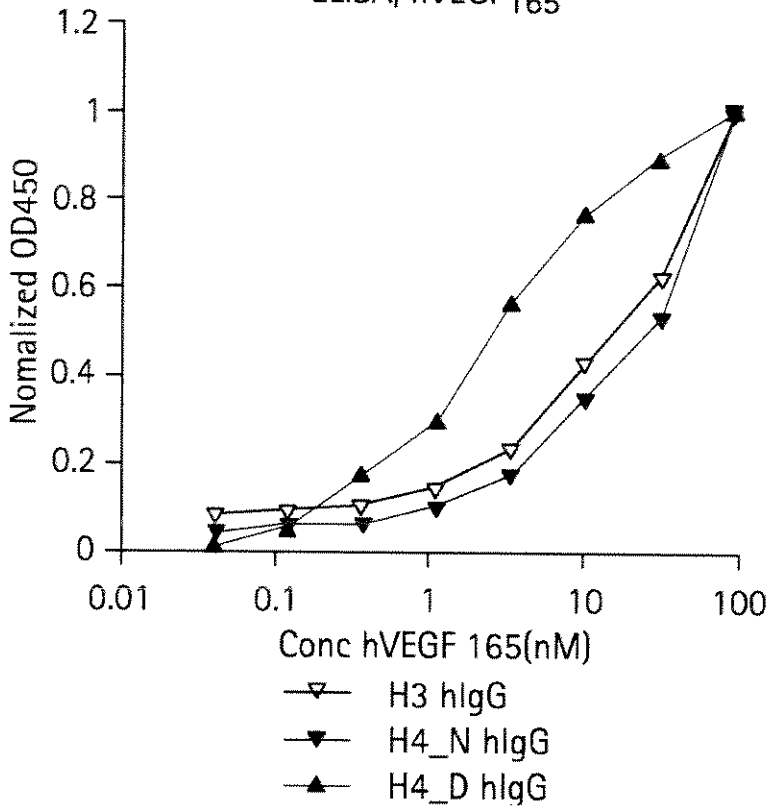


FIG. 19

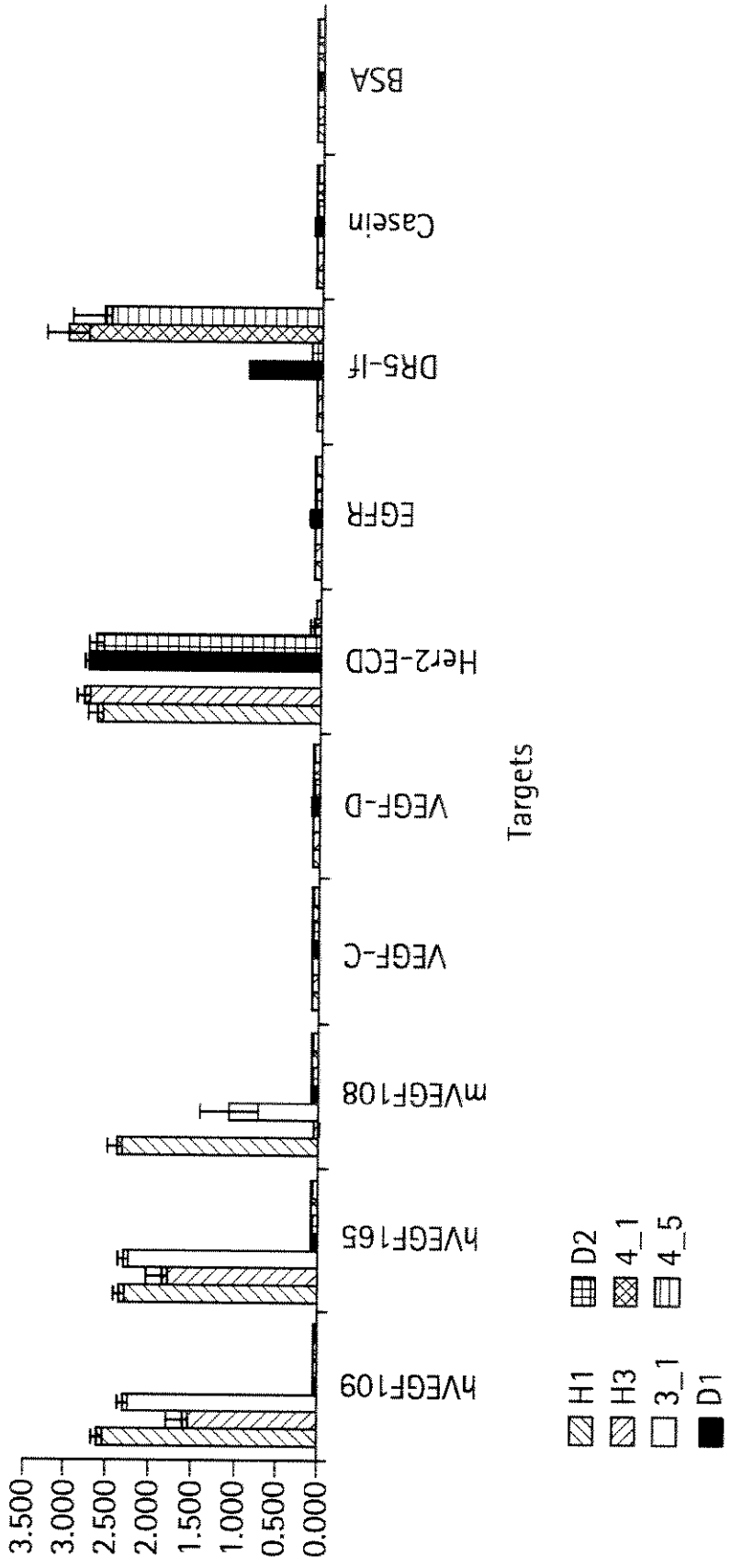

3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
hVEGF109
hVEGF165
mVEGF108
VEGF-C
VEGF-D
Her2-ECD
EGFR
DR5-If
Casein
BSA
Targets
H1
H3
3_1
D1
D2
4_1
4_5

FIG. 20

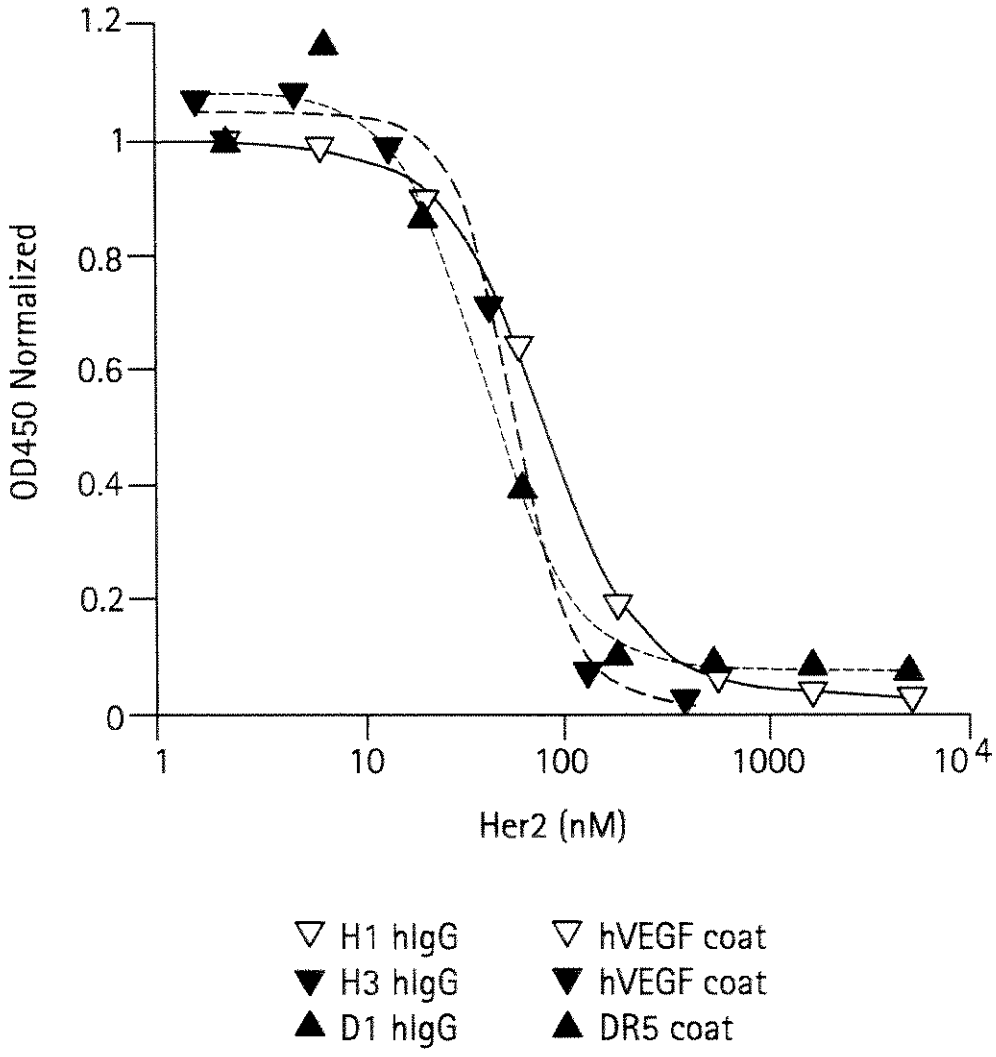

1.2
1
0.8
0.6
0.4
0.2
0
OD450 Normalized
1
10
100
1000
10 4
Her2 (nM)
H1 hIgG
H3 hIgG
D1 hIgG
hVEGF coat
hVEGF coat
DR5 coat

FIG. 21

Clone	Format	Specificity	Her2-ECD			$hVEGF_{109}$			$mVEGF_{108}$		
			k_{on}(1/Ms)	k_{off}(1/s)	K_D(nM)	k_{on}(1/Ms)	k_{off}(1/s)	k_D(nM)	k_{on}(1/Ms)	k_{off}(1/s)	K_D(nM)
3_1	Fab	hVEGF		*NB*		1.4E+05	2.2E-03	15	3.5E+04	3.6E-03	105
	IgG			*NB*		8.0E+04	9.6E-04	12	3.6E+05	1.6E-02	44
3_6	Fab	hVEGF		*NB*		4.5E+05	1.1E-03	3	8.3E+04	4.5E-03	54
	IgG			*NB*		2.0E+05	9.7E-04	5	1.8E+05	1.4E-02	78
3_7	Fab	hVEGF		*NB*		8.0E+05	2.5E-03	3	7.2E+04	5.6E-03	78
	IgG			*NB*		5.8E+05	4.2E-03	7	5.0E+05	1.5E-02	30
H1	Fab	hVEGF/Her2			59.1	2.7E+04	9.3E-03	350		/	
	IgG		170000	*0.0017*	9.8	3.4E+05	4.6E-03	140		/	
H3	Fab	hVEGF/Her2			8	1.5E+03	5.9E-03	3930		*NB*	
	IgG		1.0E+05	2.1E-03	20	*	*	**		*NB*	
H4_N	Fab	hVEGF/Her2	140000	*0.0013*	9.87	*	*	550		NB	
	IgG		230000	*0.0013*	6	*	*	**		NB	
H4_D	Fab	hVEGF/Her2		/		*	*	/		*NB*	
	IgG		1.0E+05	1.2E-03	11	*	*	2300		*NB*	

Accurate kinetic analysis not possible, use steady state binding analysis

**repeat experiment with higher analyte concentration to enable SS binding analysis

NB=No Binding detected

FIG. 22A

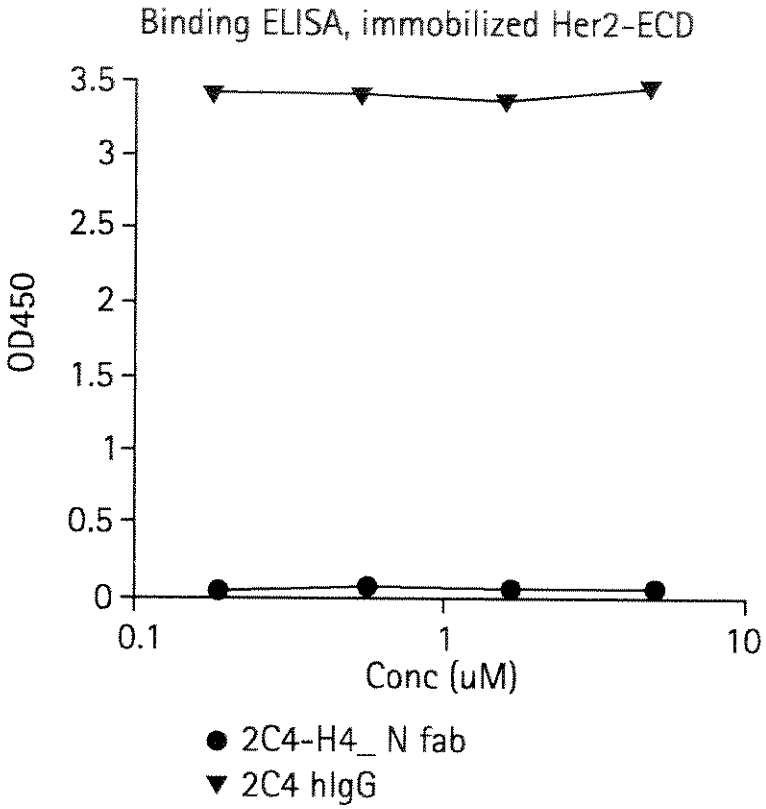


FIG. 22B

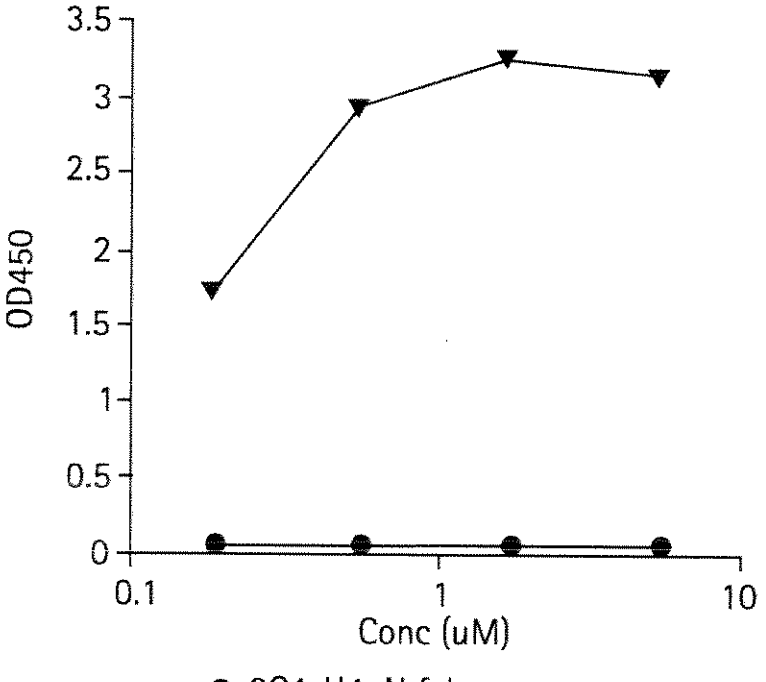


FIG. 23A

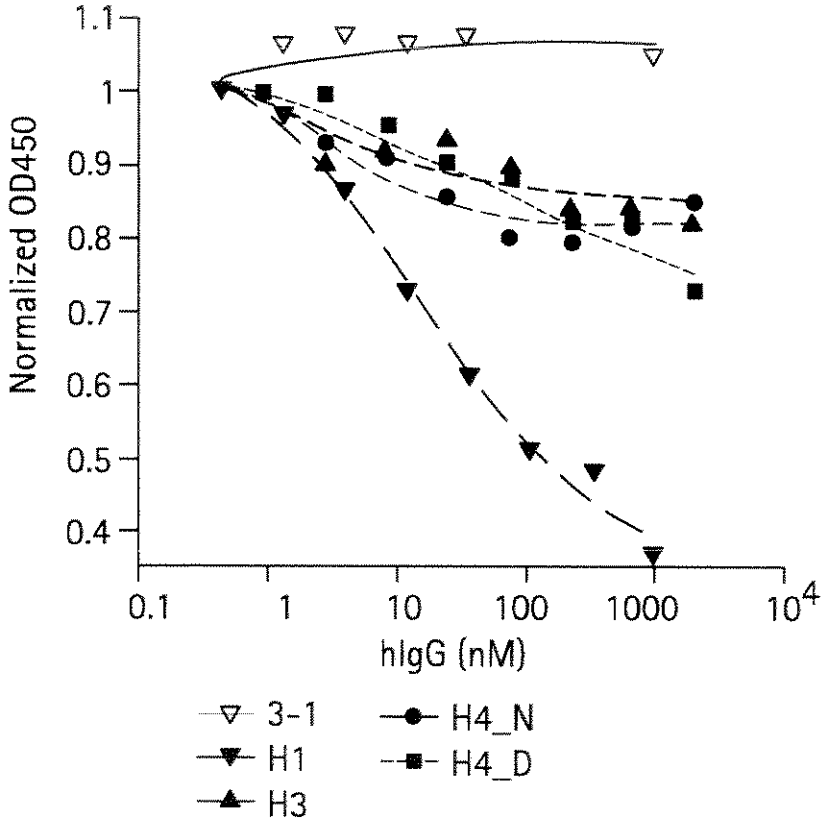


FIG. 23B

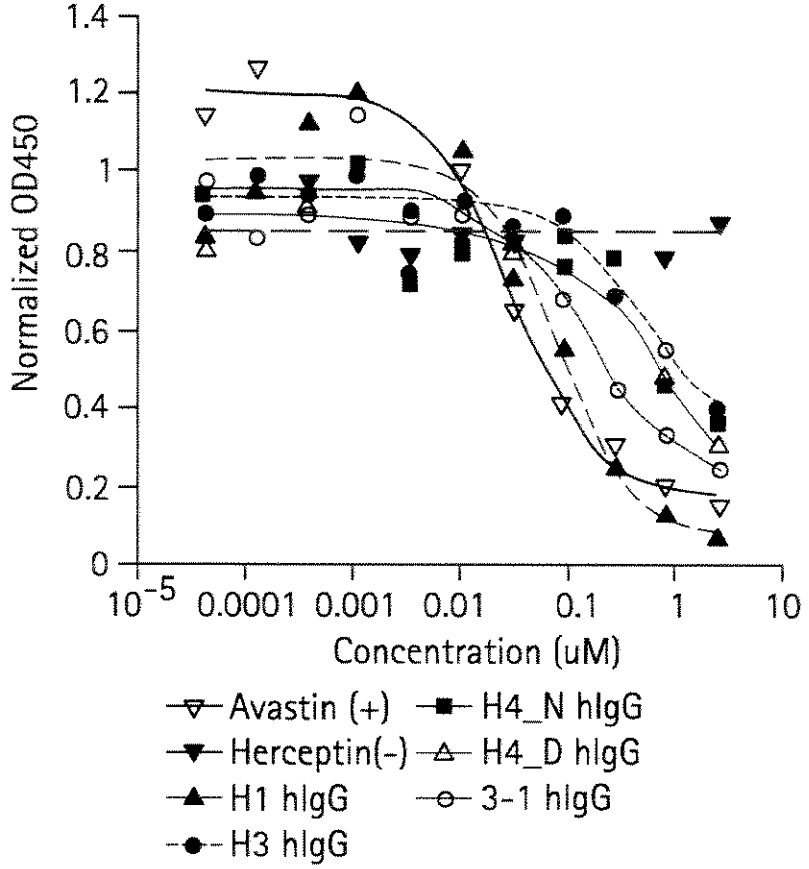


FIG. 24

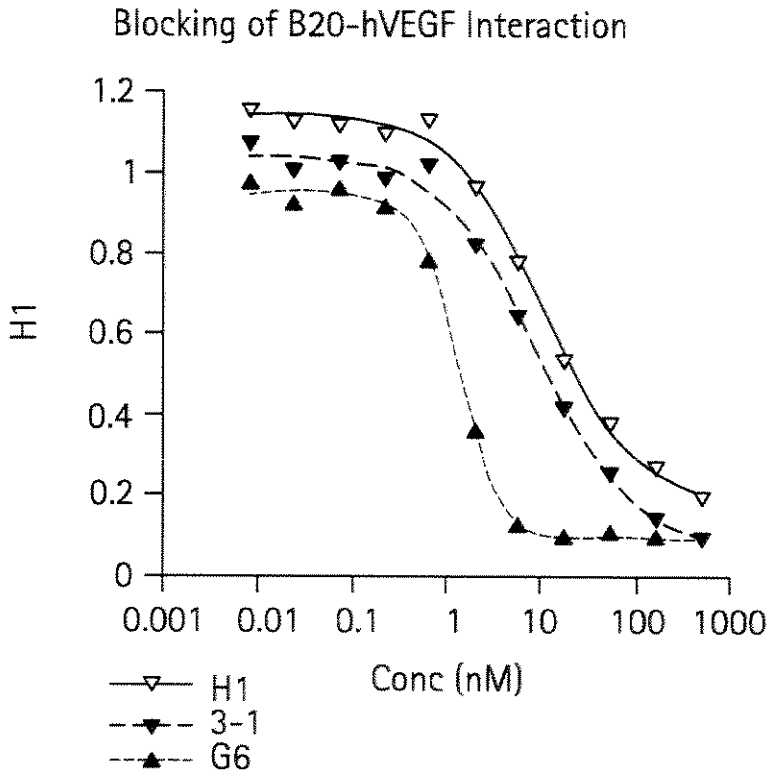

Blocking of B20-hVEGF Interaction
H1
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.01
0.1
1
10
100
1000
Conc (nM)
H1
3-1
G6

FIG. 25

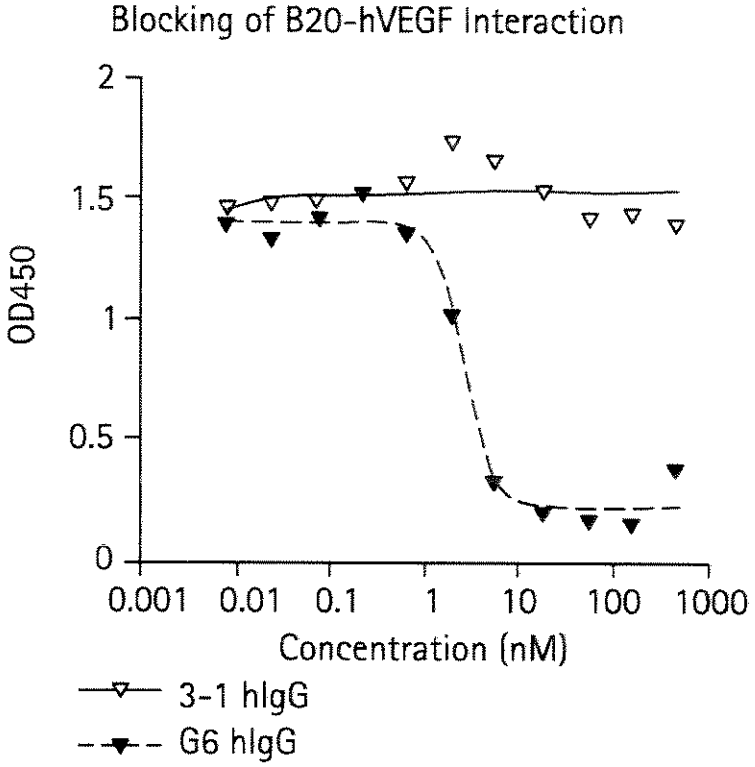
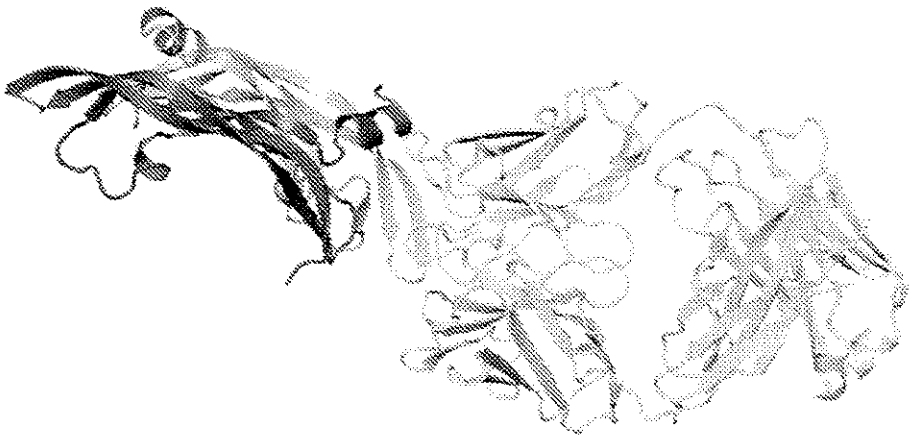
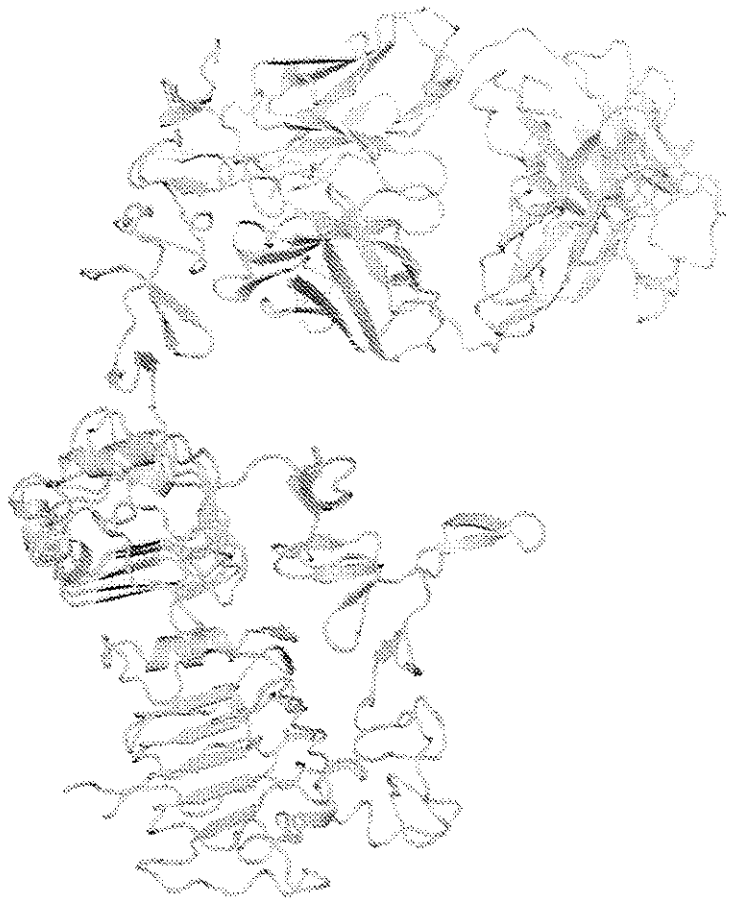

Blocking of B20-hVEGF Interaction
OD450
2
1.5
1
0.5
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)
3-1 hIgG
G6 hIgG

FIG. 26



bH1 Fab/VEGF



bH1 Fab/HER2

FIG. 27

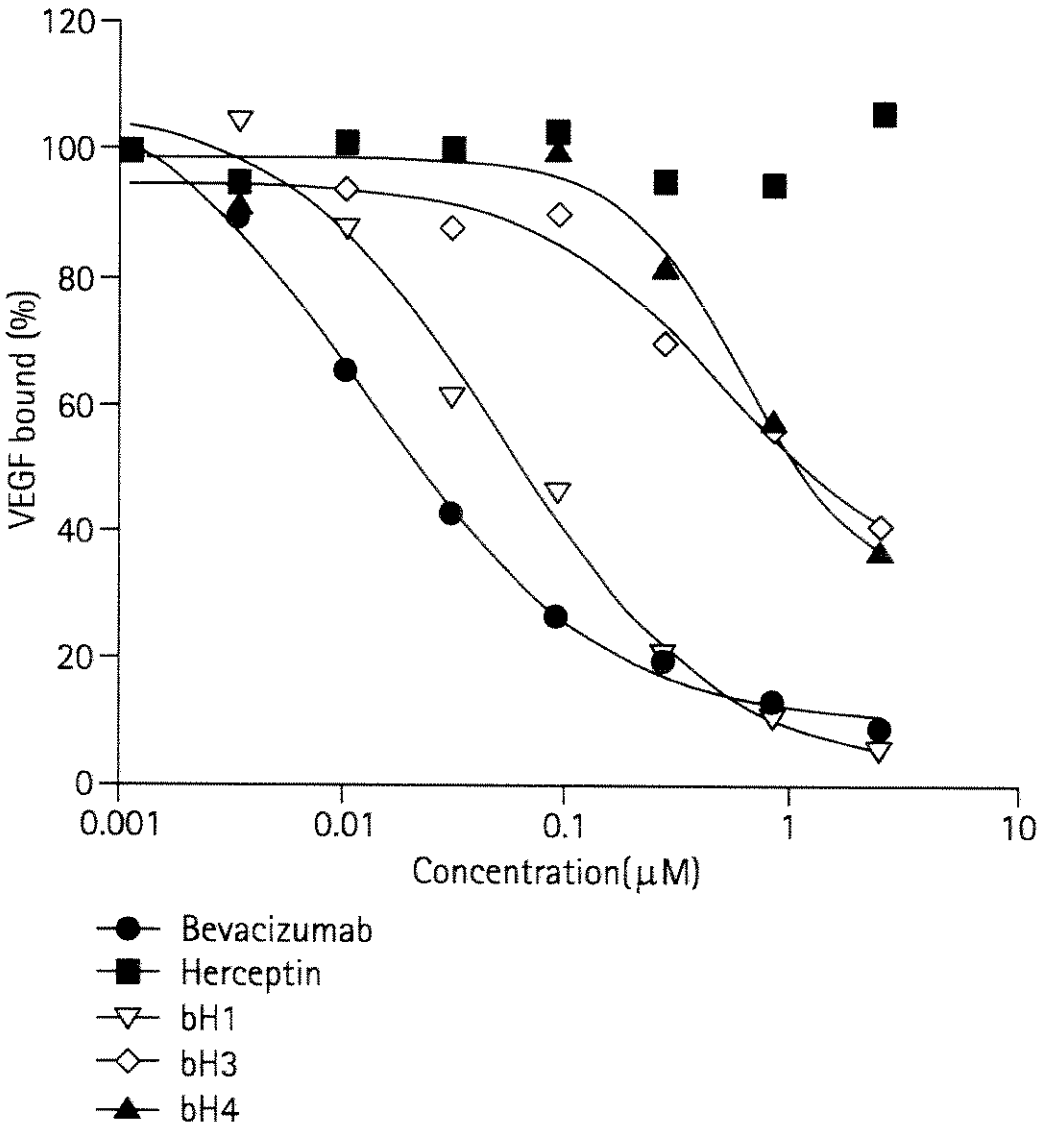
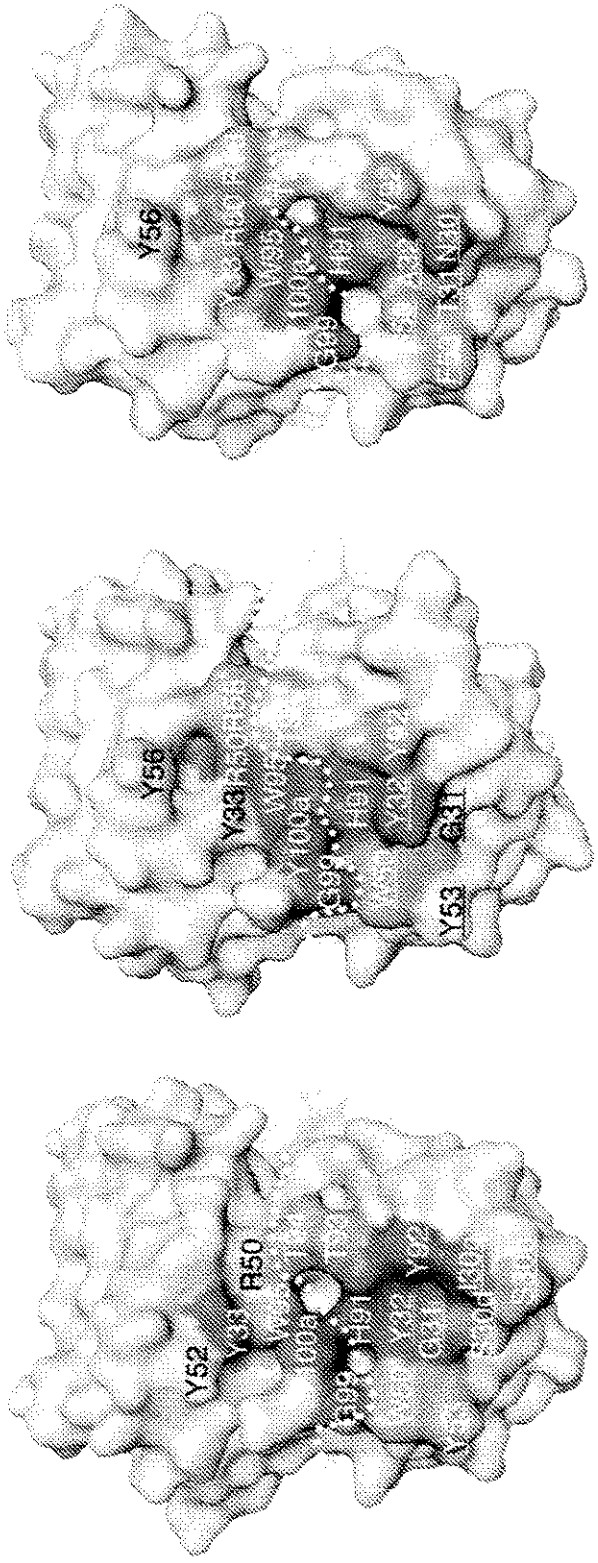

120
100
80
60
40
20
0
VEGF bound (%)
0.001
0.01
0.1
1
10
Concentration(μM)
Bevacizumab
Herceptin
bH1
bH3
bH4

FIG. 28



bH1 (VEGF bound)

bH1 (HER2 bound)

Herceptin (HER2 bound)

FIG. 29

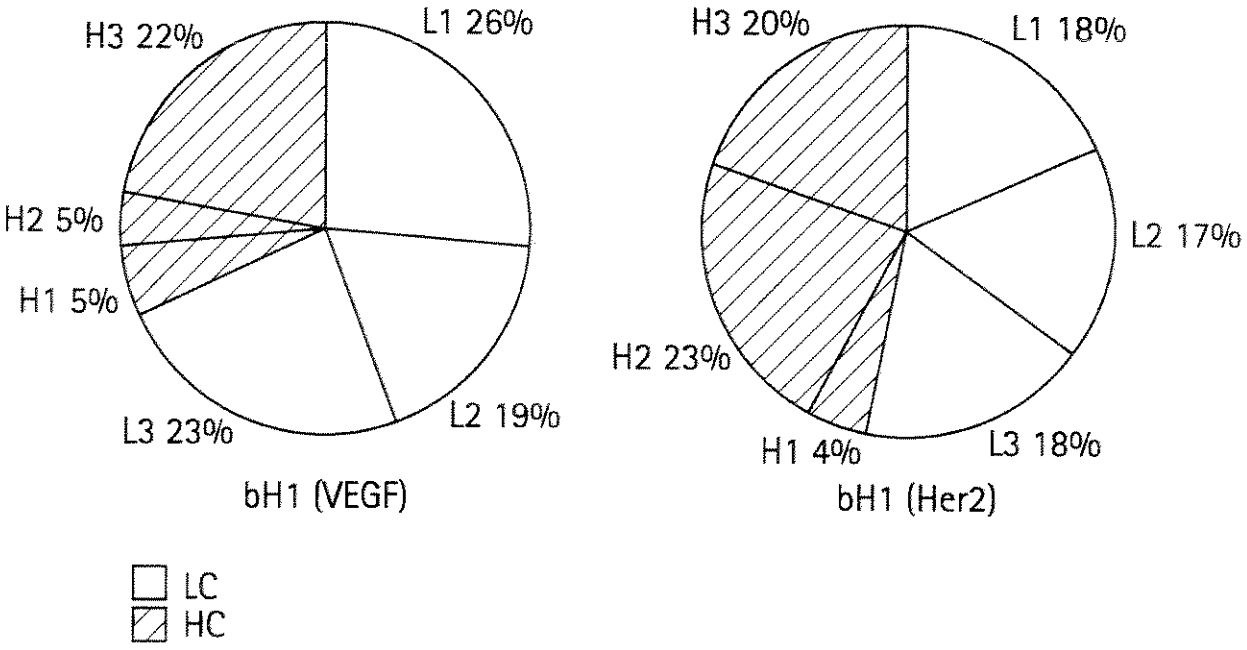

H3 22%
L1 26%
H2 5%
H1 5%
L3 23%
L2 19%
bH1 (VEGF)
H3 20%
L1 18%
L2 17%
H2 23%
H1 4%
L3 18%
bH1 (Her2)
LC
HC

FIG. 30

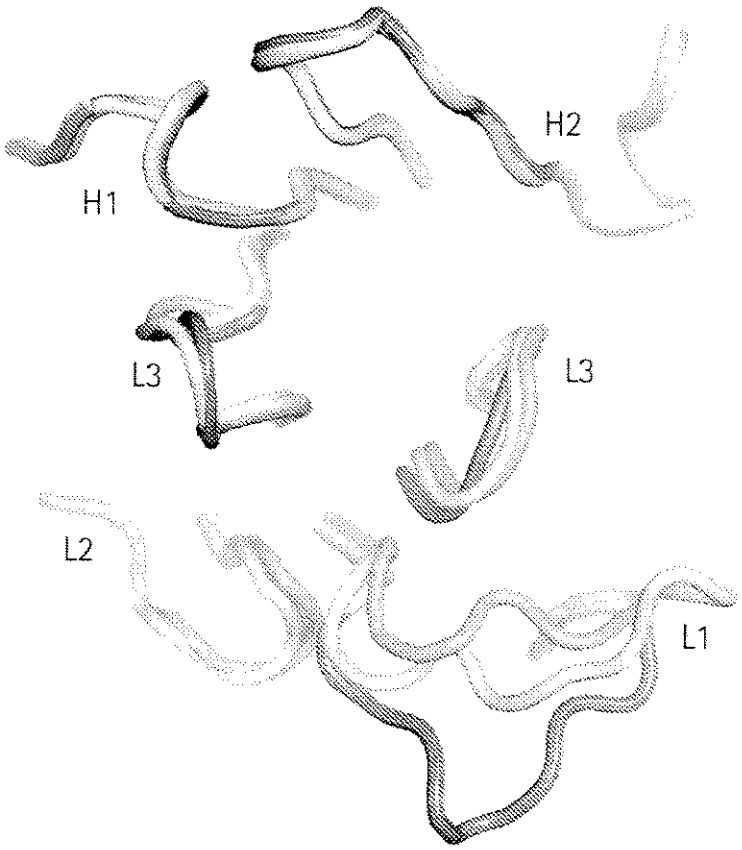


FIG. 31

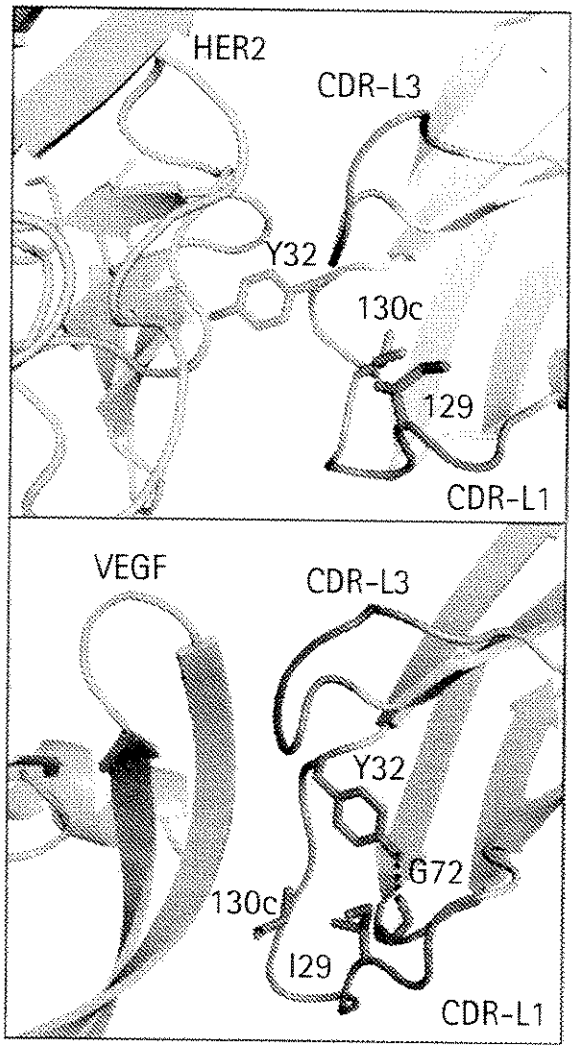
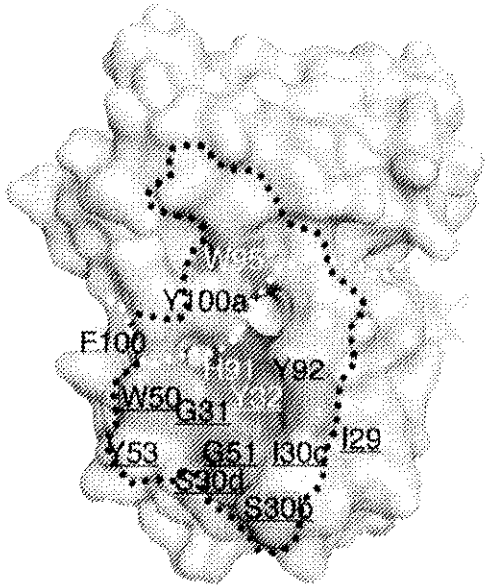
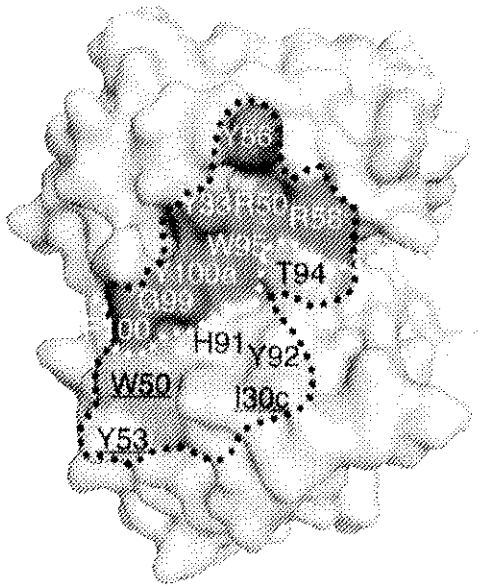

HER2
CDR-L3
Y32
130c
129
CDR-L1
VEGF
CDR-L3
Y32
G72
130c
I29
CDR-L1

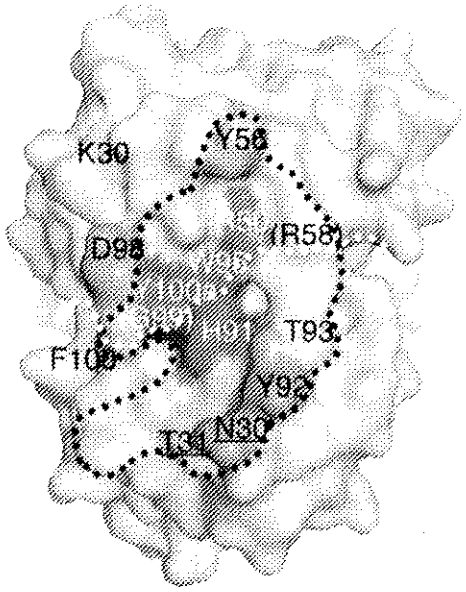
FIG. 32



bH1 (VEGF bound)



bH1 (HER2 bound)



Herceptin (HER2 bound)

FIG. 33

CDR	Alanine-scan Wild Type	Codon	m1	m2	m3
CDR-L1	D28	GMT	A		
	I29	RYT	A	T	V
	P30	SCA	A		
	R30a	SST	A	G	P
	S30b	KCC	A		
	I30c	RYT	A	T	V
	S30d	KCC	A		
	G31	GST	A		
	Y32	KMT	A	D	S
CDR-L2	W50	KSG	A	G	S
	G51	GST	A		
	S52	KCC	A		
	Y53	KMT	A	D	S
CDR-L3	H91	SMT	A	D	P
	Y92	KMT	A	D	S
	T93	RCT	A		
	T94	RCT	A		
CDR-H1	K30	RMA	A	E	T
	D31	GMT	A		
	T32	RCT	A		
	Y33	KMT	A	D	S
CDR-H2	R50	SST	A	G	P
	Y52	KMT	A	D	S
	T53	RCT	A		
	N54	RMC	A	D	T
	Y56	KMT	A	D	S
	R58	SST	A	G	P
CDR-H3	W95	KSG	A	G	S
	G96	GST	A		
	G97	GST	A		
	D98	GMT	A		
	G99	GST	A		
	F100	KYT	A	S	V
	Y100a	KMT	A	D	S

CDR	Homolog-scan Wild Type	Codon	m1 (Hom Res)	m2	m3	m4	m5
CDR-L1	Q27	SAA	E				
	D28	RAM	E	N	K		
	I29	VTY	V	L			
	P30	SCA	A				
	R30a	ARG	K				
	S30b	RST	A	G	T		
	I30c	VTY	V	L			
	S30d	RST	A	G	T		
	G31	RST	A	S	T		
	Y32	THY	F	S			
CDR-L2	W50	TKG	L				
	G51	GST	A				
	S52	KCC	A				
	Y53	TWC	F				
CDR-L3	H91	HWT	N	F	Y	L	I
	Y92	TWC	F				
	T93	ASC	S				
	T94	RST	S	A	G		
CDR-H1	K30	ARG	R				
	D31	GAM	E				
	T32	ASC	S				
	Y33	TWC	F				
CDR-H2	R50	ARG	K				
	Y52	TWC	F				
	T53	ASC	S				
	N54	RAC	D				
	Y56	TWC	F				
	R58	ARG	K				
CDR-H3	W95	TKG	L				
	G96	GST	A				
	G97	GST	A				
	D98	GAM	E				
	G99	GST	A				
	F100	TWC	Y				
	Y100a	TWC	F				

FIG. 34

Library Construction

Library	CDRs	Residues	Shotgun Codons	Oligonucleotides	Theoretical Diversity
LC-Ala	L1	D28, I29, P30, R30a, S30b, I30c, S30d, G31, Y32	Alanine	L1-ALA	3.3 e7
	L2	W50, G51, S52, Y53		L2-ALA	
	L3	H91, Y92, T93, T94		L3-ALA	
LC-Hom	L1	Q27, D28, I29, P30, R30a, S30b, I30c, S30d, G31, Y32	Homolog	L1-HOM	1.4 e7
	L2	W50, G51, S52, Y53		L2-HOM	
	L3	H91, Y92, T93, T94		L3-HOM	
HC-Ala	H1	K30, D31, T32, Y33	Alanine	H1-ALA	1.3 e8
	H2	R50, Y52, T53, N54, Y56, R58		H2-ALA	
	H3	W95, G96, G97, D98, G99, F100, Y100a		H3-ALA	
HC-Hom	H1	K30, D31, T32, Y33	Homolog	H1-HOM	1.3 e5
	H2	R50, Y52, T53, N54, Y56, R58		H2-HOM	
	H3	W95, G96, G97, D98, G99, F100, Y100a		H3-HOM	

FIG. 35

		Antigen Selection (VEGF)				Display Selection (anti-gD)					Fwt/mut values			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4		Fwt/m1	Fwt/m2	Fwt/m3	Fwt/m4
CDR-L1	Q27				1.39				0.97	Q27				1.44
	D28	2.38			(6.0)[E]	1.73			(1.29)[E]	D28	1.38			(4.65)[E]
	I29	8.33	8.33	1.32	(0.94)[L]	1.95	3.00	0.98	(1.15)[L]	I29	4.27	2.78	1.35	(0.81)[L]
	P30	1.83			4.27	1.18			1.33	P30	1.56			3.22
	R30a	1.91	3.50	2.63	0.90	2.38	0.91	0.94	1.93	R30a	0.80	3.84	2.79	0.47
	S30b	4.75			(0.85)[T]	1.06			(1.1)[T]	S30b	4.49			(0.80)[T]
	I30c	7.67	7.67	1.05	48.00	3.23	2.80	1.00	1.03	I30c	2.37	2.74	1.05	(46.8)[L]
	S30d	8.80			(12.2)[T]	1.44			(1.3)[T]	S30d	6.09			(9.36)[T]
	G31	9.20			(38.5)[A]	2.00			(2.90)[A]	G31	4.60			(13.3)[A]
	Y32	14.00	42.00	14.00	(38.5)[F]	1.44	1.89	1.13	(0.82)[F]	Y32	9.72	22.17	12.44	(47.0)[F]
CDR-L2	W50	22.0	14.7	46.0	78.0	4.20	1.20	1.68	1.77	W50	5.24	12.22	27.38	44.13
	G51	9.2			78.0	3.00			2.00	G51	3.07			39.00
	S52	1.8			7.8	1.60			1.61	S52	1.14			4.83
	Y53	5.3	32.0	5.3	0.1	2.67	2.00	1.08	0.83	Y53	2.00	16.00	4.93	0.06
CDR-L3	H91	46.0	46.0	23.0	(>79)[N]	2.67	2.09	2.09	(1.86)[N]	H91	17.25	22.04	11.02	(>42.5)[N]
	Y92	41.0	8.2	41.0	1.2	2.36	1.74	0.72	0.50	Y92	17.39	4.72	57.15	2.33
	T93	0.9			1.1	1.02			1.50	T93	0.86			0.74
	T94	0.8			(0.58)[S]	0.65			(1.28)[S]	T94	1.16			(0.45)[S]
CDR-H1	K30	4.36	1.92	3.00	1.50	3.31	1.65	1.87	1.14	K30	1.32	1.16	1.60	1.31
	D31	2.19			0.63	1.63			0.93	D31	1.35			0.68
	T32	0.82			2.10	1.50			0.64	T32	0.55			3.27
	Y33	2.50	0.26	0.21	5.86	1.48	1.31	1.55	2.47	Y33	1.69	0.20	0.14	2.37
CDR-H2	R50	0.63	0.55	11.00	1.57	1.13	0.53	9.00	0.98	R50	0.56	1.04	1.22	1.60
	Y52	4.35	74.00	9.25	1.18	2.17	1.63	1.63	0.91	Y52	2.01	45.54	5.69	1.30
	T53	0.39			0.68	0.93			0.58	T53	0.42			1.19
	N54	0.65	0.20	0.61	0.68	0.97	1.07	1.88	1.02	N54	0.67	0.19	0.33	0.67
	Y56	5.33	6.40	4.92	0.83	3.85	3.13	1.92	0.76	Y56	1.39	2.05	2.56	1.08
	R58	0.83	0.55	24.00	1.19	1.12	0.76	2.42	2.09	R58	0.74	0.71	9.93	0.57
CDR-H3	W95	>102	>102	1.56	>96	3.63	1.12	0.69	0.46	W95	>28.1	>91.4	2.27	>209
	G96	1.86			5.27	1.33			1.39	G96	1.39			3.80
	G97	1.38			4.88	1.69			1.23	G97	0.82			3.95
	D98	0.96			1.64	1.00			0.94	D98	0.96			1.74
	G99	0.30			0.52	1.17			1.89	G99	0.26			0.28
	F100	11.50	23.00	0.96	8.60	3.38	2.20	1.63	2.50	F100	3.40	10.45	0.59	3.44
	Y100a	4.75	57.00	1.90	0.27	2.11	2.35	1.43	0.46	Y100a	2.26	24.23	1.33	0.59

FIG. 36

		Antigen Selection (Her2)				Display Selection (anti-gD)					Fwt/mut values			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4		Fwt/m1	Fwt/m2	Fwt/m3	Fwt/m4
CDR-L1	Q27				2.85				0.97	Q27				2.94
	D28	1.82			(2.0)[E]	1.73			(1.29)[E]	D28	1.05			(1.55)[E]
	I29	1.47	2.00	0.78	(1.13)[L]	1.95	3.00	0.98	(1.15)[L]	I29	0.76	0.67	0.80	(0.98)[L]
	P30	1.31			1.22	1.18			1.33	P30	1.11			0.92
	R30a	1.67	1.75	1.67	2.31	2.38	0.91	0.94	1.93	R30a	0.70	1.92	1.77	1.20
	S30b	1.74			(4.25)[T]	1.06			(1.1)[T]	S30b	1.64			(3.86)[T]
	I30c	14.75	11.80	2.03	(1.41)[L]	3.23	2.80	1.00	(1.02)[L]	I30c	4.57	4.21	2.03	(1.38)[L]
	S30d	1.00			(1.0)[T]	1.44			(1.3)[T]	S30d	0.69			(0.769)[T]
	G31	3.36			(4.17)[A]	2.00			(2.9)[A]	G31	1.68			(1.43)[A]
	Y32	0.36	12.00	0.24	(1.8)[F]	1.44	1.89	1.13	(0.82)[F]	Y32	0.25	6.33	0.21	(2.20)[F]
CDR-L2	W50	45.50	22.75	>98	16.00	4.20	1.20	1.68	1.77	W50	10.83	18.96	>58.3	9.05
	G51	1.94			2.57	3.00			2.00	G51	0.65			1.29
	S52	2.88			4.67	1.60			1.61	S52	1.79			2.90
	Y53	11.29	39.50	8.78	0.65	2.67	2.00	1.08	0.83	Y53	4.23	19.75	8.12	0.78
CDR-L3	H91	12.43	0.98	>98	(3.0)[N]	2.67	2.09	2.09	(1.86)[N]	H91	4.66	0.47	>47.0	(1.61)[N]
	Y92	5.50	38.50	81.00	1.83	2.36	1.74	0.72	0.50	Y92	2.33	22.17	112.91	3.67
	T93	0.54			31.00	1.02			1.50	T93	0.53			20.67
	T94	6.46			(4.3)[S]	0.65			(1.28)[S]	T94	9.99			(3.36)[S]
CDR-H1	K30	2.13	1.68	2.13	1.13	3.31	1.65	1.87	1.14	K30	0.64	1.02	1.14	0.99
	D31	3.05			0.66	1.63			0.93	D31	1.88			0.71
	T32	0.76			0.75	1.50			0.64	T32	0.51			1.16
	Y33	>81	>81	>81	47.00	1.48	1.31	1.55	2.47	Y33	>54.8	>61.9	>52.4	19.05
CDR-H2	R50	>81	>81	>81	95.00	1.13	0.53	9.00	0.98	R50	>72	>153	>9.0	96.83
	Y52	0.55	2.67	0.53	0.60	2.17	1.63	1.63	0.91	Y52	0.25	1.64	0.33	0.66
	T53	1.03			0.70	0.93			0.58	T53	1.11			1.22
	N54	0.26	0.50	0.45	1.04	0.97	1.07	1.88	1.02	N54	0.27	0.47	0.24	1.02
	Y56	>81	>81	>81	1.21	3.85	3.13	1.92	0.76	Y56	>21.1	>25.9	>42.1	1.59
	R58	>81	80.00	>81	>96	1.12	0.76	2.42	2.09	R58	>72.6	104.83	>33.5	>46.0
CDR-H3	W95	>81	>81	>81	>96	3.63	1.12	0.69	0.46	W95	>22.3	>72.6	>117	>209
	G96	1.53			0.61	1.33			1.39	G96	1.15			0.44
	G97	3.05			3.75	1.69			1.23	G97	1.80			3.04
	D98	0.80			0.40	1.00			0.94	D98	0.80			0.42
	G99	26.00			18.00	1.17			1.89	G99	22.29			9.53
	F100	>81	>81	39.50	95.00	3.38	2.20	1.63	2.50	F100	>23.9	>36.8	24.24	38.00
	Y100a	80.00	>81	>81	6.31	2.11	2.35	1.43	0.46	Y100a	38.00	>34.4	>56.7	13.76

FIG. 37A

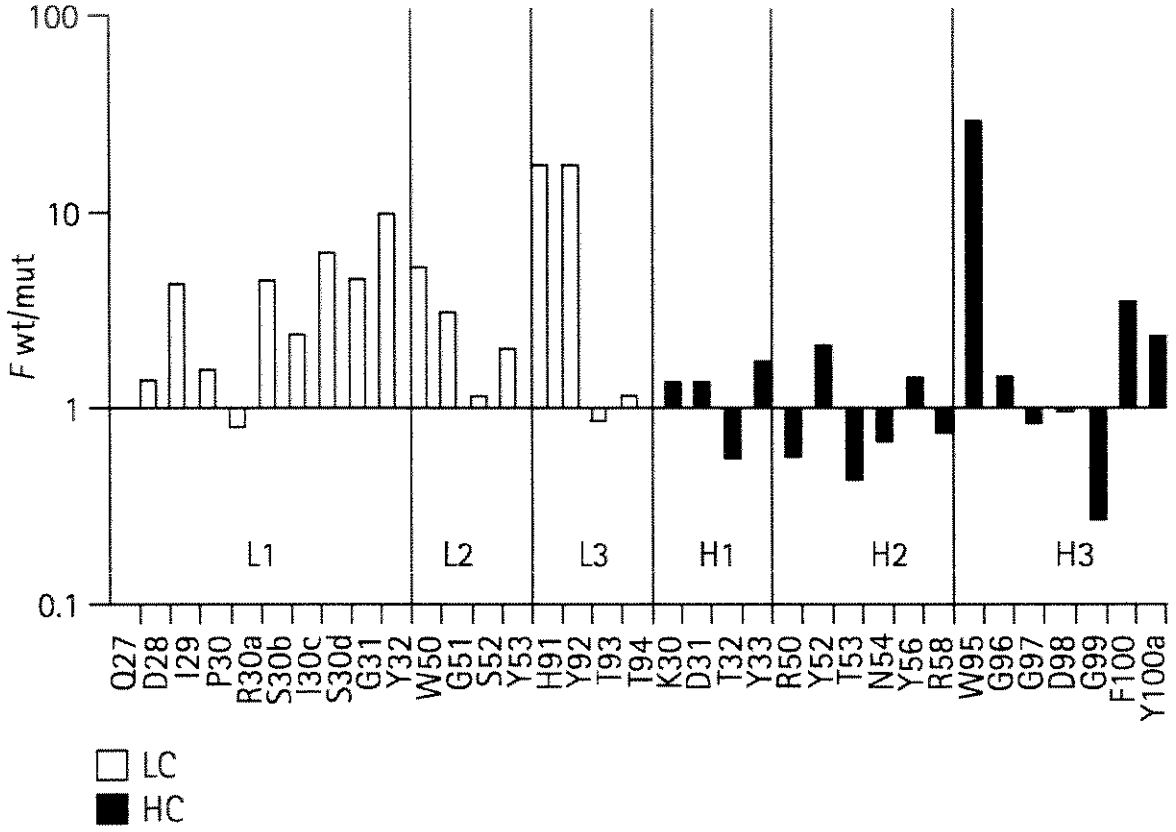


FIG. 37B

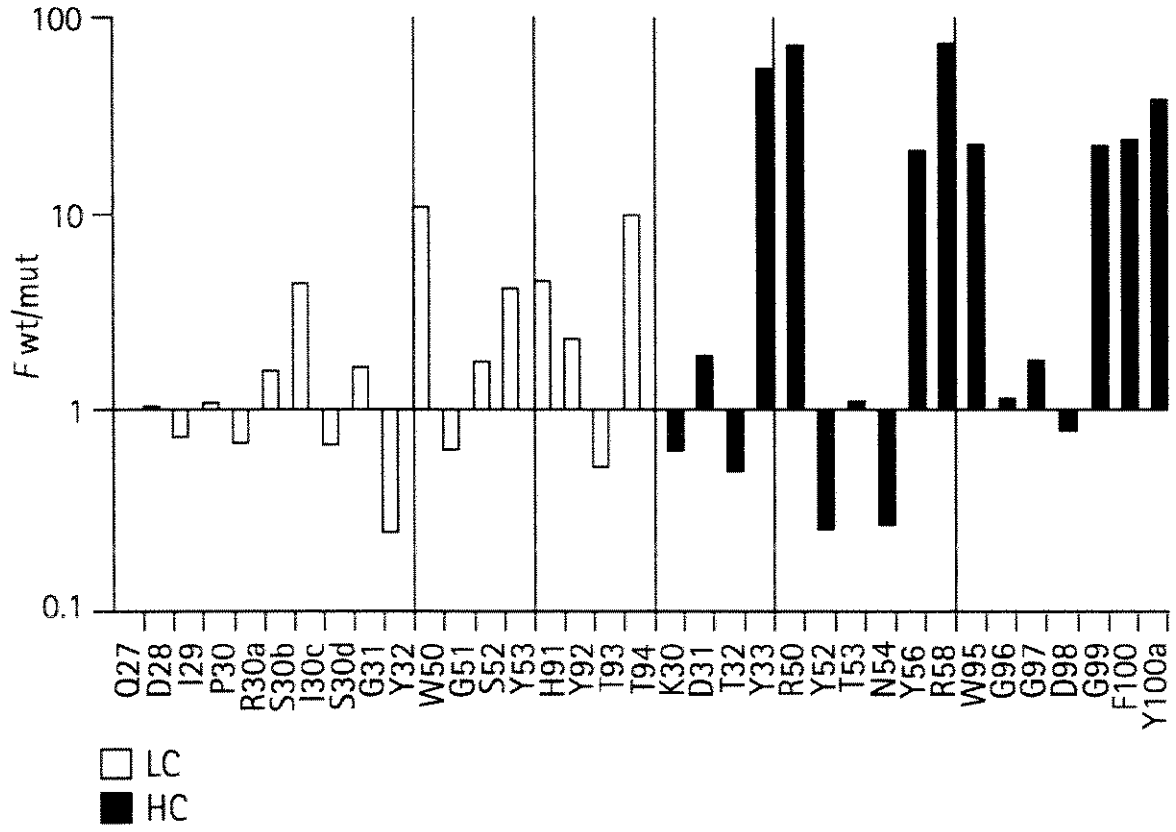


FIG. 37C

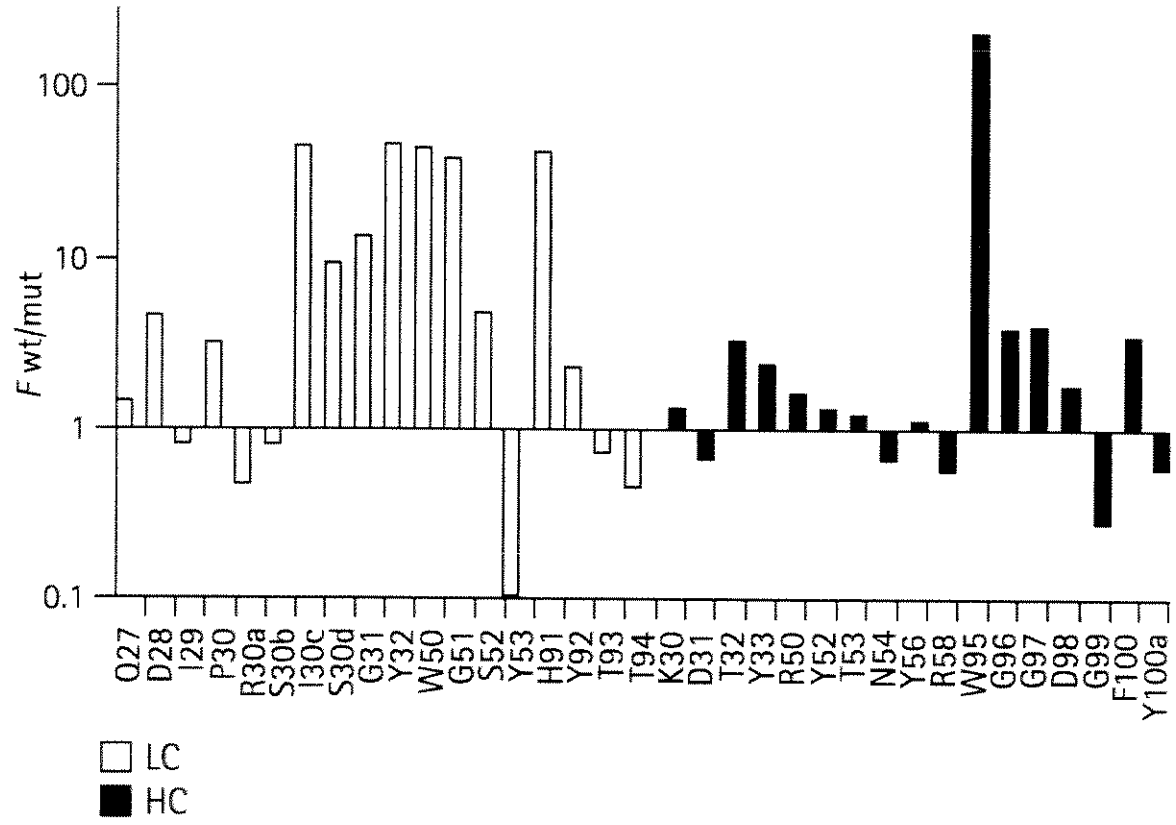


FIG. 37D

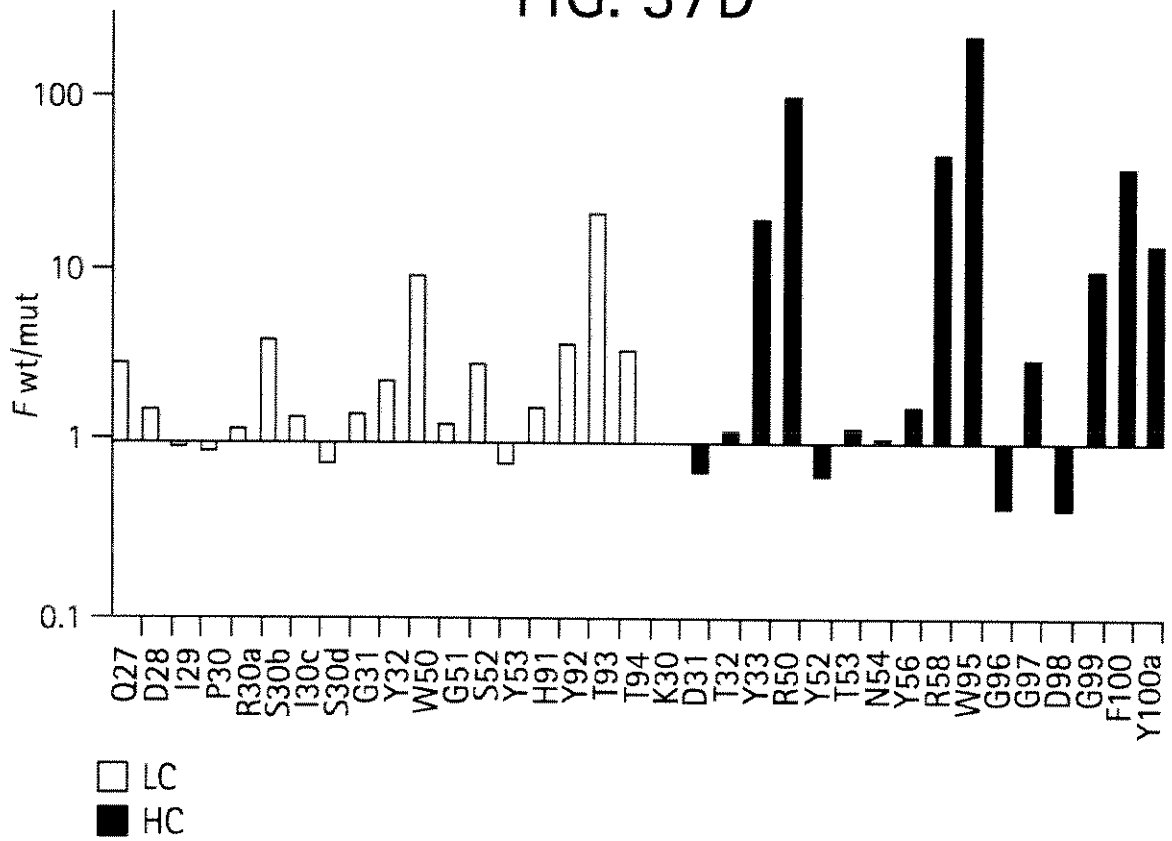


FIG. 38

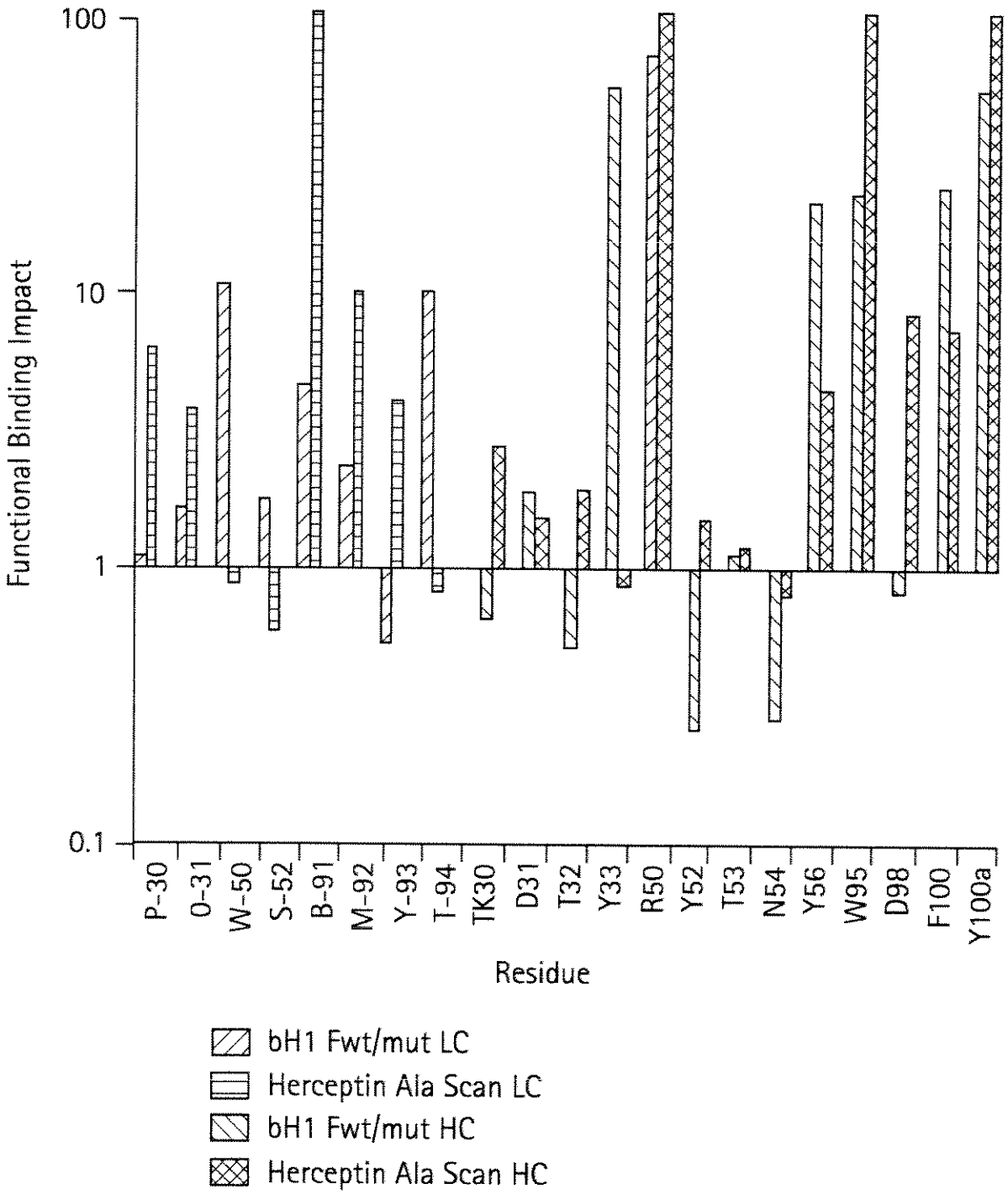

100
10
1
0.1
Functional Binding Impact
P-30
O-31
W-50
S-52
B-91
M-92
Y-93
T-94
TK30
D31
T32
Y33
R50
Y52
T53
N54
Y56
W95
D98
F100
Y100a
Residue
bH1 Fwt/mut LC
Herceptin Ala Scan LC
bH1 Fwt/mut HC
Herceptin Ala Scan HC

FIG. 39A-1

		Antigen Selection (VEGF)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-L1	Q27				1.4				1.0				1.4^{E}			0.2^{E}	
	D28	1.8			3.5	1.7			1.3	1.1^{A}			2.7^{E}	0^{A}			0.6^{E}
	I29	12.8	17.0	1.9	1.4	2.0	3.0	1.0	1.2	6.5^{A}	5.7^{T}	1.9^{V}	1.2^{L}	1.1^{A}	1.0^{T}	0.4^{V}	0.1^{L}
	P30	1.7			3.8	1.2			1.3	1.5^{A}			2.9^{A}	0.2^{A}			0.6^{A}
	R30a	1.9	4.1	1.9	0.8	2.4	0.9	0.9	1.9	0.8^{A}	4.5^{G}	2.1^{P}	0.4^{K}	-0.2^{A}	0.9^{G}	0.4^{P}	-0.5^{K}
	S30b**	5.5			0.7	1.1			1.1	5.2^{A}			0.6^{T}	1.0^{A}			-0.3^{T}
	I30c***	12.7	7.6	1.0	23.8	3.2	2.8	1.0	1.0	3.9^{A}	2.7^{T}	1.0^{V}	23.2^{L}	0.8^{A}	0.6^{T}	0^{V}	1.9^{L}
	S30d**	13.2			16.9	1.4			1.3	9.1^{A}			13.0^{T}	1.3^{A}			1.5^{T}
	G31***	16.0			35.5	2.0			2.9	8.0^{A}			12.2^{A}	1.2^{A}			1.5^{A}
	Y32***	26.0	78.0	16.0	23.0	1.4	1.9	1.1	0.8	18.1^{A}	41.2^{D}	23.1^{S}	$>28^{F}$	1.7^{A}	2.2^{D}	1.9^{S}	$>2.0^{F}$
CDR-L2	W50**	39.0	26.0	39.0	20.4	4.2	1.2	1.7	1.8	9.3^{A}	21.7^{G}	23.2^{S}	11.6^{L}	1.3^{A}	1.8^{G}	1.9^{S}	1.5^{L}
	G51	16.0			20.4	3.0			2.0	5.3^{A}			10.2^{A}	1.0^{A}			1.4^{A}
	S52	2.0			6.5	1.6			1.6	1.3^{A}			4.0^{A}	0.1^{A}			0.8^{A}
	Y53**	9.0	63.0	4.5	0.1	2.7	2.0	1.1	0.8	3.4^{A}	31.5^{D}	4.2^{S}	0.1^{F}	0.7^{A}	2.0^{D}	0.8^{S}	-1.4^{F}

FIG. 39A-2

		Antigen Selection (VEGF)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-L3	H91***	80.0	40.0	40.0	>150	2.7	2.1	2.1	1.9	30^{A}	19.2^{D}	19.2^{P}	$>80^{N}$	2.0^{A}	1.7^{D}	1.7^{P}	$>2.6^{N}$
	Y92***	25.0	12.5	75.0	1.0	2.4	1.7	0.7	0.5	10.6^{A}	7.2^{D}	104.5^{S}	2.0^{F}	1.4^{A}	1.2^{D}	2.8^{S}	0.4^{F}
	T93**	0.7			0.9	1.0			1.5	0.7^{A}			0.6^{S}	-0.3^{A}			-0.3^{S}
	T94**	0.7			0.6	0.6			1.3	1.1^{A}			0.4^{S}	0^{A}			-0.5^{S}
CDR-H1	K30	4.4	1.9	3.0	1.5	3.3	1.7	1.9	1.1	1.3^{A}	1.2^{E}	1.6^{T}	1.3^{R}	0.2^{A}	0.1^{E}	0.3^{T}	0.2^{R}
	D31	2.2			0.6	1.6			0.9	1.3^{A}			0.7^{E}	0.2^{A}			-0.2^{E}
	T32	0.8			2.1	1.5			0.6	0.5^{A}			3.3^{S}	-0.4^{A}			0.7^{S}
	Y33***	2.5	0.3	0.2	5.9	1.5	1.3	1.5	2.5	1.7^{A}	0.2^{D}	0.1^{S}	2.4^{F}	0.3^{A}	-1.0^{D}	-1.2^{S}	0.5^{F}

FIG. 39A-3

		Antigen Selection (VEGF)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-H2	R50*	0.6	0.6	11.0	1.6	1.1	0.5	9.0	1.0	0.6^{A}	1.0^{G}	1.2^{P}	1.6^{K}	-0.3^{A}	0^{G}	0.1^{P}	0.3^{K}
	Y52*	4.4	74.0	9.3	1.2	2.2	1.6	1.6	0.9	2.0^{A}	45.5^{D}	5.7^{S}	1.3^{F}	0.4^{A}	2.3^{D}	1.0^{S}	0.2^{F}
	T53	0.4			0.7	0.9			0.6	0.4^{A}			1.2^{S}	-0.5^{A}			0.1^{S}
	N54	0.6	0.2	0.6	0.7	1.0	1.1	1.9	1.0	0.7^{A}	0.2^{D}	0.3^{T}	0.7^{D}	-0.2^{A}	-1.0^{D}	-0.7^{T}	-0.2^{D}
	Y56	5.3	6.4	4.9	0.8	3.8	3.1	1.9	0.8	1.4^{A}	2.0^{D}	2.6^{S}	1.1^{F}	0.4^{A}	0.2^{A}	0.6^{S}	0^{F}
	R58	0.8	0.5	24.0	1.2	1.1	0.8	2.4	2.1	0.7^{A}	0.7^{G}	9.9^{P}	0.6^{K}	-0.2^{A}	-0.2^{G}	1.4^{P}	-0.3^{K}
CDR-H3	W95**	>102	>102	1.6	>96	3.6	1.1	0.7	0.5	$>28^{A}$	$>91^{G}$	2.3^{S}	$>209^{L}$	$>2.0^{A}$	$>2.7^{G}$	0.5^{S}	$>3.2^{L}$
	G96	1.9			5.3	1.3			1.4	1.4^{A}			3.8^{A}	0.2^{A}			0.8^{A}
	G97	1.4			4.9	1.7			1.2	0.8^{A}			4.0^{A}	-0.1^{A}			0.8^{A}
	D98	1.0			1.6	1.0			0.9	1.0^{A}			1.7^{E}	0^{A}			0.3^{E}
	G99***	0.3			0.5	1.2			1.9	0.3^{A}			0.3^{A}	-0.8^{A}			-0.8^{A}
	F100	11.5	23.0	1.0	8.6	3.4	2.2	1.6	2.5	3.4^{A}	10.5^{S}	0.6^{V}	3.4^{Y}	0.7^{A}	1.4^{S}	-0.3^{V}	0.7^{Y}
	Y100a***	4.8	57.0	1.9	0.3	2.1	2.4	1.4	0.5	2.3^{A}	24.2^{D}	1.3^{S}	0.6^{F}	0.5^{A}	1.9^{D}	0.2^{S}	-0.3^{F}

FIG. 39B-1

		Antigen Selection (Her2)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-L1	Q27				1.6				1.0				1.6^{E}				0.3^{E}
	D28	1.8			1.7	1.7			1.3	1.1^{A}			1.3^{E}	0^{A}			0.2^{E}
	I29	1.5	2.0	0.8	1.3	2.0	3.0	1.0	1.2	0.8^{A}	0.7^{T}	0.8^{V}	1.1^{L}	-0.2^{A}	-0.2^{T}	-0.1^{V}	0.1^{L}
	P30	1.3			0.8	1.2			1.3	1.1^{A}			0.6^{A}	0.1^{A}			-0.3^{A}
	R30a	1.7	1.8	1.7	1.4	2.4	0.9	0.9	1.9	0.7^{A}	1.9^{G}	1.8^{P}	0.7^{K}	-0.2^{A}	0.4^{G}	0.3^{P}	-0.2^{K}
	S30b	1.7			1.7	1.1			1.1	1.6^{A}			1.6^{T}	0.3^{A}			0.3^{T}
	I30c	14.8	11.8	2.0	1.3	3.2	2.8	1.0	1.0	4.6^{A}	4.2^{T}	2.0^{V}	1.2^{L}	0.9^{A}	0.9^{T}	0.4^{V}	0.1^{L}
	S30d	1.0			2.2	1.4			1.3	0.7^{A}			1.7^{T}	-0.2^{A}			0.3^{T}
	G31*	3.4			5.1	2.0			2.9	1.7^{A}			1.8^{A}	0.3^{A}			0.3^{A}
	Y32***	0.4	12.0	0.2	1.8	1.4	1.9	1.1	0.8	0.3^{A}	6.3^{D}	0.2^{S}	2.1^{F}	-0.8^{A}	1.1^{D}	-1.0^{S}	0.4^{F}
CDR-L2	W50**	45.5	22.8	>98	17.0	4.2	1.2	1.7	1.8	10.8^{A}	19^{G}	$>58^{S}$	9.6^{L}	1.4^{A}	1.7^{G}	$>2.4^{S}$	1.3^{L}
	G51	1.9			1.7	3.0			2.0	0.6^{A}			0.9^{A}	-0.3^{A}			-0.1^{A}
	S52	2.9			3.0	1.6			1.6	1.8^{A}			1.9^{A}	0.3^{A}			0.4^{A}
	Y53*	11.3	39.5	8.8	0.6	2.7	2.0	1.1	0.8	4.2^{A}	19.8^{D}	8.1^{S}	0.8^{F}	0.9^{A}	1.8^{D}	1.2^{S}	-0.1^{F}

FIG. 39B-2

		Antigen Selection (Her2)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-L3	H91***	12.4	1.0	>98	2.8	2.7	2.1	2.1	1.9	4.7^{A}	0.5^{D}	$>47^{P}$	1.5^{N}	0.9^{A}	-0.4^{D}	$>2.3^{P}$	0.2^{N}
	Y92**	5.5	38.5	81.0	1.8	2.4	1.7	0.7	0.5	2.3^{A}	22.2^{D}	112.9^{S}	3.5^{F}	0.5^{A}	1.8^{D}	2.8^{S}	0.7^{F}
	T93	0.5			0.7	1.0			1.5	0.5^{A}			0.5^{S}	-0.4^{A}			-0.4^{S}
	T94**	6.5			1.4	0.6			1.3	10^{A}			1.1^{S}	1.4^{A}			0.1^{S}
CDR-H1	K30	2.1	1.7	2.1	1.1	3.3	1.7	1.9	1.1	0.6^{A}	1.0^{E}	1.1^{T}	1.0^{R}	-0.3^{A}	0^{E}	0.1^{T}	0^{R}
	D31	3.1			0.7	1.6			0.9	1.9^{A}			0.7^{E}	0.4^{A}			-0.2^{E}
	T32	0.8			0.7	1.5			0.6	0.5^{A}			1.2^{S}	-0.4^{A}			0.1^{S}
	Y33*	>81	>81	>81	47.0	1.5	1.3	1.5	2.5	$>55^{A}$	$>62^{D}$	$>52^{S}$	19.1^{F}	$>2.4^{A}$	$>2.4^{D}$	$>2.3^{S}$	1.7^{F}

FIG. 39B-3

		Antigen Selection (HER2)				Display Selection (anti-gD)				$F_{wt/mut}$ values				$\Delta\Delta G_{wt/mut}$ (kcal/mol)			
		wt/m1	wt/m2	wt/m3	wt/m4	wt/m1	wt/m2	wt/m3	wt/m4	$F_{wt/m1}$	$F_{wt/m2}$	$F_{wt/m3}$	$F_{wt/m4}$	$\Delta\Delta G_{wt/m1}$	$\Delta\Delta G_{wt/m2}$	$\Delta\Delta G_{wt/m3}$	$\Delta\Delta G_{wt/m4}$
CDR-H2	R50***	>81	>81	>81	95.0	1.1	0.5	9.0	1.0	$>72^{A}$	$>153^{G}$	$>9^{P}$	98.6^{K}	$>2.5^{A}$	$>3.0^{G}$	$>1.3^{P}$	2.7^{K}
	Y52	0.6	2.7	0.5	0.6	2.2	1.6	1.6	0.9	0.3^{A}	1.6^{D}	0.3^{S}	0.7^{F}	-0.8^{A}	0.3^{D}	-0.7^{S}	-0.2^{F}
	T53	1.0			0.7	0.9			0.6	1.1^{A}			1.2^{S}	0.1^{A}			0.1^{S}
	N54	0.3	0.5	0.5	1.0	1.0	1.1	1.9	1.0	0.3^{A}	0.5^{D}	0.2^{T}	1.0^{D}	-0.8^{A}	-0.5^{D}	-0.9^{T}	0^{D}
	Y56*	>81	>81	>81	1.2	3.8	3.1	1.9	0.8	$>21^{A}$	$>26^{D}$	$>42^{S}$	1.6^{F}	$>1.8^{A}$	$>1.9^{D}$	$>2.2^{S}$	0.3^{F}
	R58**	>81	80.0	>81	>96	1.1	0.8	2.4	2.1	$>73^{A}$	104.8^{G}	$>33^{P}$	$>46^{K}$	$>2.5^{A}$	2.8^{G}	$>2.1^{P}$	$>2.3^{K}$
CDR-H3	W95***	>81	>81	>81	>96	3.6	1.1	0.7	0.5	$>22^{A}$	$>73^{G}$	$>117^{S}$	$>209^{L}$	$>1.8^{A}$	$>2.5^{G}$	$>2.8^{S}$	$>3.2^{L}$
	G96	1.5			0.6	1.3			1.4	1.1^{A}			0.4^{A}	0.1^{A}			-0.5^{A}
	G97	3.1			3.8	1.7			1.2	1.8^{A}			3.0^{A}	0.3^{A}			0.7^{A}
	D98	0.8			0.4	1.0			0.9	0.8^{A}			0.4^{E}	-0.1^{A}			-0.5^{E}
	G99***	26.0			18.0	1.2			1.9	22.3^{A}			9.5^{A}	1.8^{A}			1.3^{A}
	F100	>81	>81	39.5	95.0	3.4	2.2	1.6	2.5	$>24^{A}$	$>37^{S}$	24.2^{V}	38.0^{Y}	$>1.9^{A}$	$>2.1^{S}$	1.9^{V}	2.2^{Y}
	Y100a***	80.0	>81	>81	6.3	2.1	2.4	1.4	0.5	38^{A}	$>34^{D}$	$>56.7^{S}$	13.8^{F}	2.2^{A}	$>2.1^{D}$	$>2.4^{S}$	-1.6^{F}

FIG. 40A

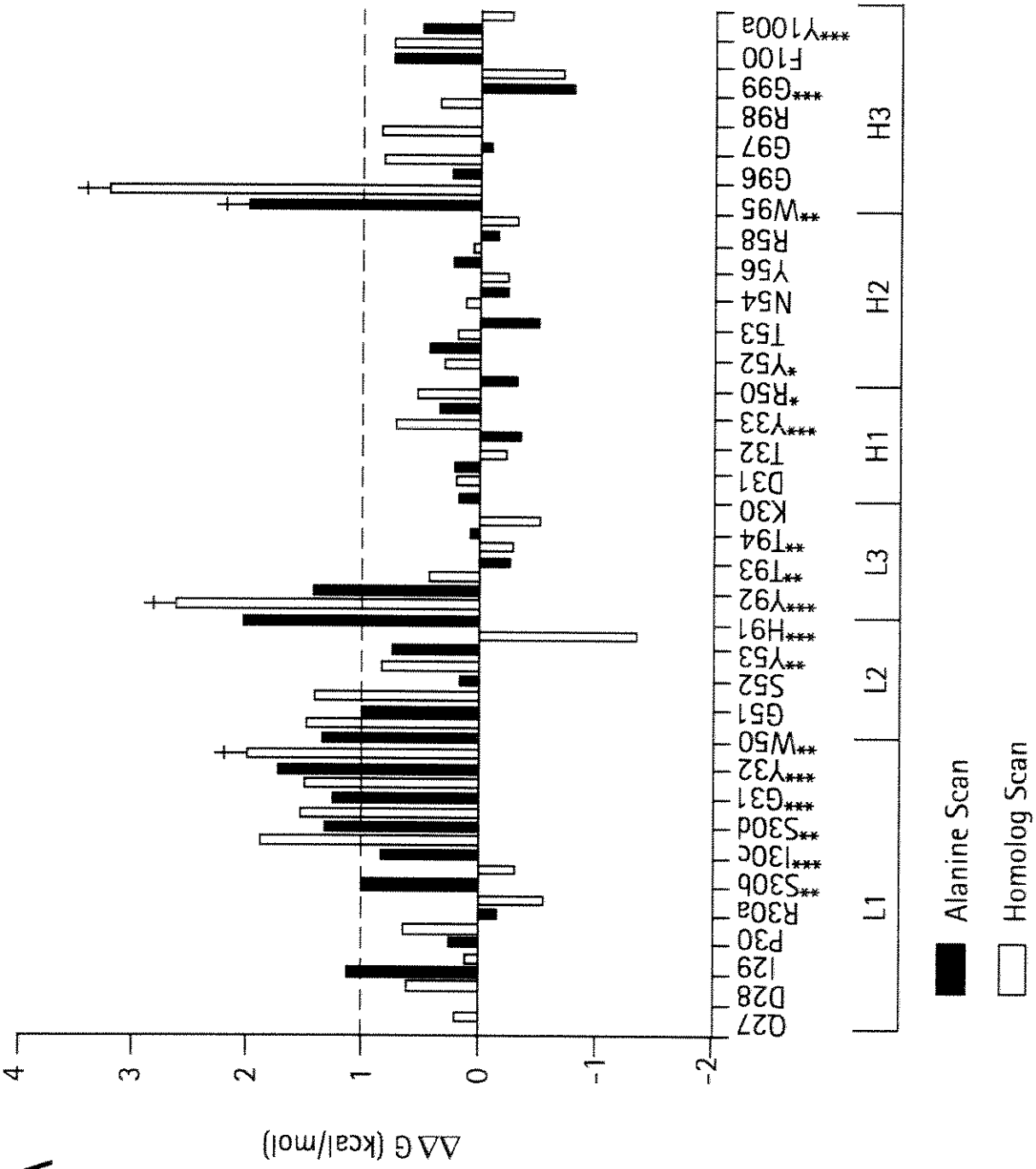

ΔΔG (kcal/mol)
4
3
2
1
0
-1
-2
Q27
D28
I29
P30
R30a
**S30b
***I30c
**S30d
***G31
***Y32
**W50
G51
S52
**Y53
***H91
***Y92
**T93
**T94
K30
D31
T32
***Y33
*R50
*Y52
T53
N54
Y56
R58
**W95
G96
G97
R98
***G99
F100
***Y100a
L1
L2
L3
H1
H2
H3
Alanine Scan
Homolog Scan

FIG. 40B

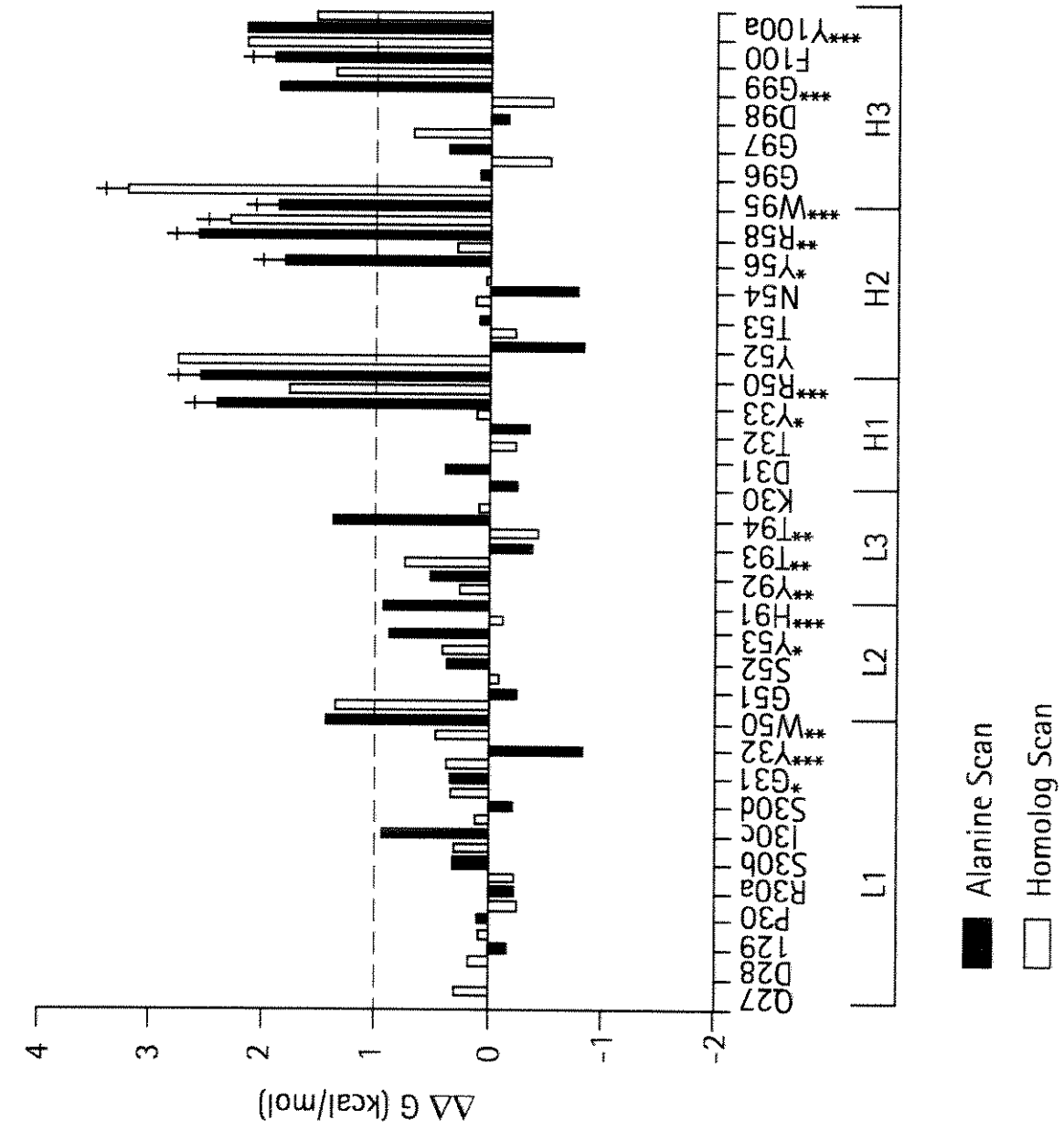

ΔΔ G (kcal/mol)
4
3
2
1
0
-1
-2
Q27
D28
I29
P30
R30a
S30b
I30c
S30d
*G31
***Y32
**W50
G51
S52
*Y53
***H91
**Y92
**T93
**T94
K30
D31
T32
*Y33
***R50
Y52
T53
N54
*Y56
**R58
***W95
G96
G97
D98
***G99
F100
***Y100a
L1
L2
L3
H1
H2
H3
Alanine Scan
Homolog Scan

FIG. 41

Clone	IC50 (nM) hVEGF109	IC50 (nM) HER2-ECD	Number of Mutation	CDR-L1														CDR 2				CDR 3						
				24	25	26	27	28	29	30	30a	30b	30c	30d	31	32		52	52a	53	54	91	92	93	94	95	96	97
H1 fab	127	2.1		C	R	A	S	Q	D	I	P	R	S	I	S	G	Y	V	A	W	G	S	Y	H	Y	T	T	P	P	T
35.74	4.0	0.7	9	C	R	A	I	Q	D	I	P	K	T	I	T	G	Y	V	A	W	G	S	F	H	F	N	S	P	P	K
35.28	7.0	0.6	11	C	R	A	I	Q	N	I	A	K	T	I	T	G	Y	V	A	W	G	S	F	H	F	N	S	P	P	K
35.56	9.0	1.7	9	C	R	A	V	Q	N	L	P	K	T	I	T	G	Y	V	A	W	G	S	F	H	Y	T	I	P	P	K
35.2	10.0	3.5	9	C	R	A	I	E	D	L	P	K	T	V	S	G	Y	V	A	W	G	S	F	H	Y	S	S	P	P	T
35.25	11.0	0.4	7	C	R	A	I	Q	K	V	P	R	T	I	T	G	Y	V	A	W	G	S	F	H	Y	T	S	P	P	T
20.49	12.0	2.6	7	C	R	A	S	V	D	L	P	K	S	V	S	G	Y	V	A	W	G	S	F	H	F	T	S	P	P	T
35.45	12.0	1.1	8	C	R	A	S	E	D	I	P	K	T	V	S	G	Y	V	A	W	G	S	F	H	F	S	S	P	P	T
20.52	15.0	3.1	10	C	R	A	I	E	W	L	P	K	T	I	S	G	Y	V	A	W	G	S	F	H	F	S	S	P	P	T
20.74	17.0	0.4	6	C	R	A	S	Q	K	I	A	R	T	I	S	G	Y	V	A	W	G	S	F	H	Y	S	S	P	P	T
20.80	17.0	2.2	6	C	R	A	S	E	D	I	P	K	S	I	S	G	Y	V	A	W	G	S	F	H	F	S	S	P	P	T
35.21	18.0	1.4	6	C	R	A	S	Q	D	L	P	K	S	I	S	G	Y	V	A	W	G	S	F	H	F	S	S	P	P	T
35.61	19.0	7.1	10	C	R	A	I	E	N	L	P	K	S	I	T	G	Y	V	A	W	G	S	F	H	Y	S	T	T	P	K
35.95	19.0	4.3	8	C	R	A	I	E	N	L	P	K	S	V	S	G	Y	V	A	W	G	S	F	H	Y	T	S	P	P	T
20.93	20.0	3.8	9	C	R	A	V	E	D	L	P	K	T	I	T	G	Y	V	A	W	G	S	F	H	F	T	T	P	P	K
20.57	22.0	not tested	8	C	R	A	S	Q	E	I	A	K	T	I	S	G	Y	V	A	W	G	S	F	H	F	S	S	P	P	T
35.87	34.0	2.2	7	C	R	A	S	Q	N	I	P	K	S	V	S	G	Y	V	A	W	G	S	F	H	F	N	S	P	P	T
20.38	52.0	not tested	7	C	R	A	S	E	D	L	P	K	S	V	S	G	Y	V	A	W	G	S	F	H	Y	S	S	P	P	T
20.38	200.0	not tested	7	C	R	A	S	E	N	I	P	R	T	V	S	G	Y	V	A	W	G	S	F	H	F	T	S	P	P	T

FIG. 42

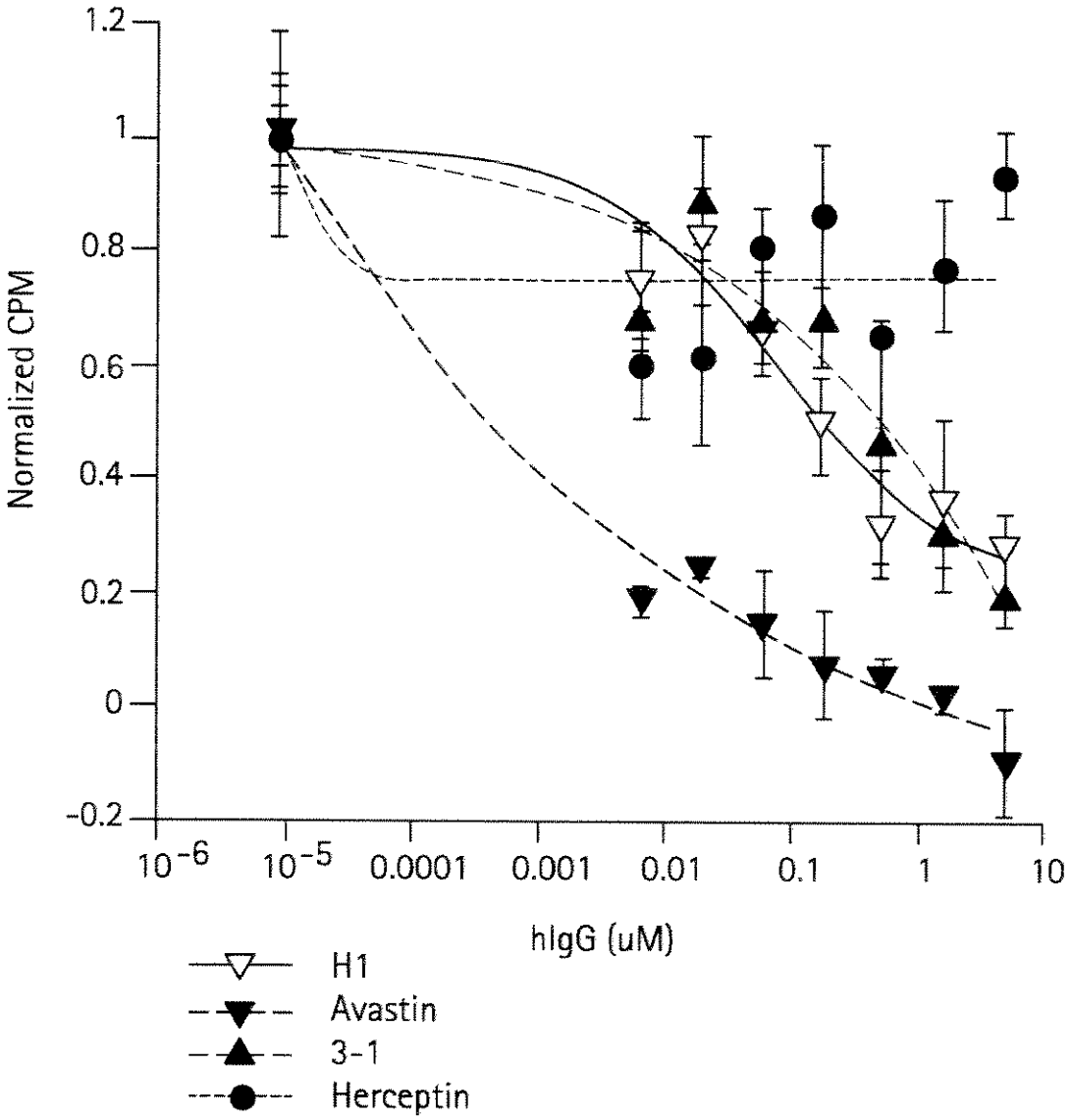

1.2
1
0.8
0.6
0.4
0.2
0
-0.2
Normalized CPM
10^-6
10^-5
0.0001
0.001
0.01
0.1
1
10
hIgG (uM)
H1
Avastin
3-1
Herceptin

FIG. 43A

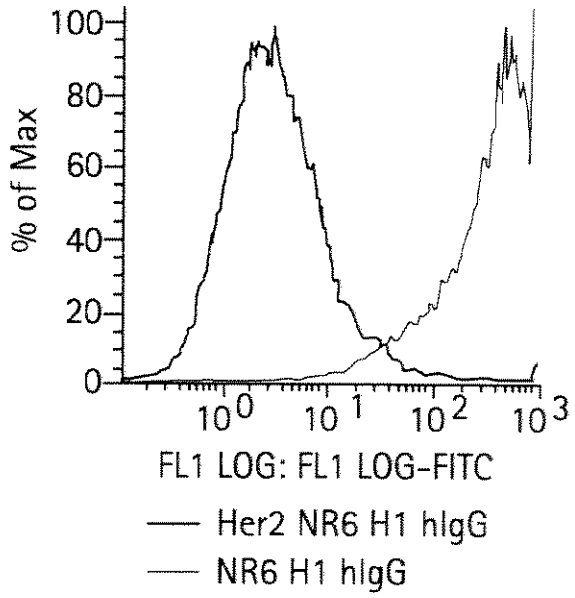


FIG. 43B

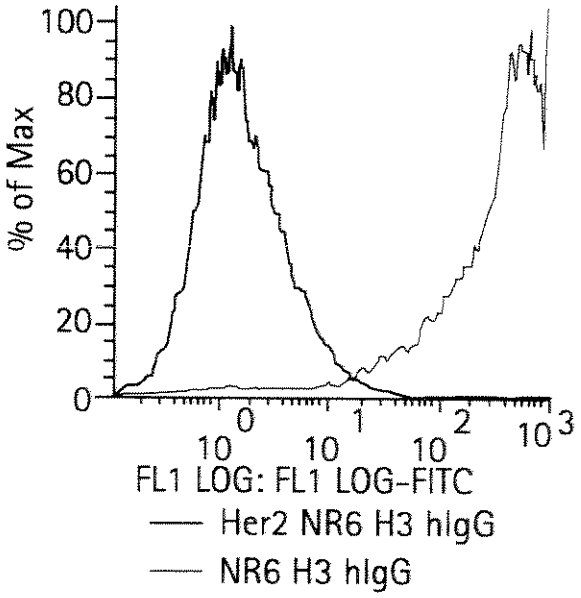


FIG. 43C

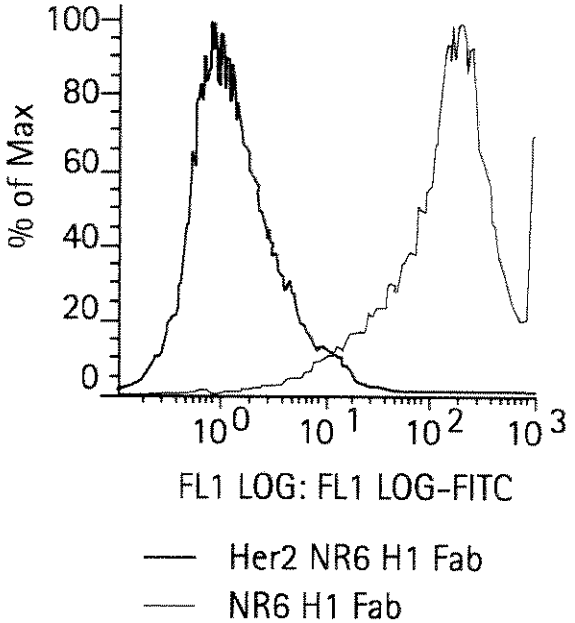


FIG. 43D

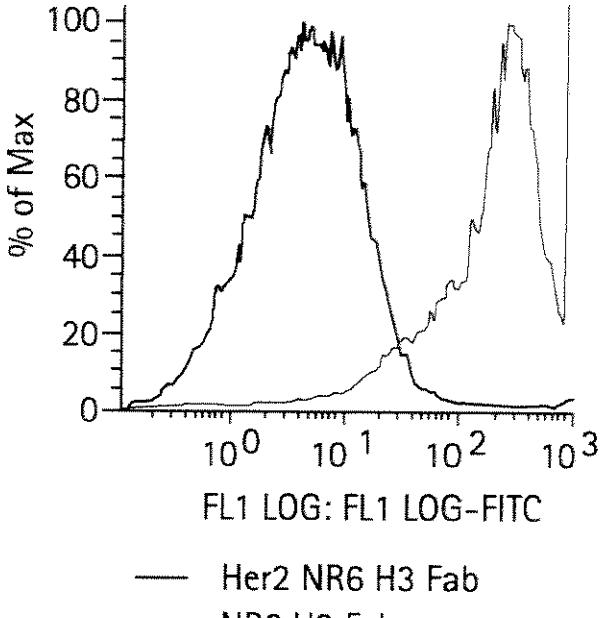


FIG. 44

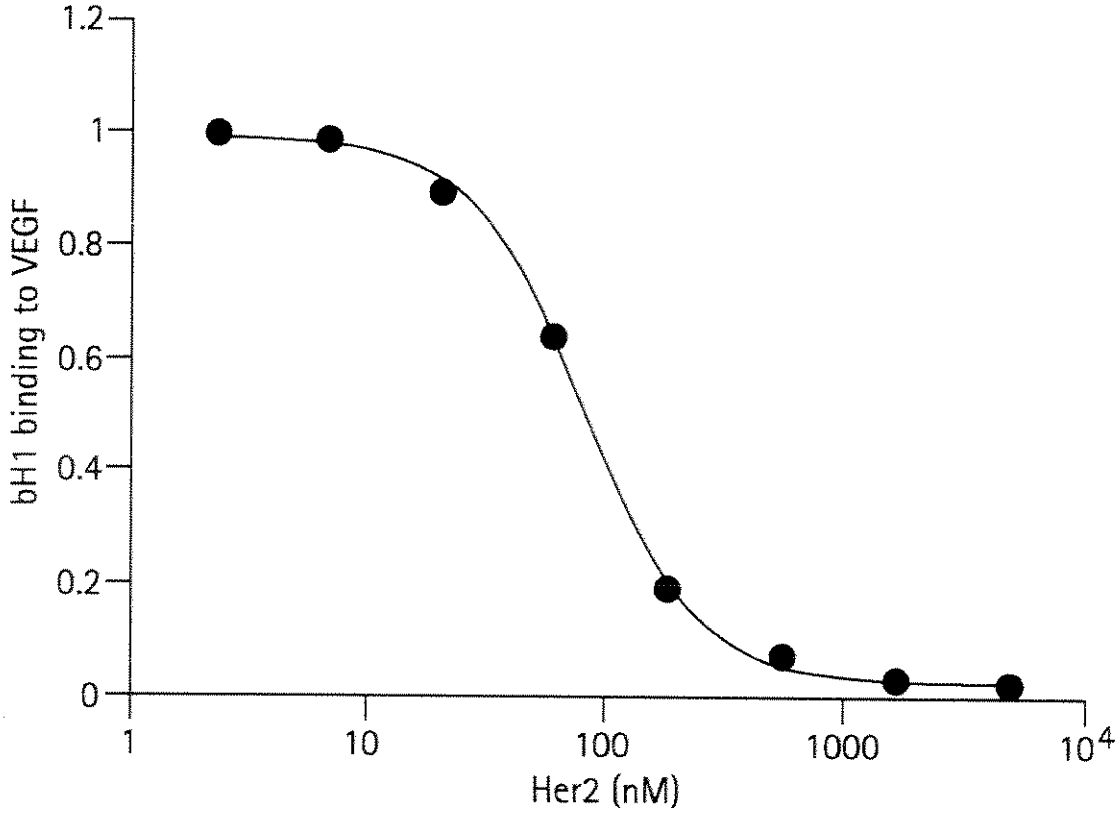


FIG. 45A

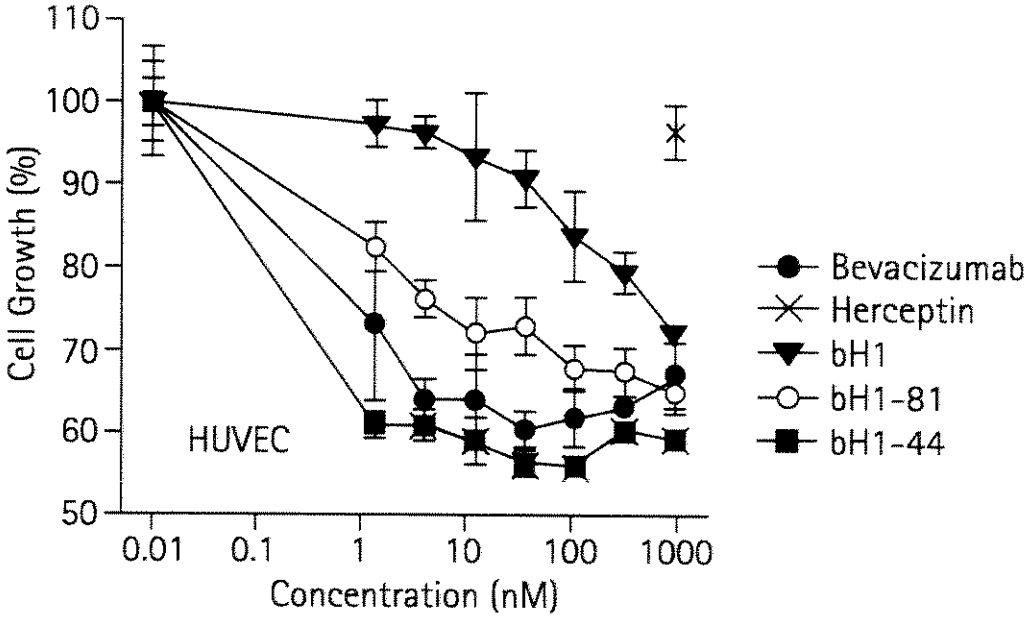


FIG. 45B

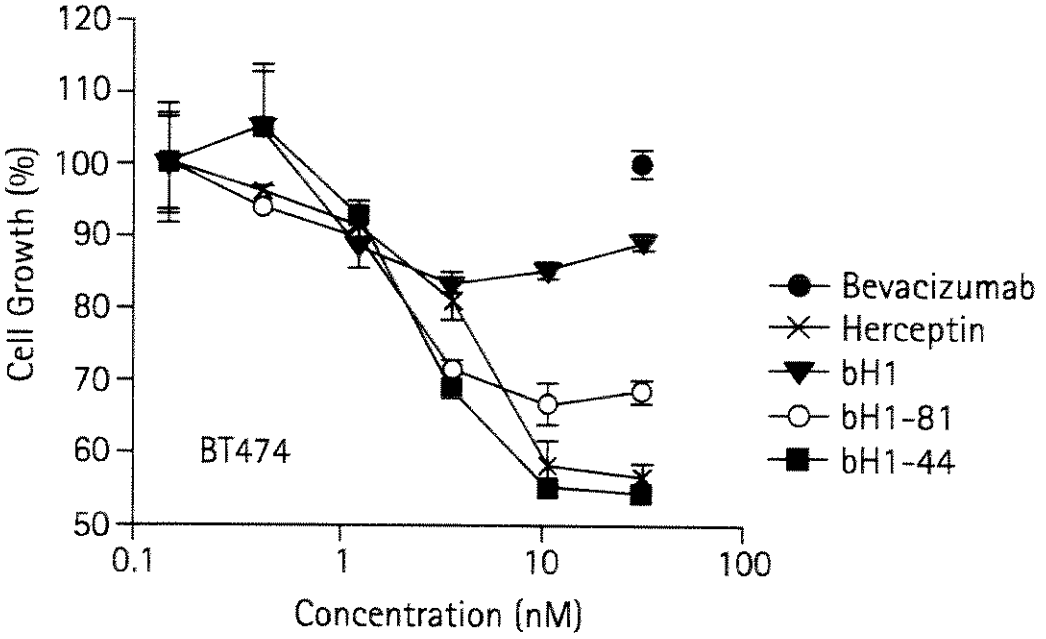


FIG. 46

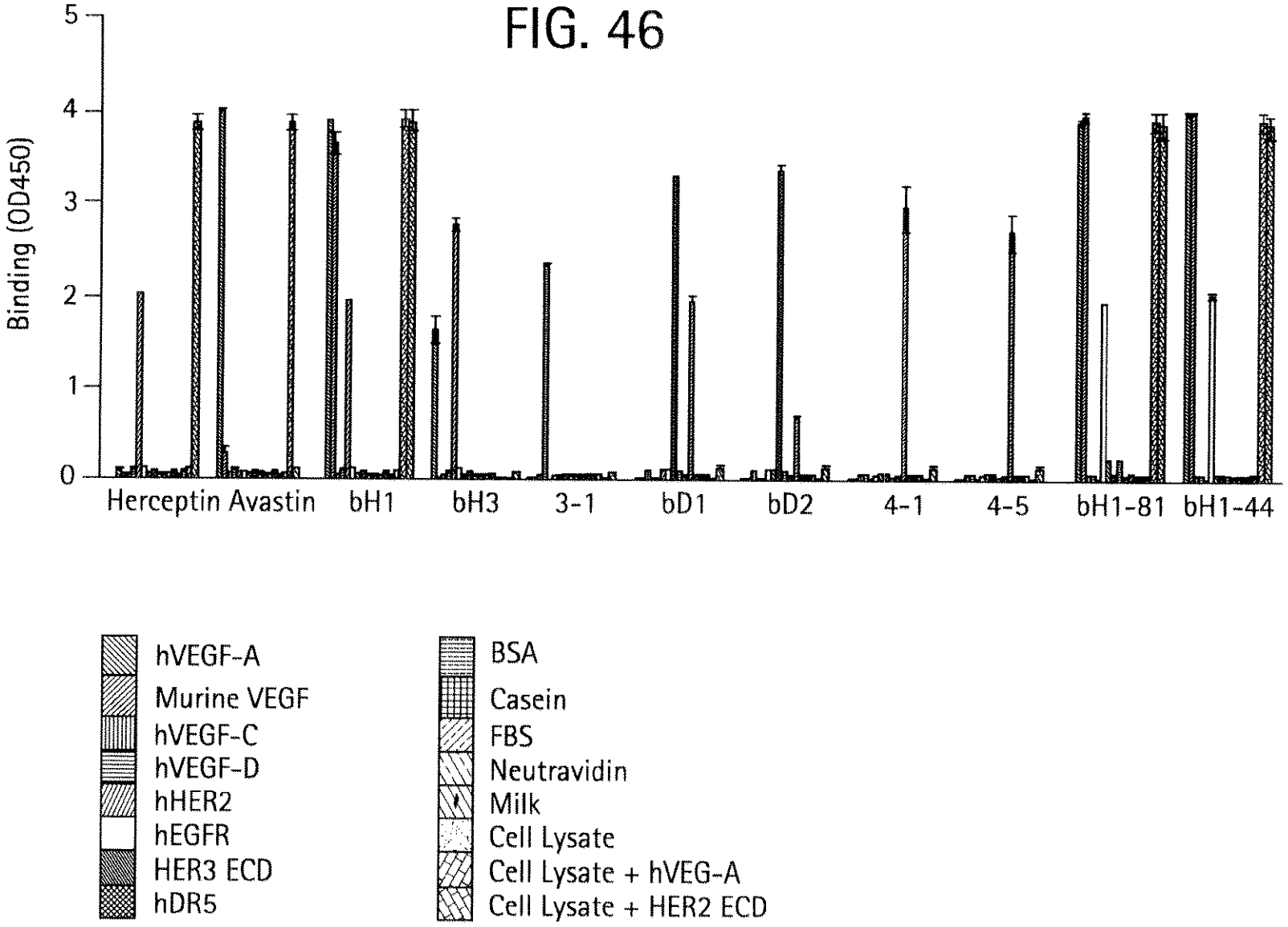

Binding (OD450)
5
4
3
2
1
0
Herceptin
Avastin
bH1
bH3
3-1
bD1
bD2
4-1
4-5
bH1-81
bH1-44
hVEGF-A
Murine VEGF
hVEGF-C
hVEGF-D
hHER2
hEGFR
HER3 ECD
hDR5
BSA
Casein
FBS
Neutravidin
Milk
Cell Lysate
Cell Lysate + hVEG-A
Cell Lysate + HER2 ECD

FIG. 47A

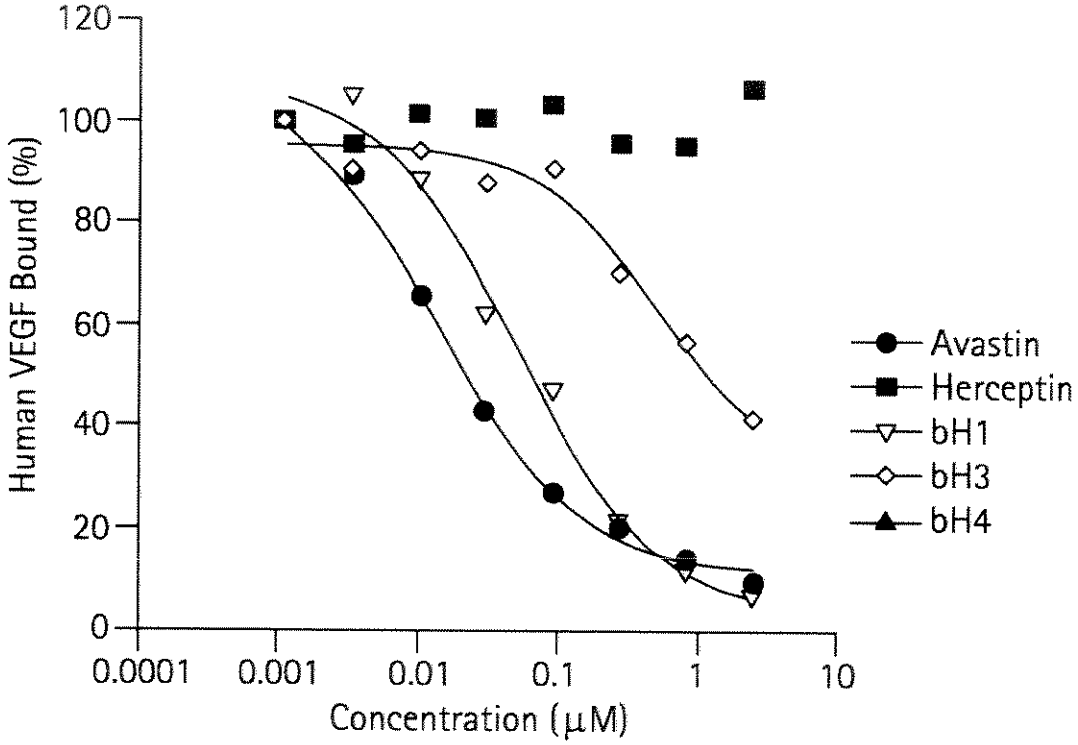


FIG. 47B

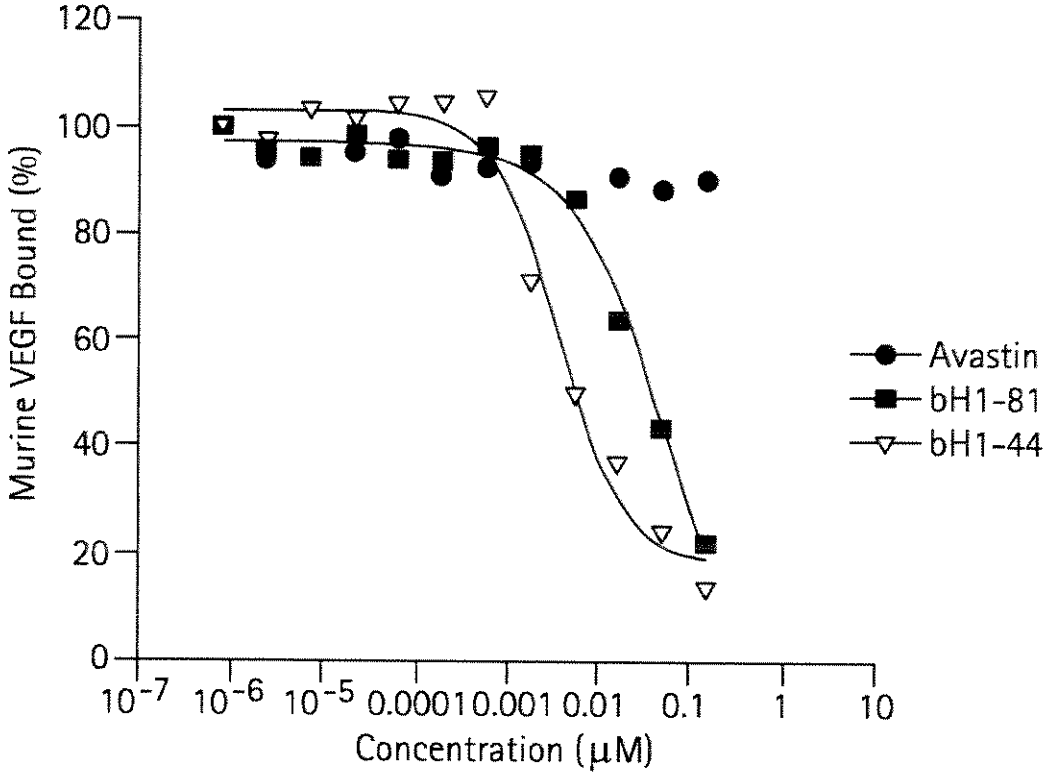


FIG. 48A

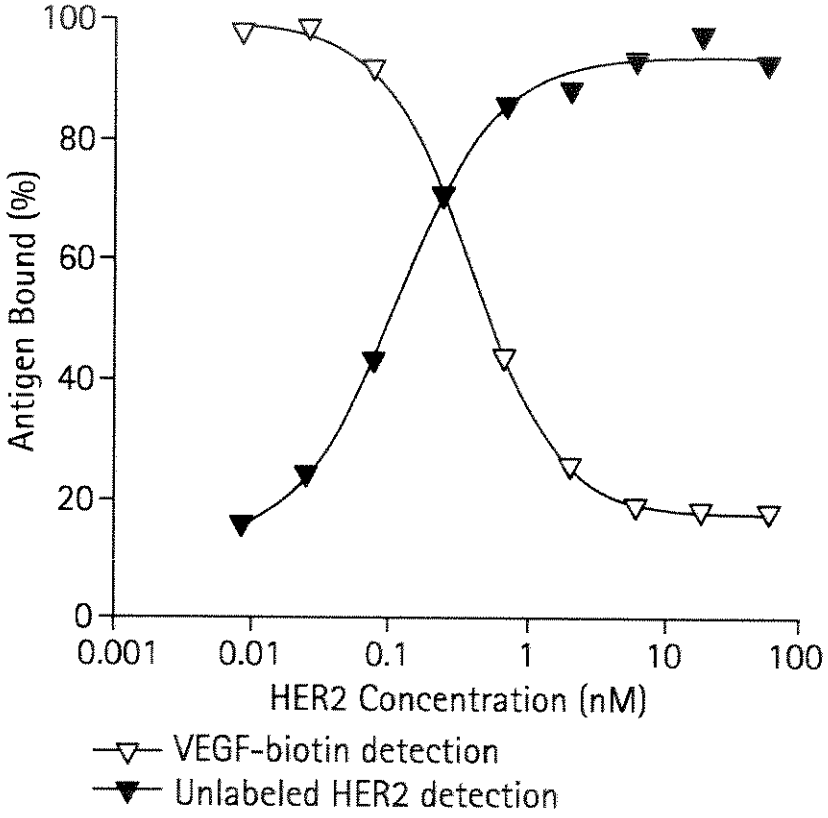


FIG. 48B

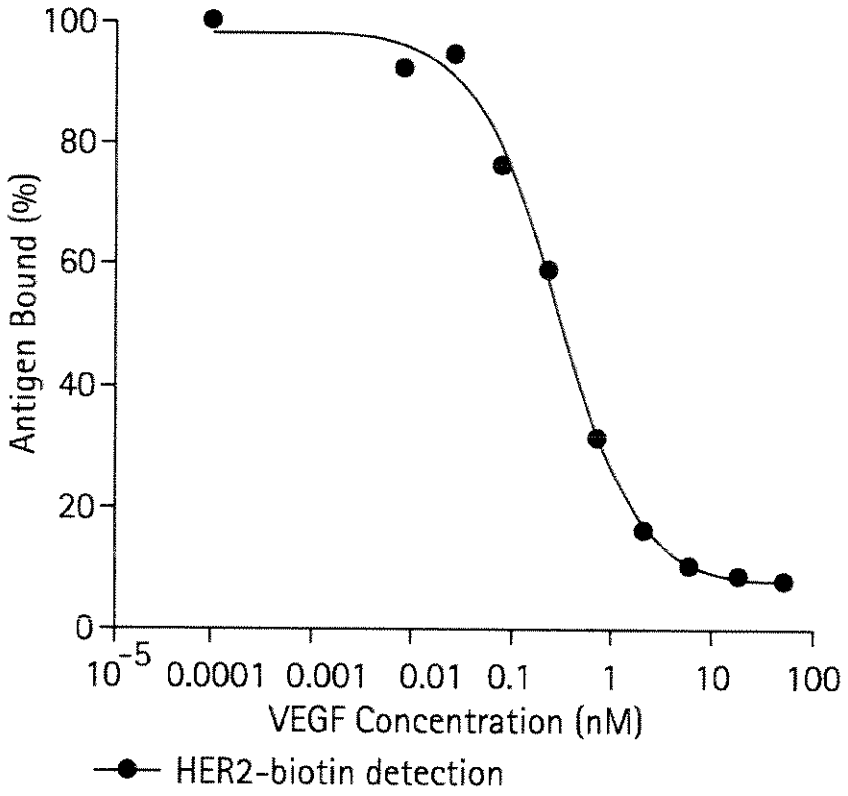


FIG. 49A

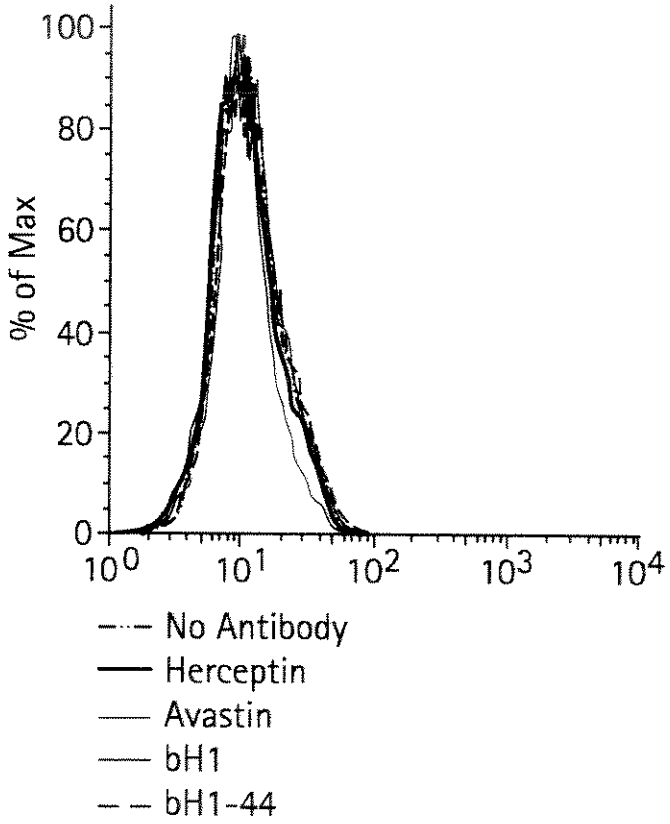


FIG. 49B

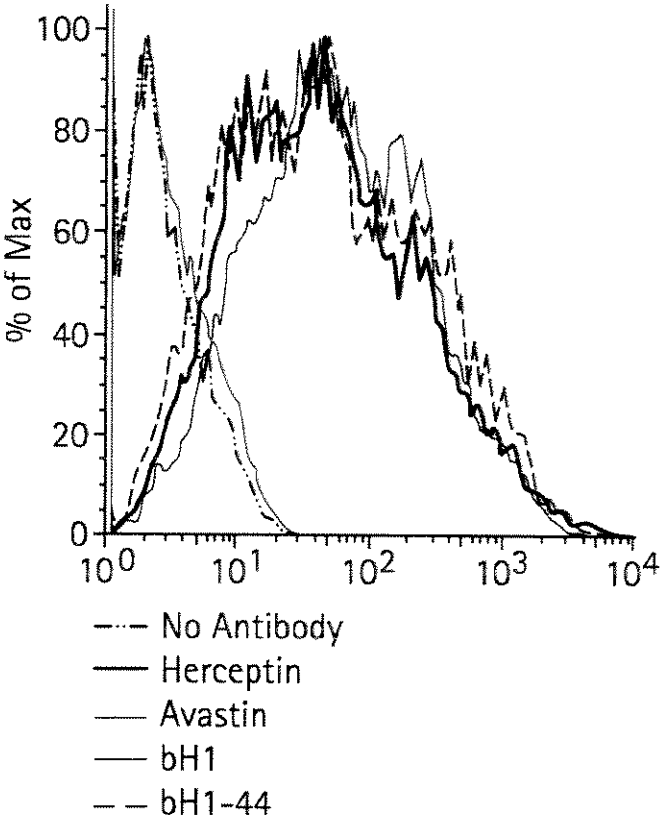


FIG. 50

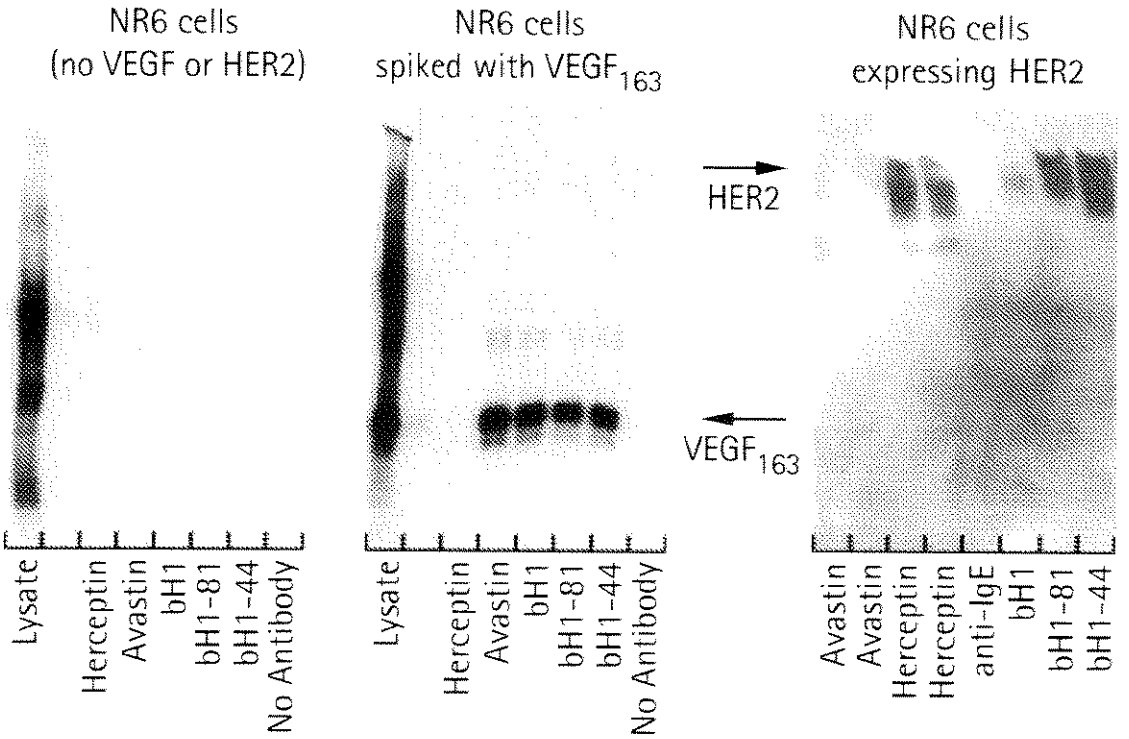

NR6 cells
(no VEGF or HER2)
NR6 cells
spiked with VEGF163
NR6 cells
expressing HER2
HER2
VEGF163
Lysate
Herceptin
Avastin
bH1
bH1-81
bH1-44
No Antibody
Lysate
Herceptin
Avastin
bH1
bH1-81
bH1-44
No Antibody
Avastin
Avastin
Herceptin
Herceptin
anti-IgE
bH1
bH1-81
bH1-44

FIG. 51A

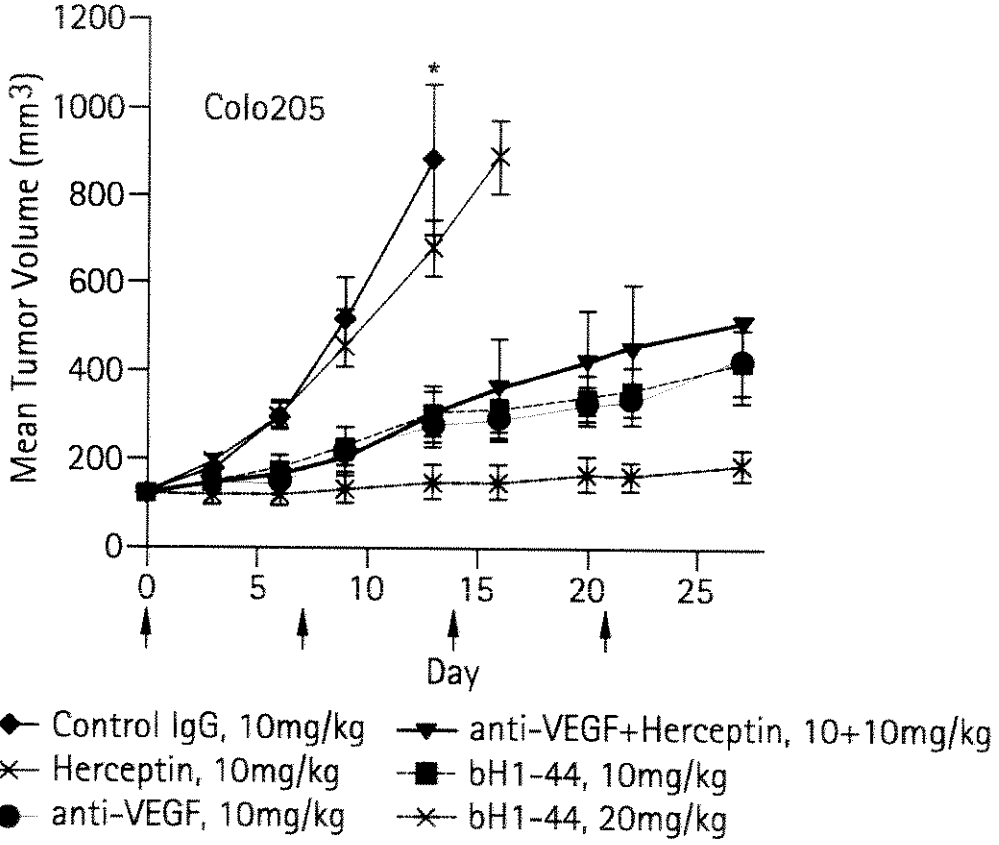


FIG. 51B

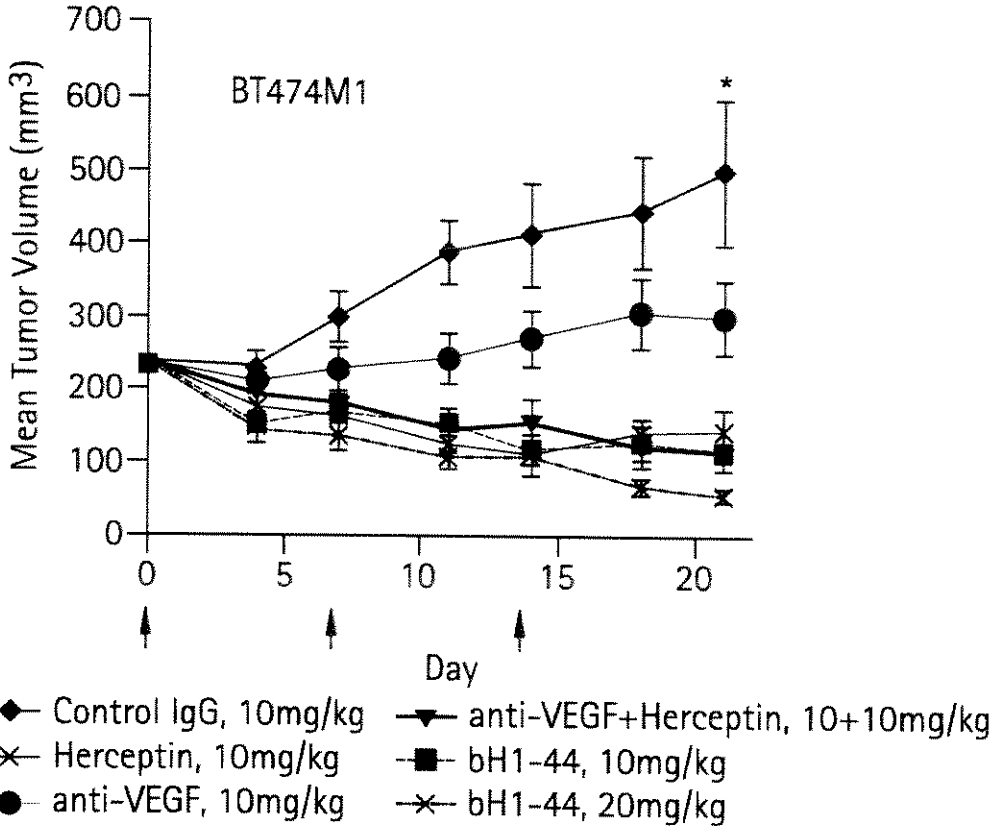


FIG. 52A

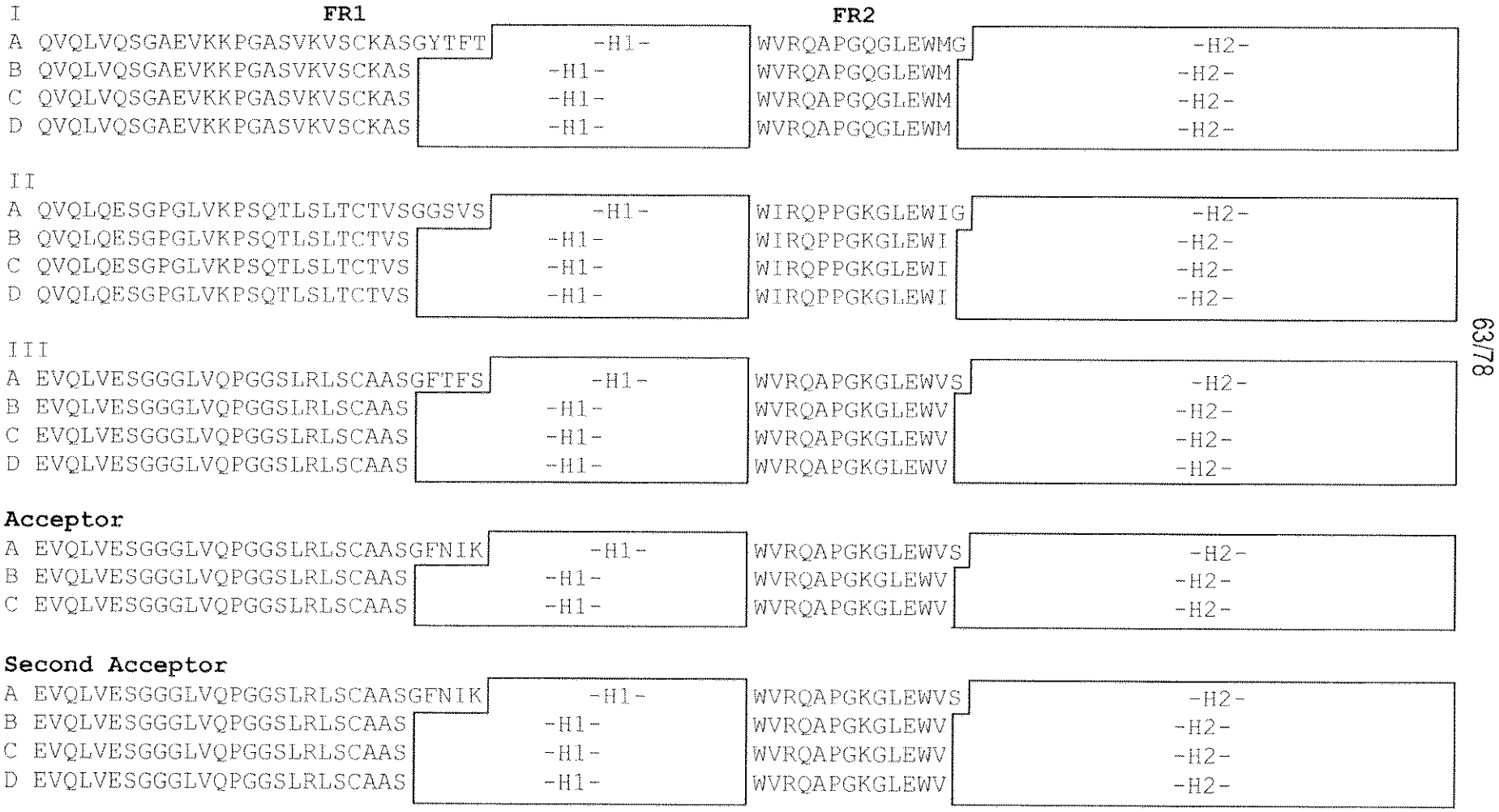

I
FR1
FR2
A QVQLVQSGAEVKKPGASVKVSCKASGYTFT -H1- WVRQAPGQGLEWMG -H2-
B QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2-
C QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2-
D QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2-
II
A QVQLQESGPGLVKPSQTLSLTCTVSGGSVS -H1- WIRQPPGKGLEWIG -H2-
B QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2-
C QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2-
D QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2-
III
A EVQLVESGGGLVQPGGSLRLSCAASGFTFS -H1- WVRQAPGKGLEWVS -H2-
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
Acceptor
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2-
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
Second Acceptor
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2-
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2-

FIG. 52B

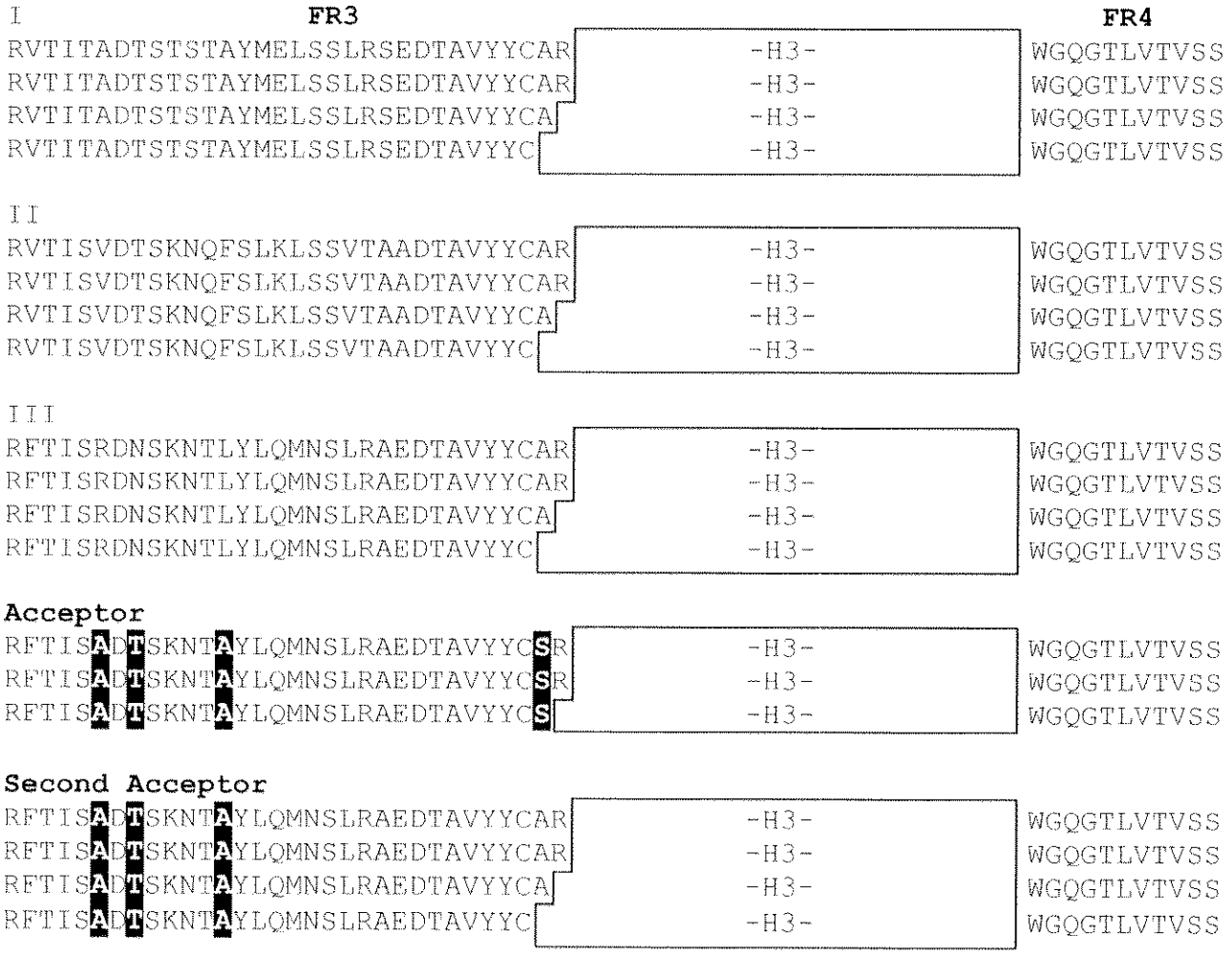

I
FR3
FR4
RVTITADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS
RVTITADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS
RVTITADTSTSTAYMELSSLRSEDTAVYYCA -H3- WGQGTLVTVSS
RVTITADTSTSTAYMELSSLRSEDTAVYYC -H3- WGQGTLVTVSS
II
RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS
RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS
RVTISVDTSKNQFSLKLSSVTAADTAVYYCA -H3- WGQGTLVTVSS
RVTISVDTSKNQFSLKLSSVTAADTAVYYC -H3- WGQGTLVTVSS
III
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS
RFTISRDNSKNTLYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS
Acceptor
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS
RFTISADTSKNTAYLQMNSLRAEDTAVYYCS -H3- WGQGTLVTVSS
Second Acceptor
RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS
RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS
RFTISADTSKNTAYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS
RFTISADTSKNTAYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS

FIG. 53

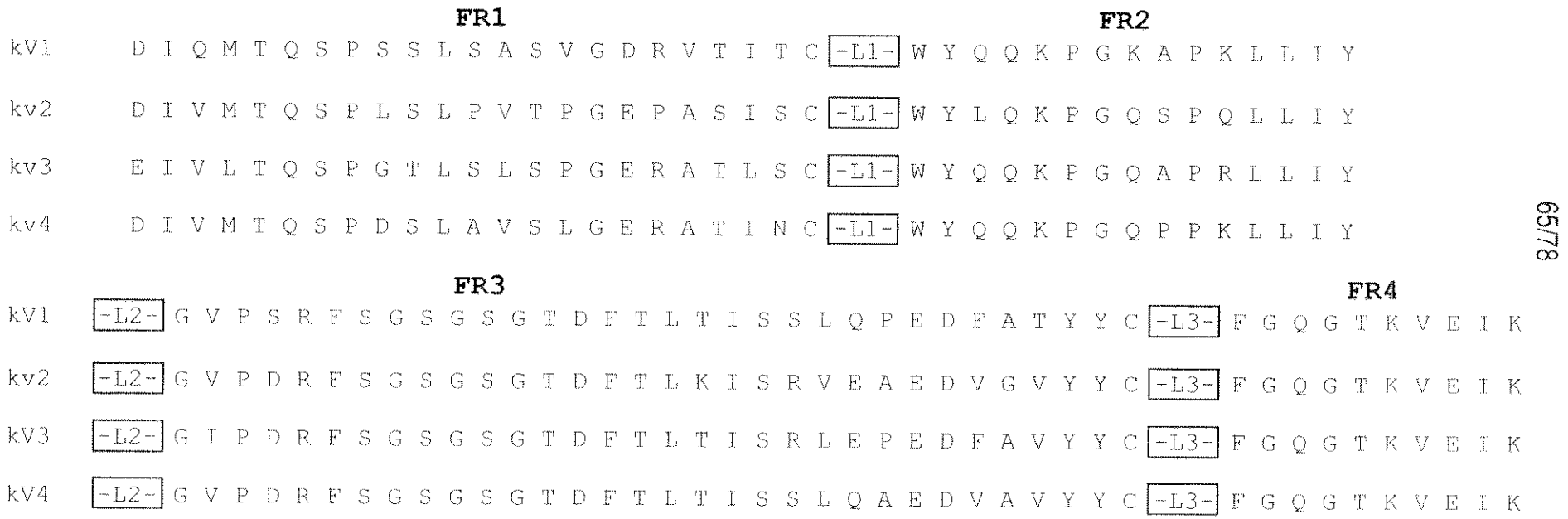

FR1
FR2
kV1 D I Q M T Q S P S S L S A S V G D R V T I T C -L1- W Y Q Q K P G K A P K L L I Y
kv2 D I V M T Q S P L S L P V T P G E P A S I S C -L1- W Y L Q K P G Q S P Q L L I Y
kv3 E I V L T Q S P G T L S L S P G E R A T L S C -L1- W Y Q Q K P G Q A P R L L I Y
kv4 D I V M T Q S P D S L A V S L G E R A T I N C -L1- W Y Q Q K P G Q P P K L L I Y
FR3
FR4
kV1 -L2- G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y C -L3- F G Q G T K V E I K
kv2 -L2- G V P D R F S G S G S G T D F T L K I S R V E A E D V G V Y Y C -L3- F G Q G T K V E I K
kV3 -L2- G I P D R F S G S G S G T D F T L T I S R L E P E D F A V Y Y C -L3- F G Q G T K V E I K
kV4 -L2- G V P D R F S G S G S G T D F T L T I S S L Q A E D V A V Y Y C -L3- F G Q G T K V E I K

FIG. 54

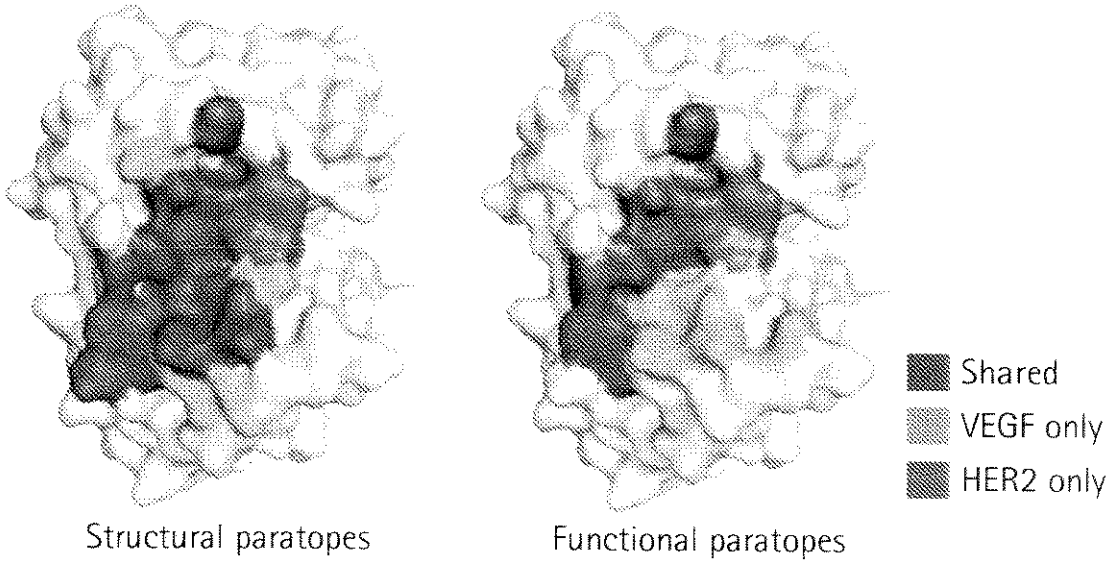

Shared
VEGF only
HER2 only
Structural paratopes
Functional paratopes

FIG. 55A

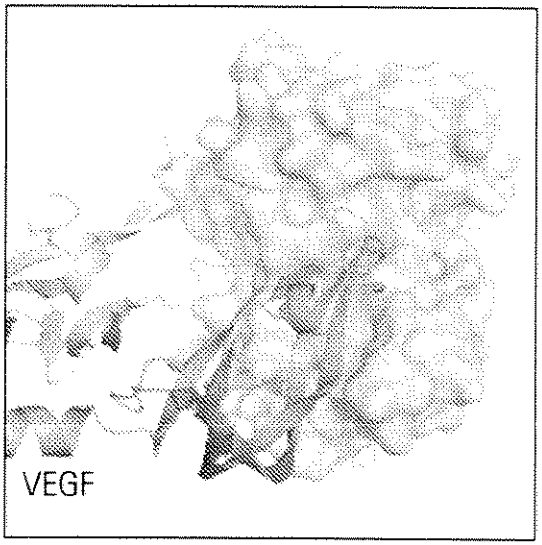


FIG. 55B

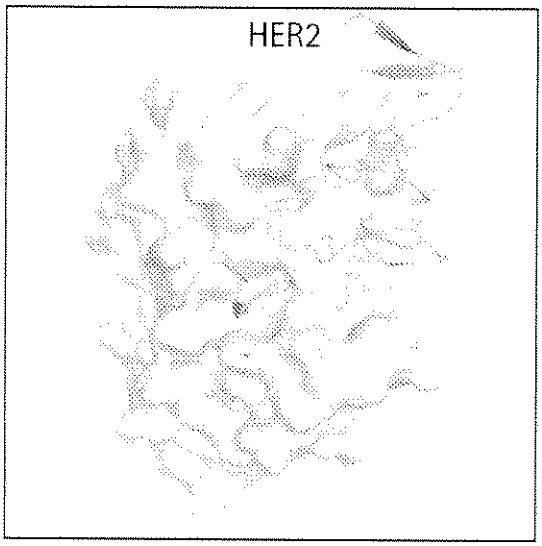


FIG. 55C

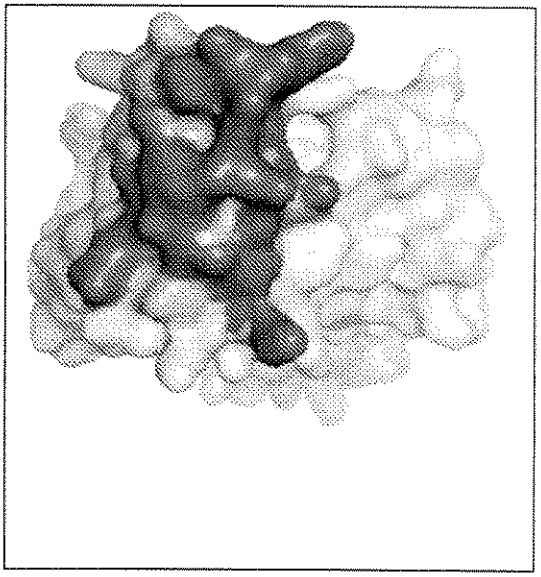


FIG. 55D

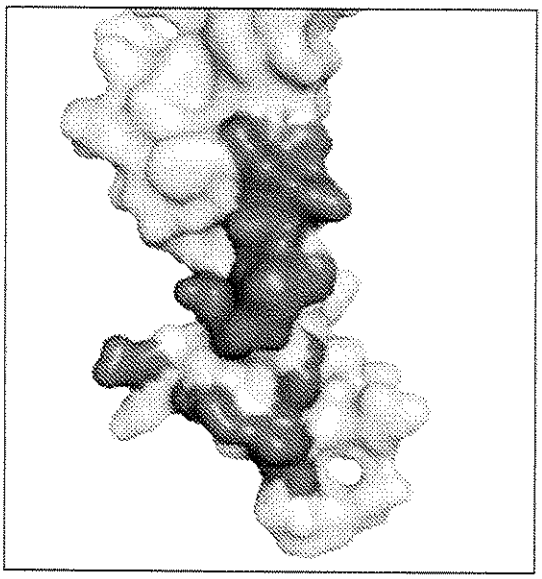


FIG. 56

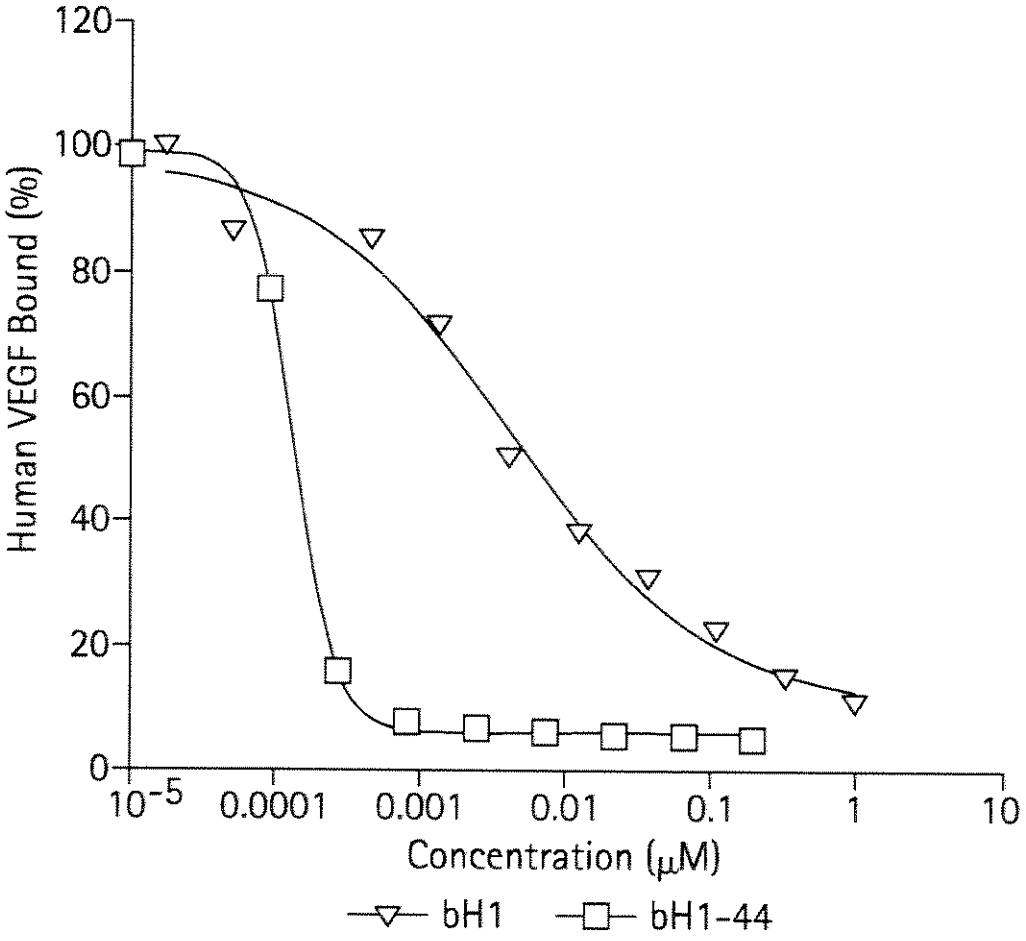

120
100
80
60
40
20
0
Human VEGF Bound (%)
10-5
0.0001
0.001
0.01
0.1
1
10
Concentration (μM)
bH1
bH1-44

FIG. 57

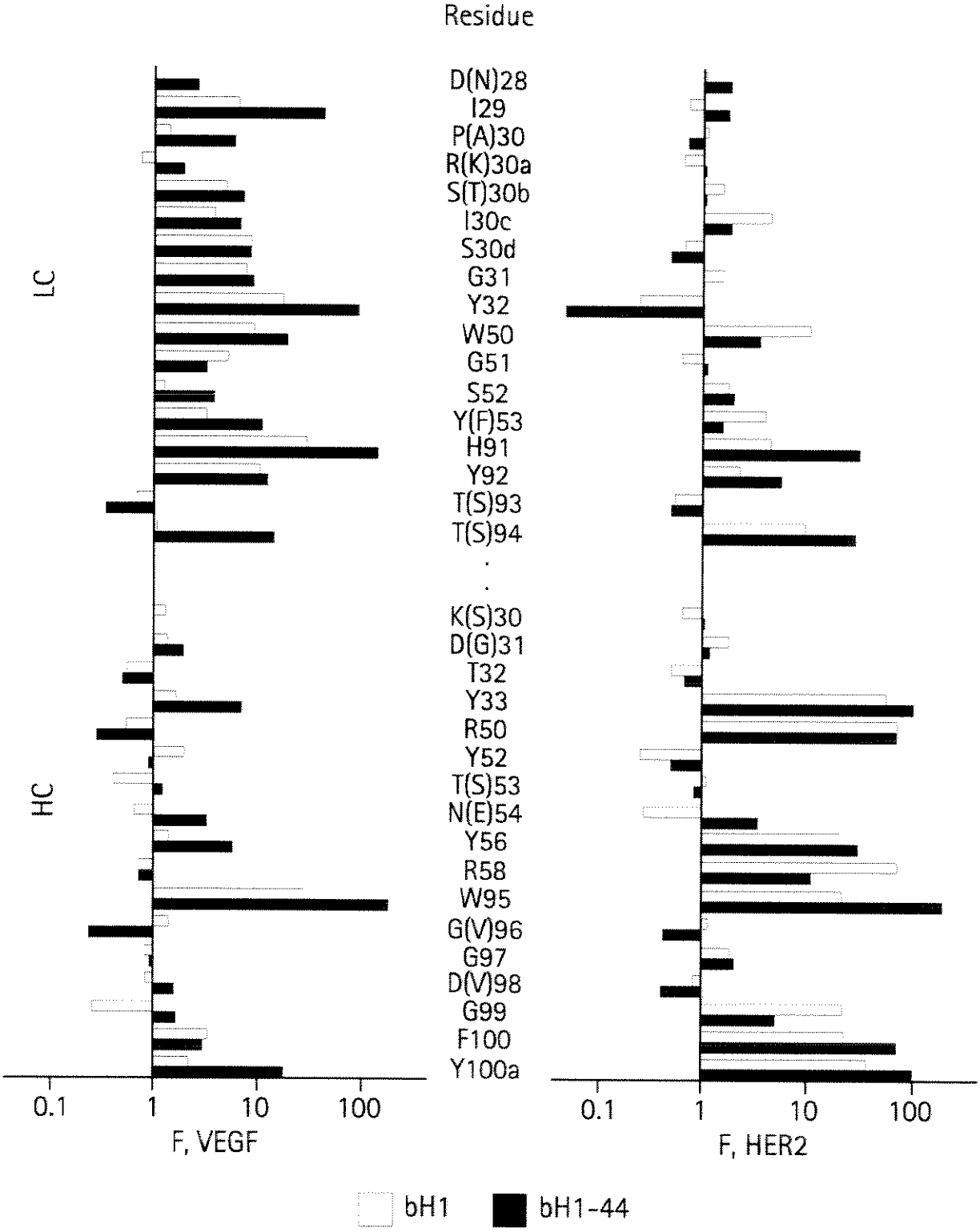

Residue
LC
HC
D(N)28
I29
P(A)30
R(K)30a
S(T)30b
I30c
S30d
G31
Y32
W50
G51
S52
Y(F)53
H91
Y92
T(S)93
T(S)94
K(S)30
D(G)31
T32
Y33
R50
Y52
T(S)53
N(E)54
Y56
R58
W95
G(V)96
G97
D(V)98
G99
F100
Y100a
0.1
1
10
100
F, VEGF
0.1
1
10
100
F, HER2
bH1
bH1-44

FIG. 58A

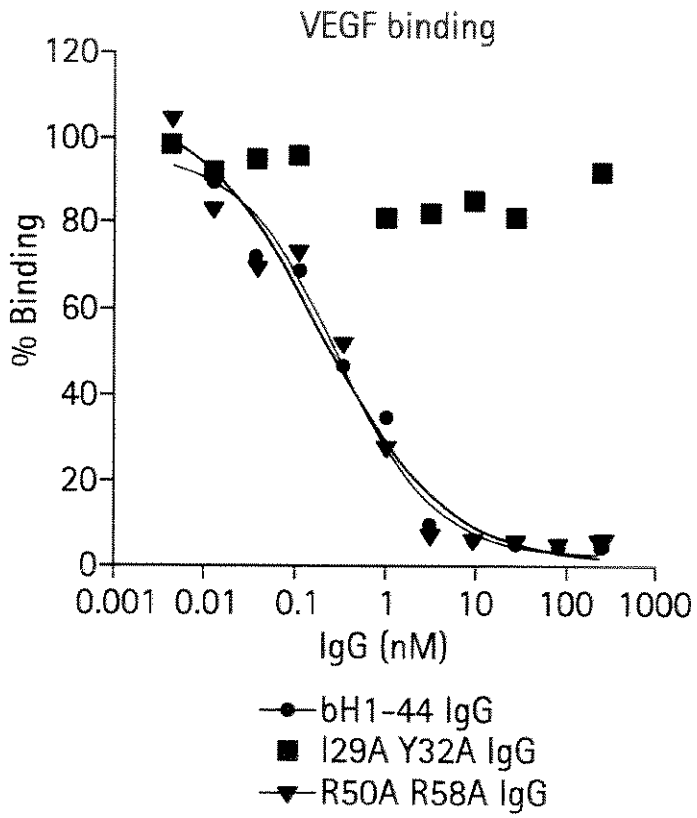


FIG. 58B

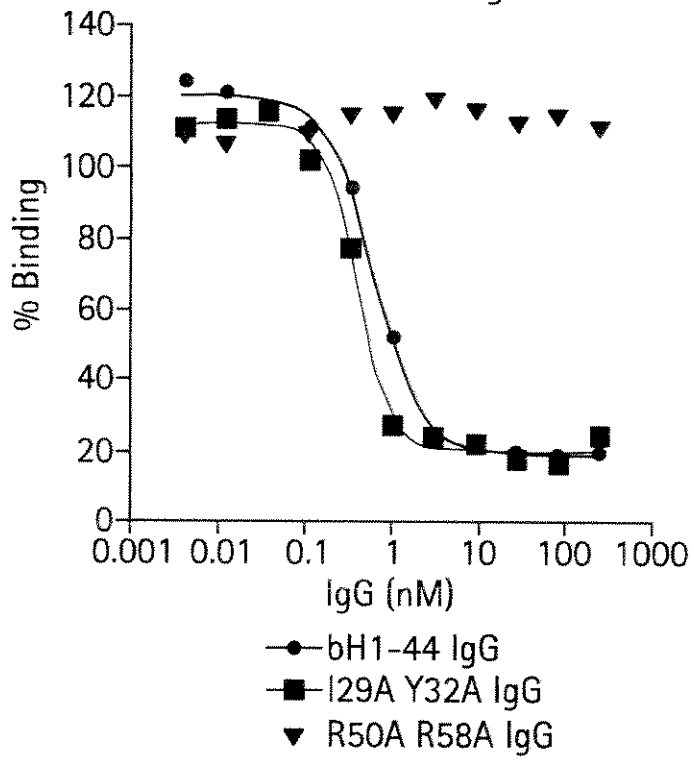


FIG. 59A

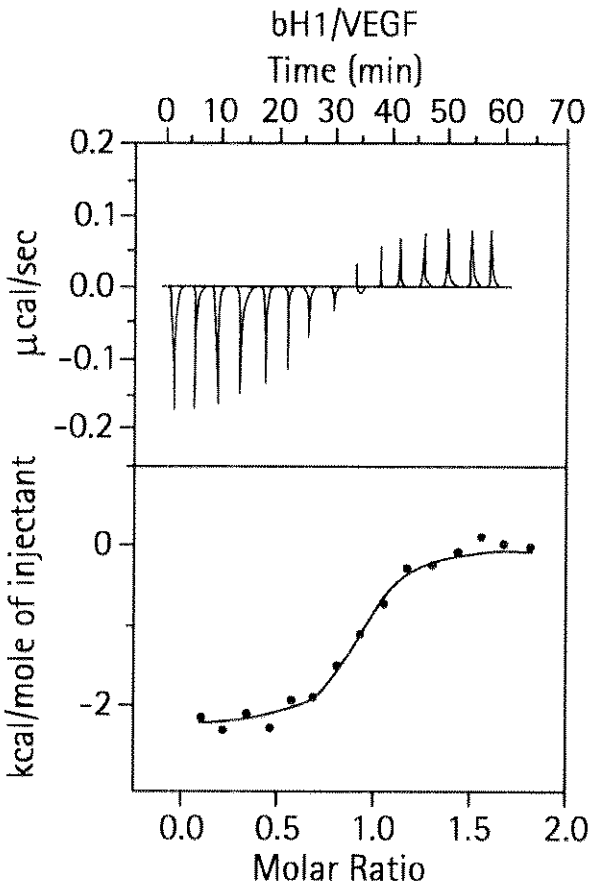


FIG. 59B

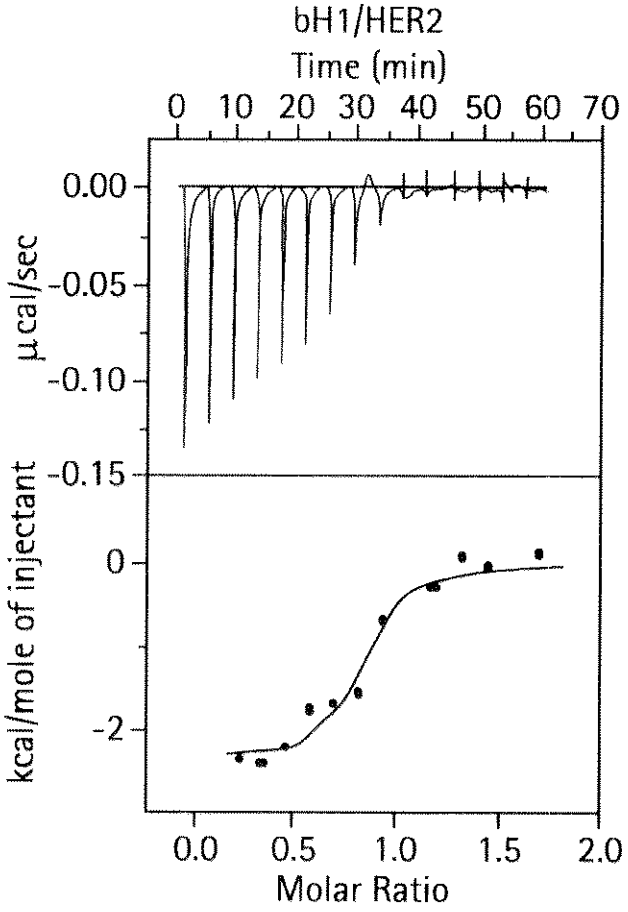


FIG. 59C

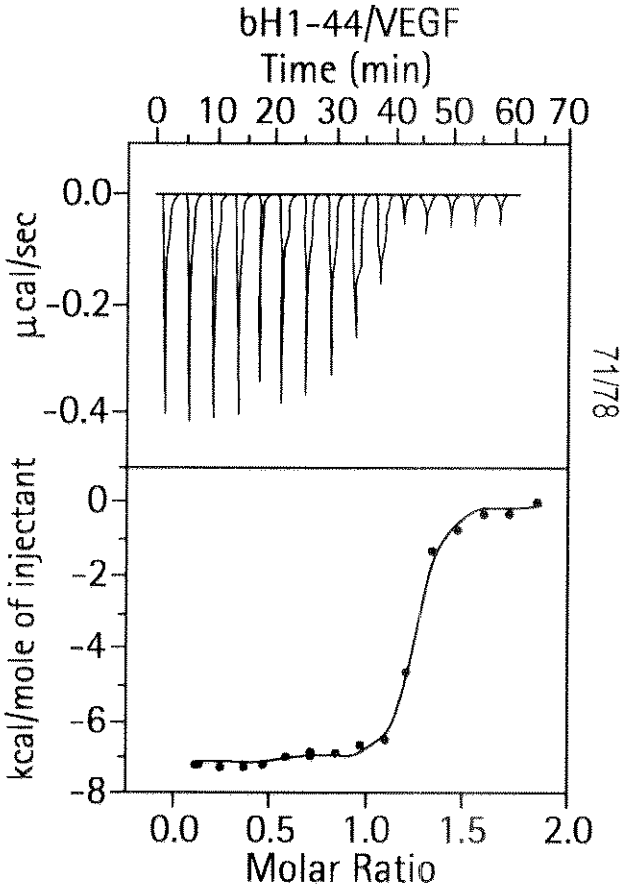


FIG. 59D

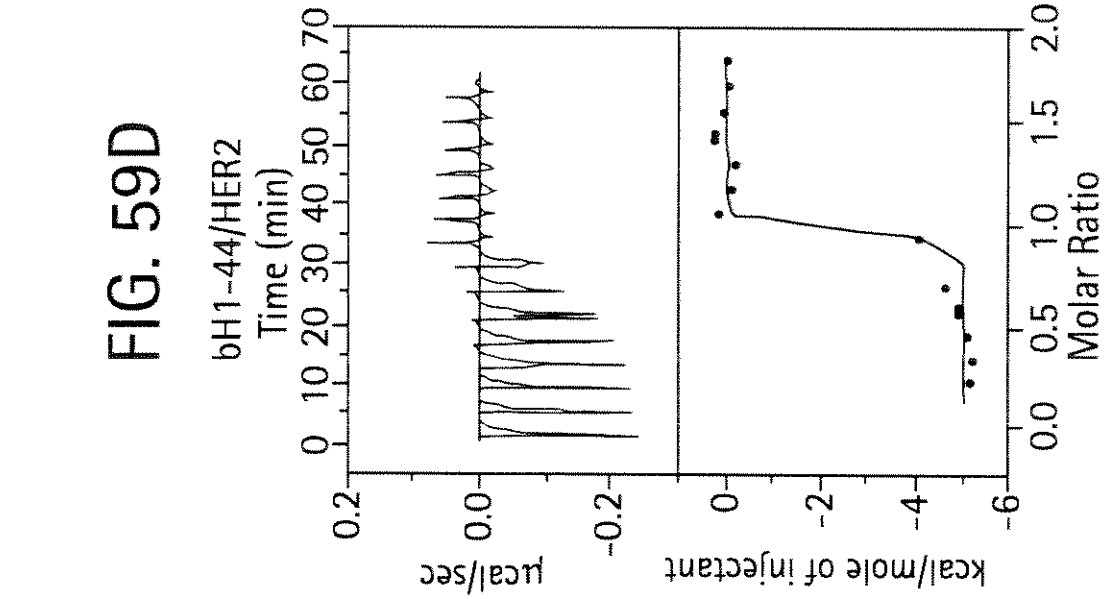


FIG. 59E

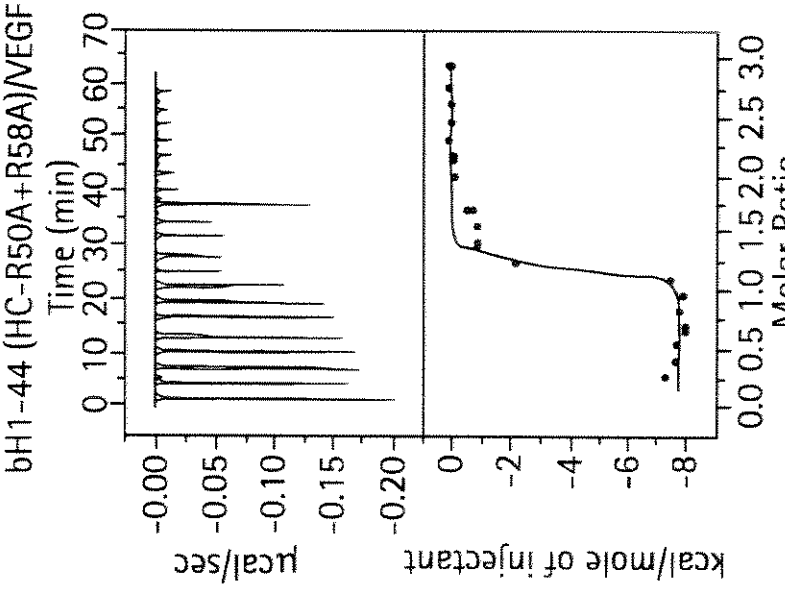


FIG. 59F

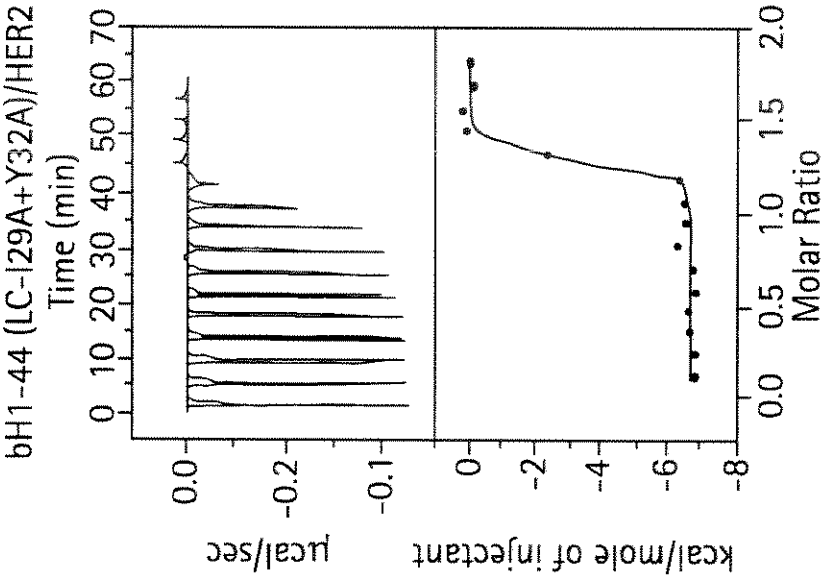


FIG. 60

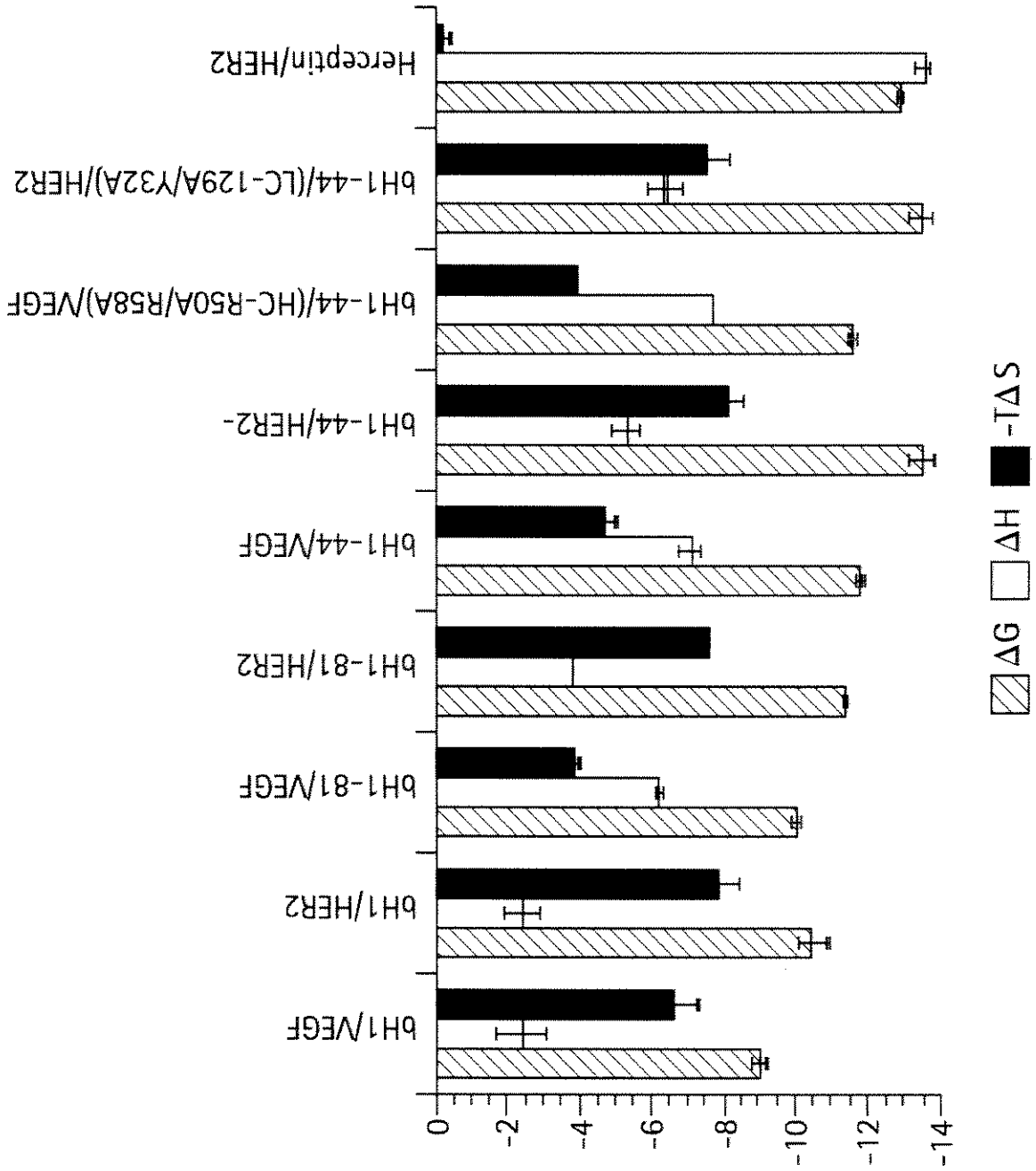

0
-2
-4
-6
-8
-10
-12
-14
ΔG
ΔH
-TΔS
bH1/VEGF
bH1/HER2
bH1-81/VEGF
bH1-81/HER2
bH1-44/VEGF
bH1-44/HER2-
bH1-44/(HC-R50A/R58A)/VEGF
bH1-44/(LC-129A/Y32A)/HER2
Herceptin/HER2

FIG. 61

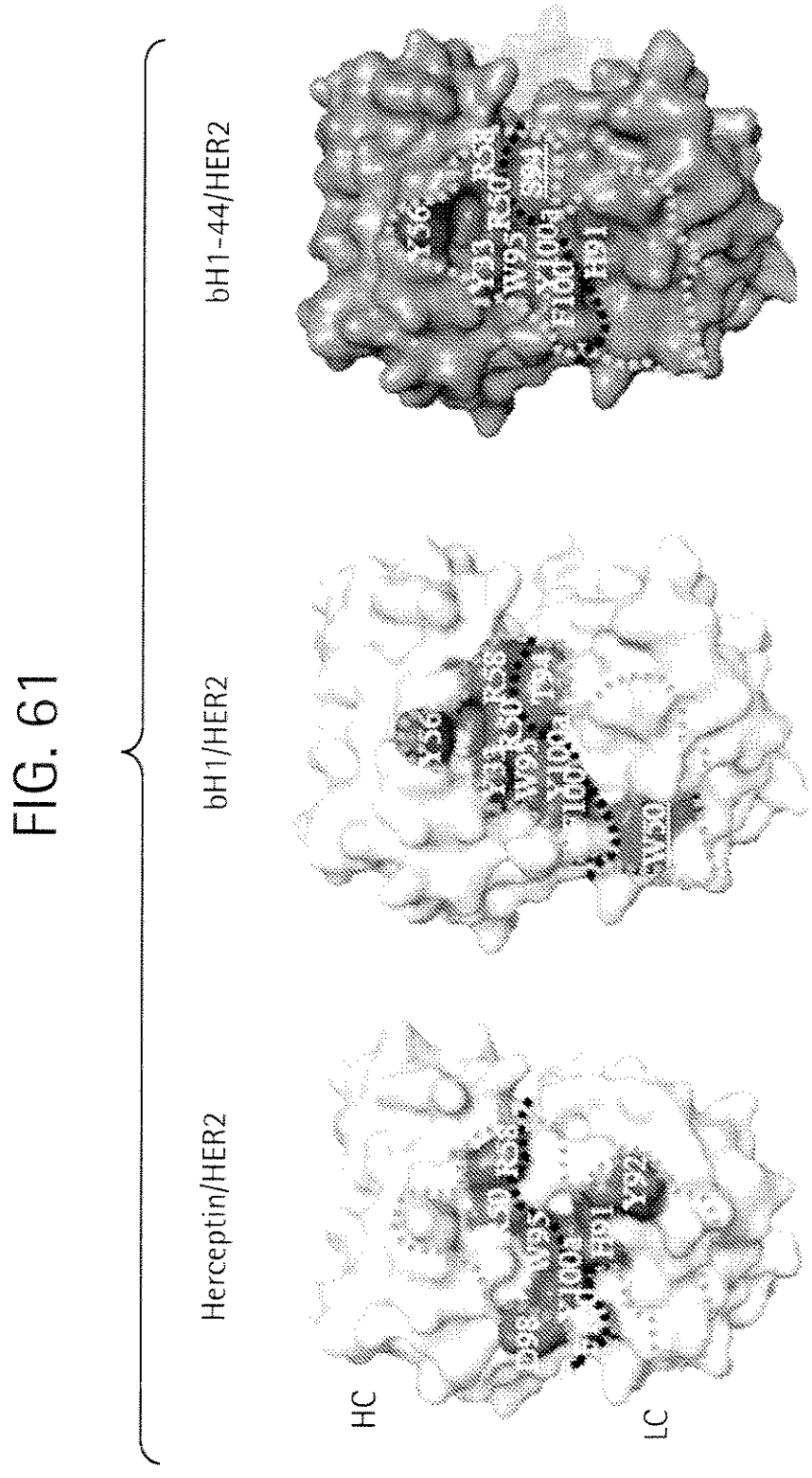


FIG. 62

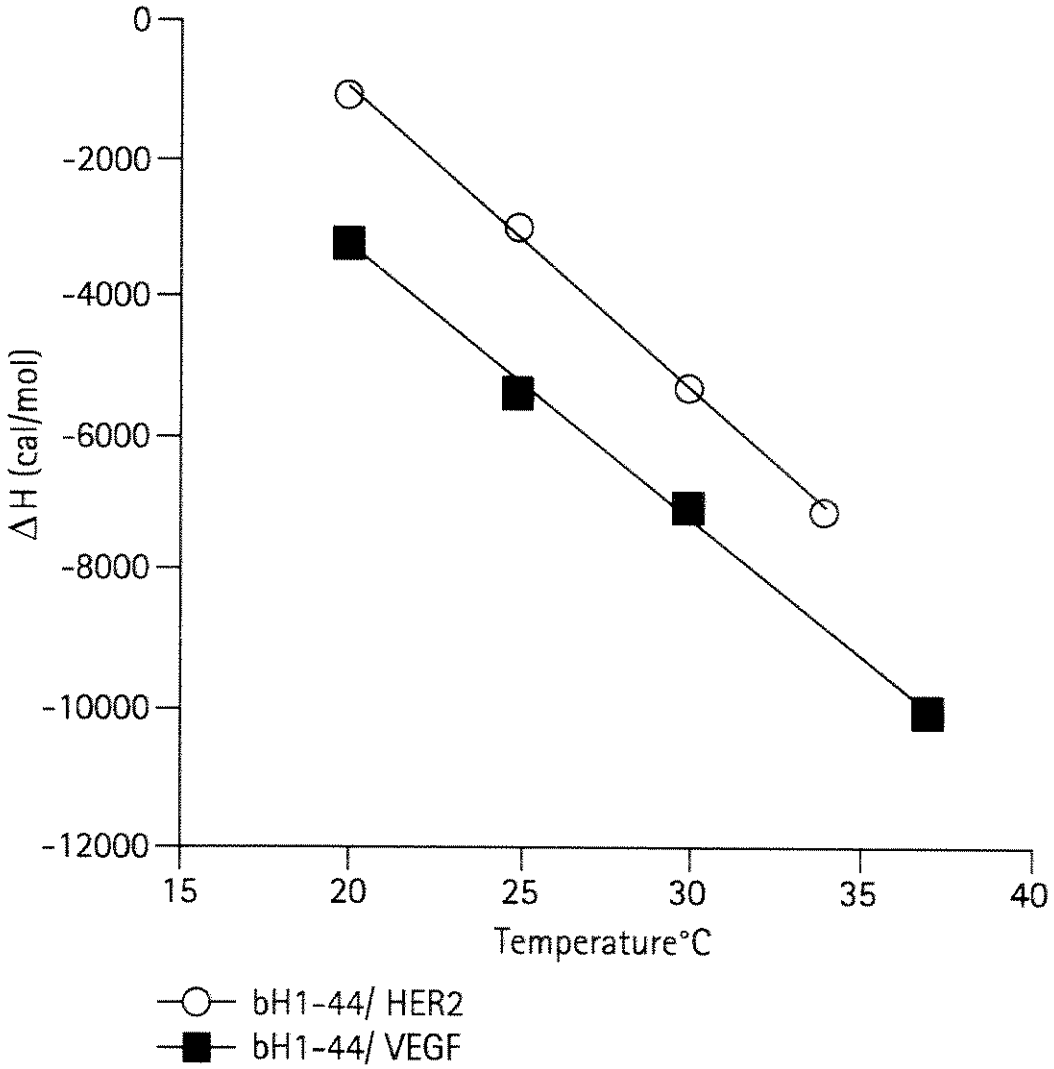

0
-2000
-4000
-6000
-8000
-10000
-12000
ΔH (cal/mol)
15
20
25
30
35
40
Temperature°C
bH1-44/ HER2
bH1-44/ VEGF

FIG. 63A

VEGF

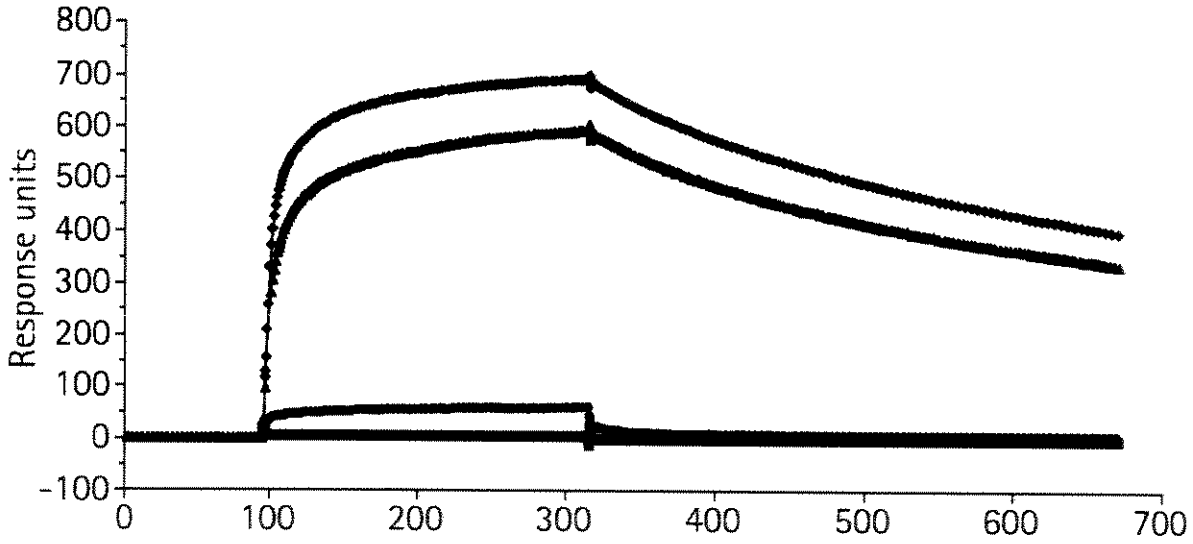


FIG. 63B

HER2

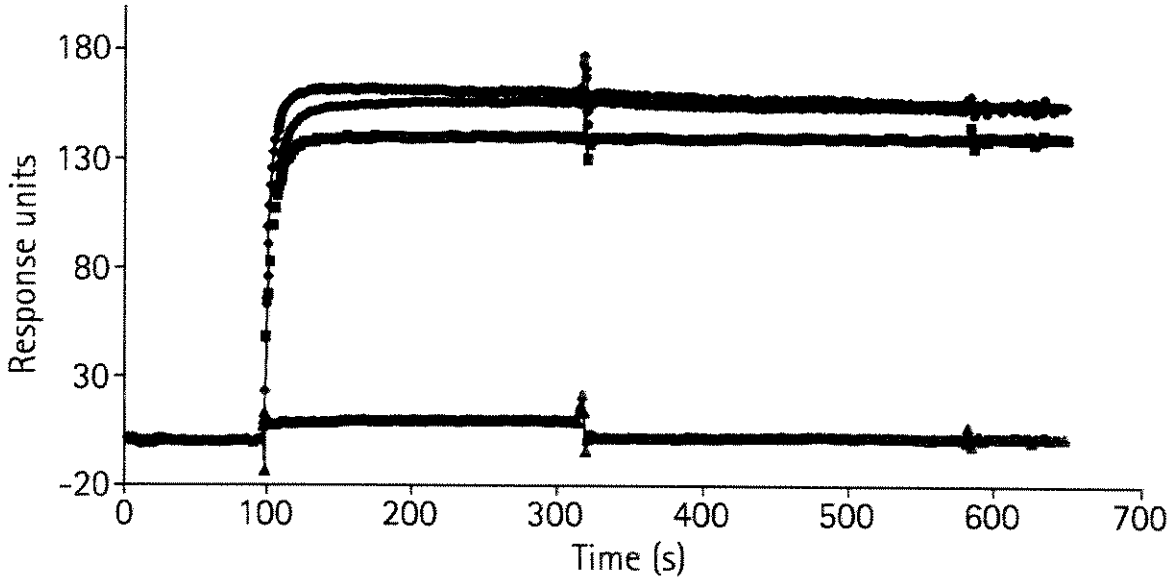


FIG. 64A

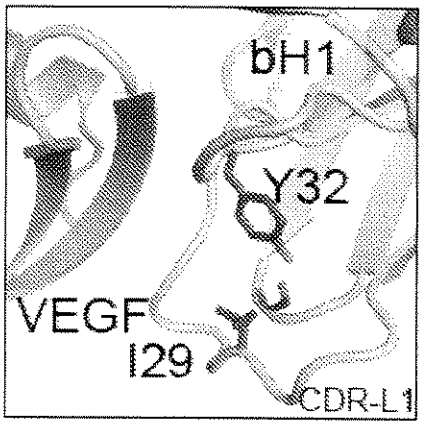


FIG. 64B

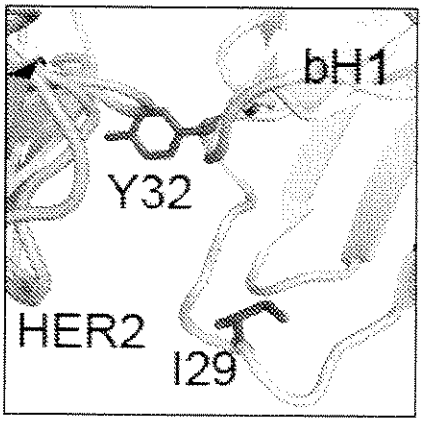


FIG. 64C

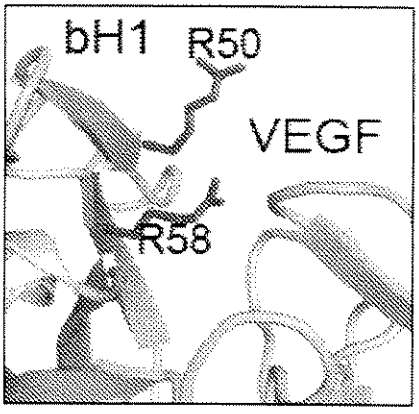


FIG. 64D

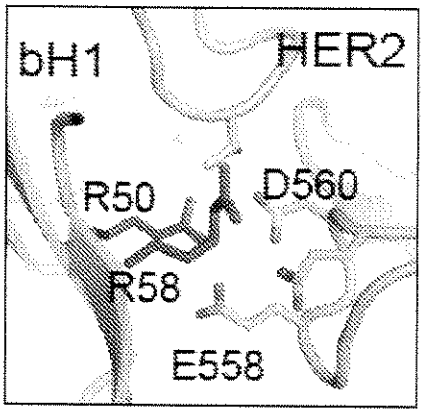
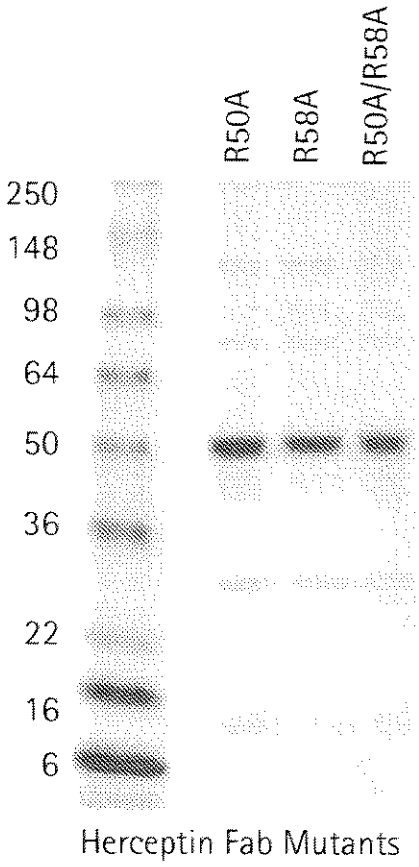


FIG. 65


R50A
R58A
R50A/R58A
250
148
98
64
50
36
22
16
6
Herceptin Fab Mutants