

Figura 1A

RESONANCIA DE PLASMÓN SUPERFICIAL (K_D)

	HUMANO		CINOMOLGO		RATÓN	RATA	
	CTLA-4		CTLA-4		CTLA-4	CTLA-4	
	DÍMERO	MONÓMERO	DÍMERO	MONÓMERO	DÍMERO	DÍMERO	MONÓMERO
AGEN1884	0.6 nM	0.8 nM	6.2 nM	1.6 nM	9.9 nM	No hay unión	No hay unión
Anticuerpo de referencia (IgG1)	0.54 nM	1.2 nM	1.2 nM	0.75 nM	1.2 nM	26 nM	22 nM

Figura 1B

CTLA-4 Células que expresan

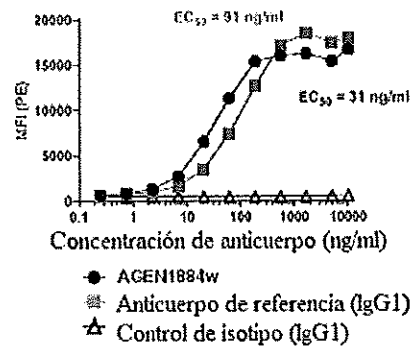


Figura 1C

ENSAYO DE SELECTIVIDAD DEL CTLA-4

	Conc. en ng/ml	rhCTLA-4 Fo (R&D)	rcCTLA-4 Fo (Sino)	rhCD28 Fo (R&D)	rhICOS Fo (R&D)	rhBTLA Fo (Sino)	rhPD-1 Fo (R&D)	rcPD-1 Fo (JAB)
AGEN1884	1000	22729	15557	46	43	87	15	32
	100	20359	12676	48	47	115	25	41
	10	7820	4096	77	60	95	32	46
Anticuerpo de referencia (IgG1)	1000	20345	21850	52	46	87	24	40
	100	19131	19810	55	53	87	28	41
	10	6825	7245	66	57	92	35	41
Control de isotipo (IgG1)	1000	79	154	57	34	73	31	49
	100	75	168	67	67	123	39	45
	10	88	175	52	48	91	31	30

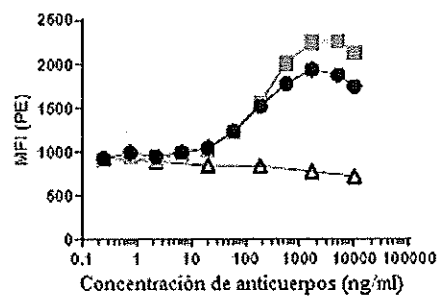
Figura 1D

Afinidad de unión relativa

	AGEN1884	Anticuerpo de referencia (IgG1)	Control de isotipo (IgG1)
rhCTLA-4	+++	+++	—
rcCTLA-4	+++	+++	—
rhCD28	—	—	—
rhICOS	—	—	—
rhBTLA	—	—	—
rhPD-1	—	—	—
rcPD-1	—	—	—

Figura 2A

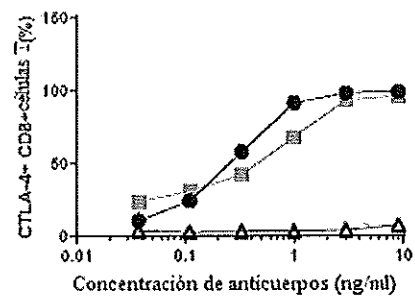
CÉLULAS T PRIMARIAS HUMANAS
(SUPERFICIE)



- AGEN1894w
- Anticuerpo de referencia (IgG1)
- △ Control de isotipo (IgG1)

Figura 2B

CÉLULAS T PRIMARIAS HUMANAS
(INTRACELULAR)



- AGEN1884
- Anticuerpo de referencia (IgG1)
- △ Control de isotipo (IgG1)

Figura 2C

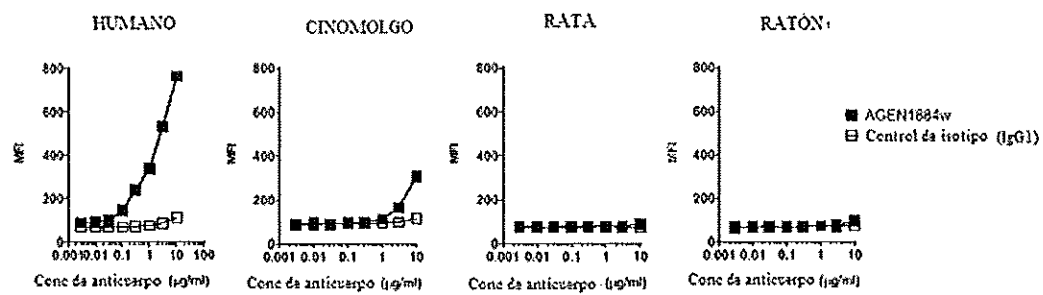


Figura 3A

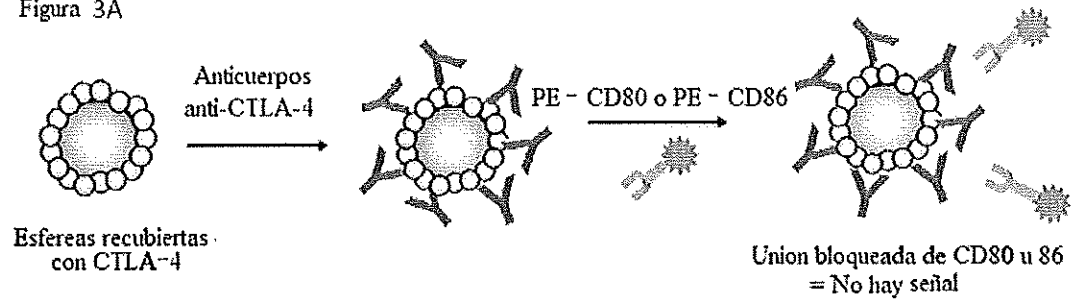


Figura 3B

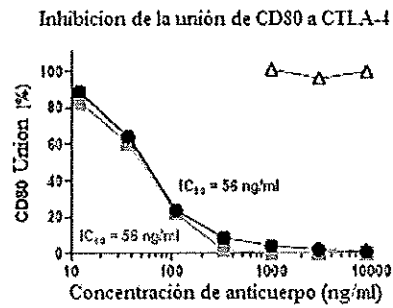
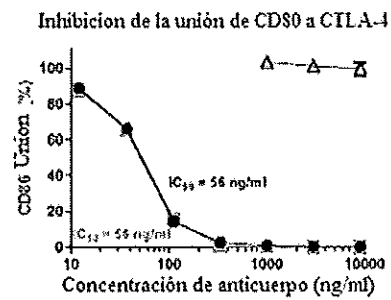


Figura 3C



- ◆ AGEN1884
- ◻ Anticuerpo de referencia (IgG1)
- △ Control de isotipo (IgG1)

Figura 3D

Células que expresan CTLA-4

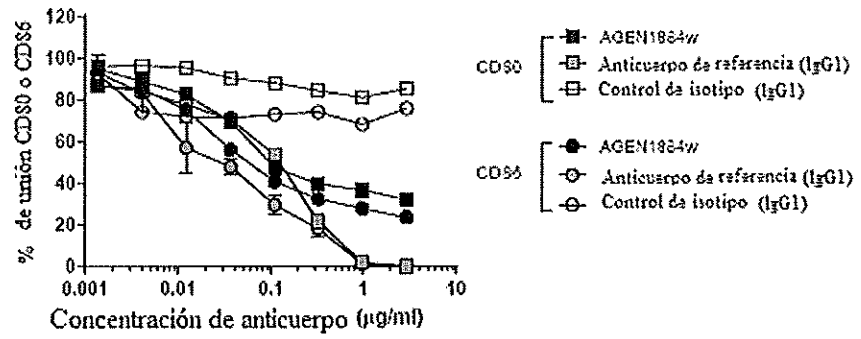


Figura 3E

Células que expresan CTLA-4

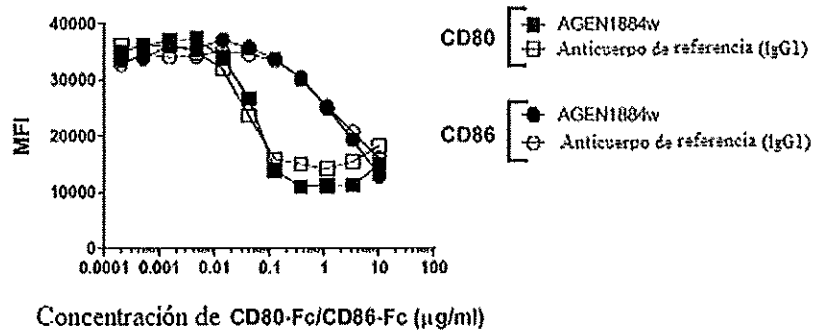


Figura 4A

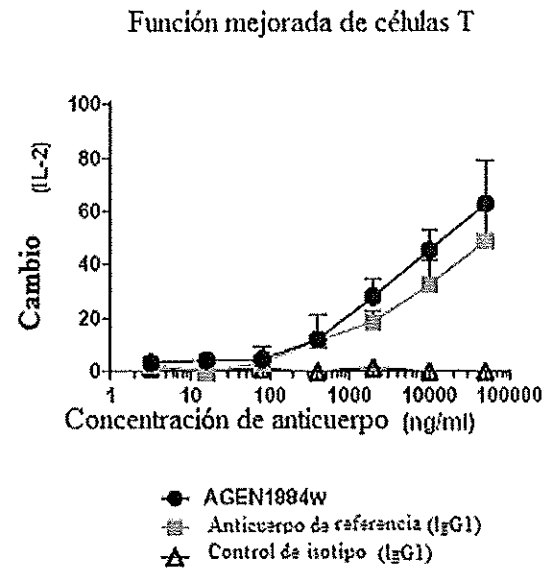


Figura 4B

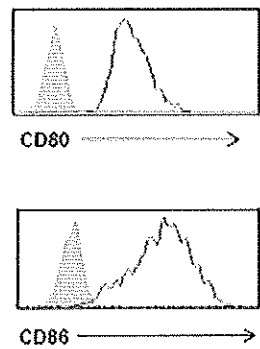


Figura 4C

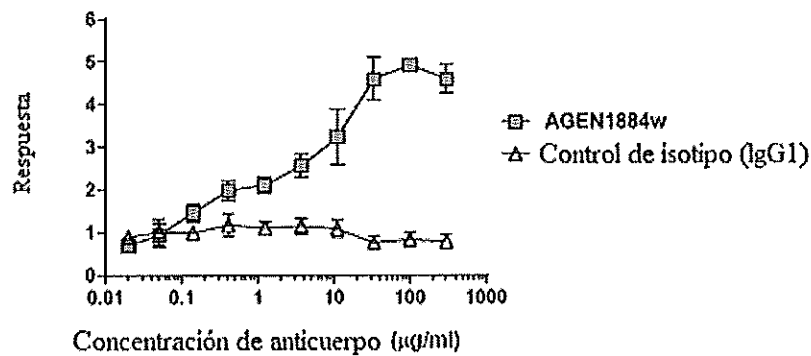


Figura 4D

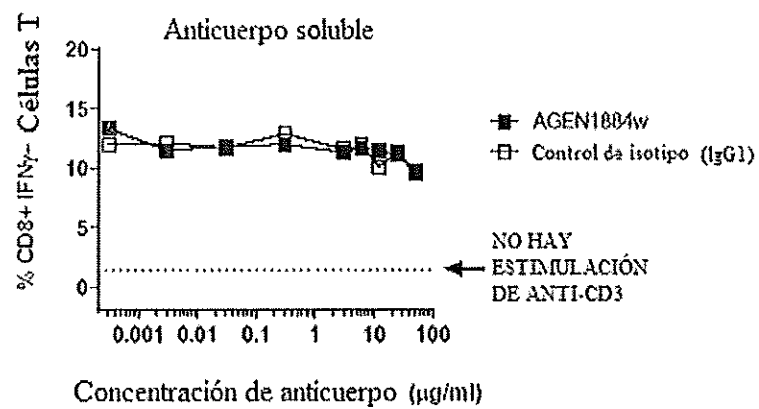


Figura 4E

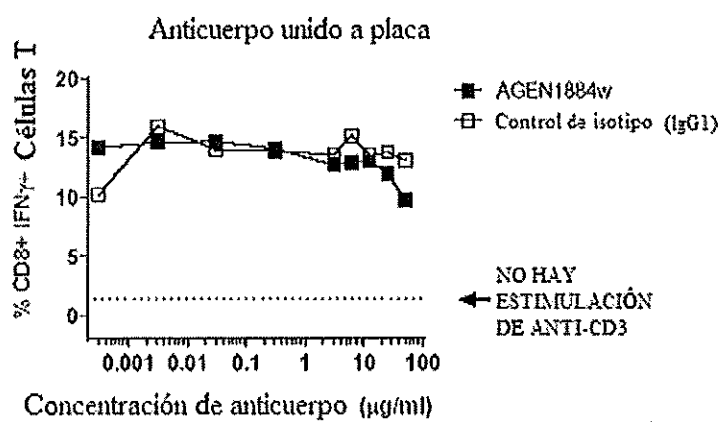


Figura 4 F

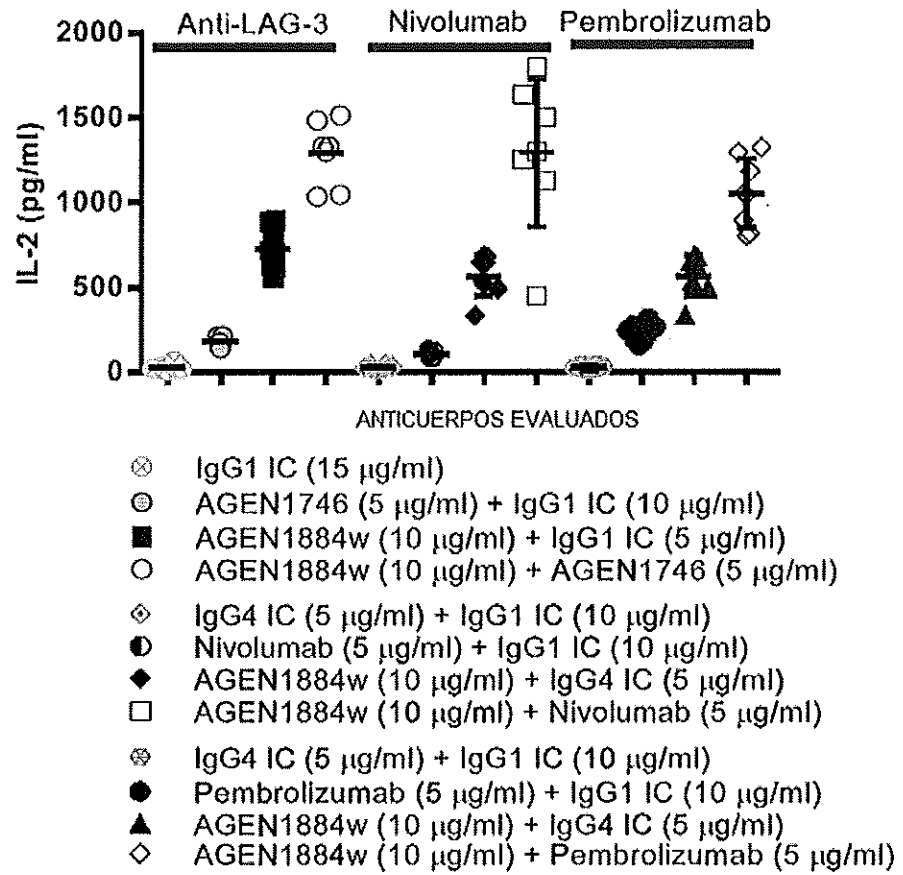


Figura 5A

Resonancia de plasmón superficial (K_D)

	Humano		Cinomolgo	
	CTLA-4		CTLA-4	
	dímero	monómero	dímero	monómero
AGEN2041w	1.7 nM	2.0 nM	4.4 nM	2.1 nM
Anticuerpo de referencia (IgG1)	0.5 nM	1.2 nM	1.2 nM	0.8 nM
Anticuerpo de referencia (IgG2)	1.5 nM	1.6 nM	3.1 nM	1.5 nM

Figura 5B

Células que expresan CTLA-4

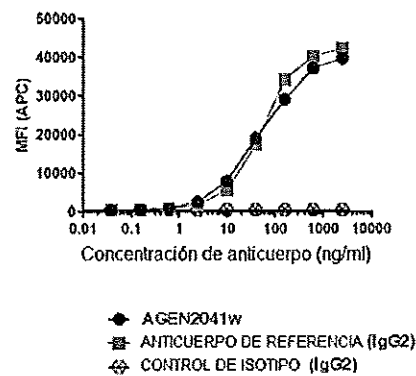


Figura 5C

Inhibición de la unión de CD80 y CD86 a CTLA-4

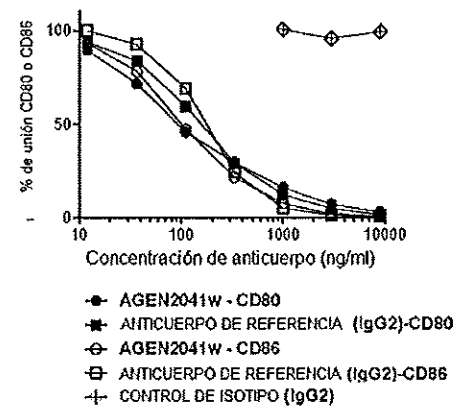


Figura 5D

ENSAYO DE SELECTIVIDAD DE CTLA-4

	CONC. EN NG/ML	rhCTLA-4 Fc (R&D)	rcCTLA-4 Fc (Sino)	rhCD28 Fc (R&D)	rhICOS Fc (R&D)	rhBTLA Fc (Sino)	rhPD-1 Fc (R&D)	rcPD-1 Fc (4AB)
AGEN2041w	1000	16693	13885	58	48	95	30	31
	100	15890	9949	42	43	104	19	27
	10	7737	3684	73	55	89	32	29
ANTICUERPO DE REFERENCIA (IGG2)	1000	17288	16442	58	49	106	33	39
	100	13932	13721	62	47	102	43	50
	10	4846	5143	71	59	111	36	60
CONTROL DE ISOTIPO (IGG2)	1000	79	158	64	41	93	36	41
	100	83	184	52	57	88	94	96
	10	80	187	55	58	99	79	70

Figura 5E

AFINIDAD DE UNIÓN RELATIVA

	AGEN2041w	ANTICUERPO DE REFERENCIA (IGG2)	CONTROL DE ISOTIPO (IGG2)
rhCTLA-4	+++	+++	—
rcCTLA-4	+++	+++	—
rhCD28	—	—	—
rhICOS	—	—	—
rhBTLA	—	—	—
rhPD-1	—	—	—
rcPD-1	—	—	—

Figura 6A

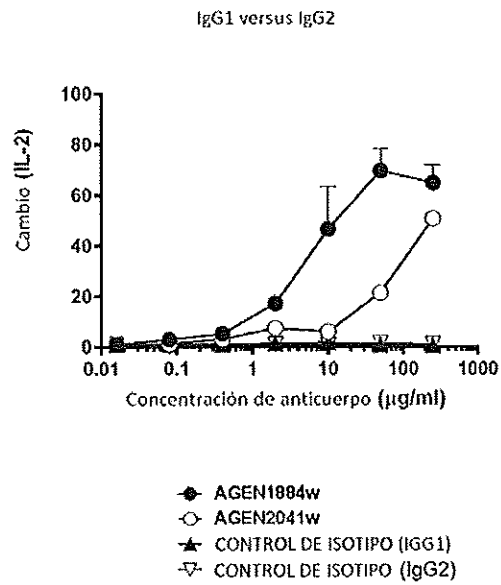


Figura 6B

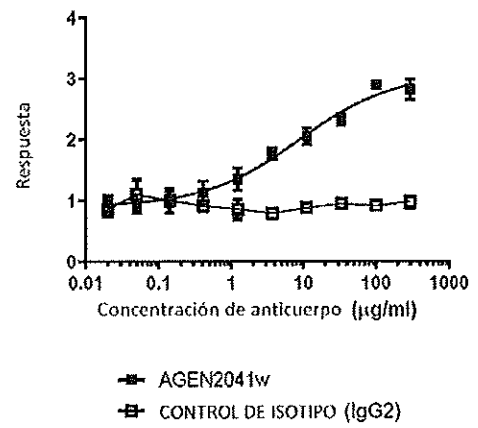


Figura 7A

Células que expresan CTLA-4

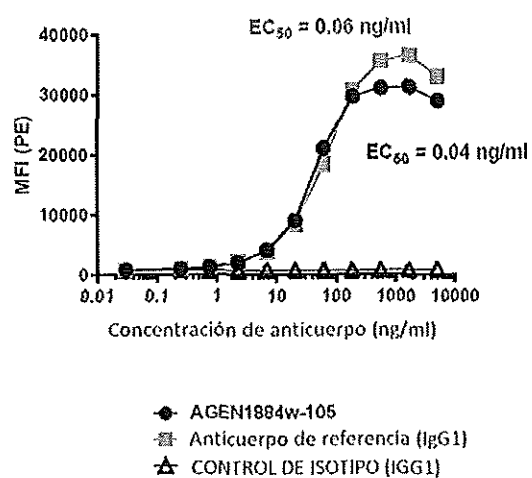


Figura 7B

Función mejorada de las células T

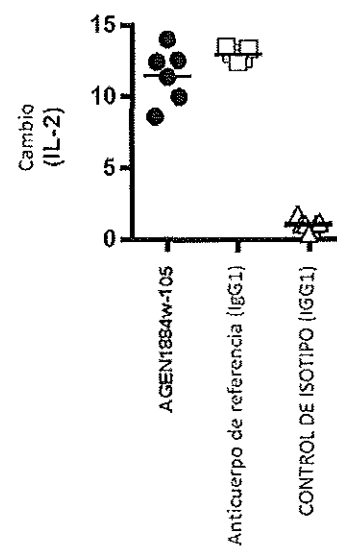


Figura 8A

Unión a células rCHO-huFcγRIIA+

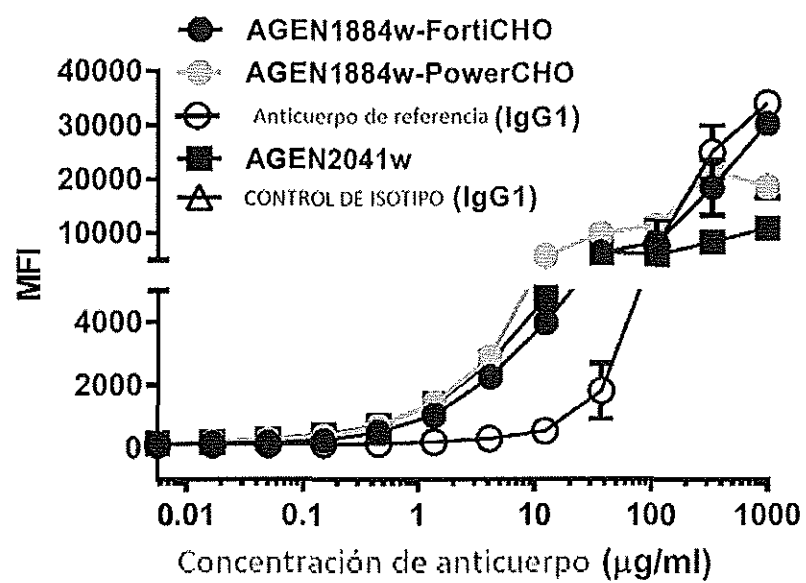


Figura 8B

Unión a células rCHO-huFcγRIIb+

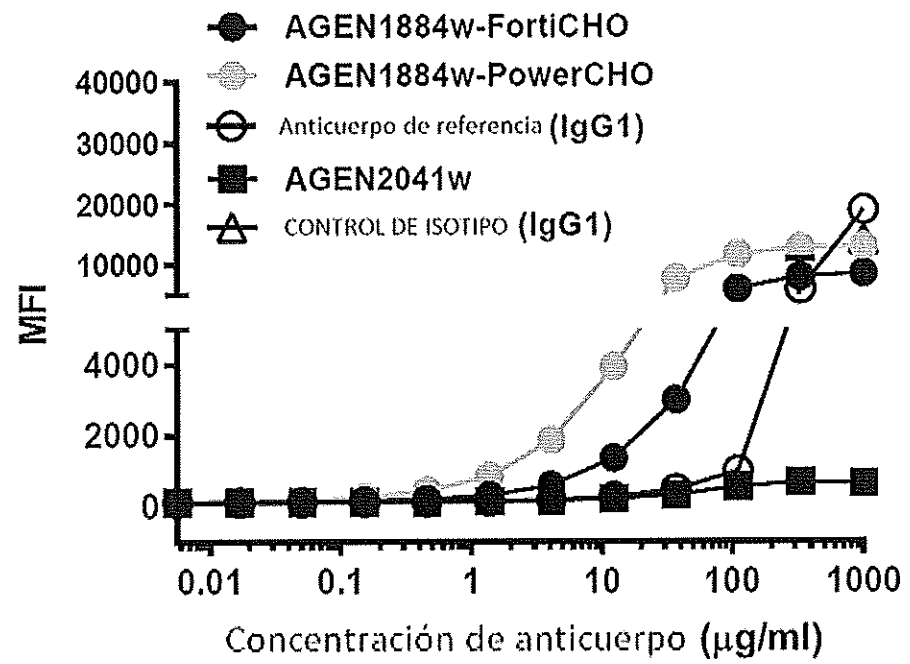


Figura 8C

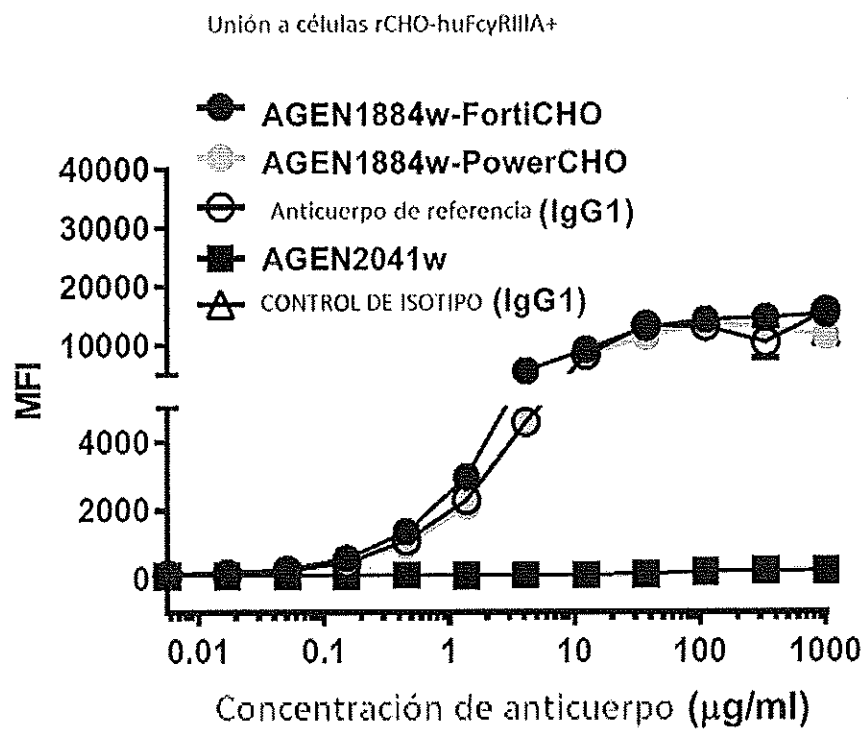


Figura 9A

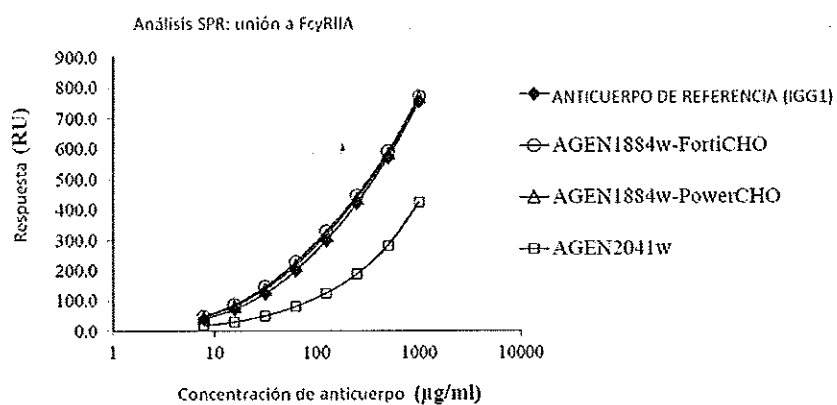


Figura 9B

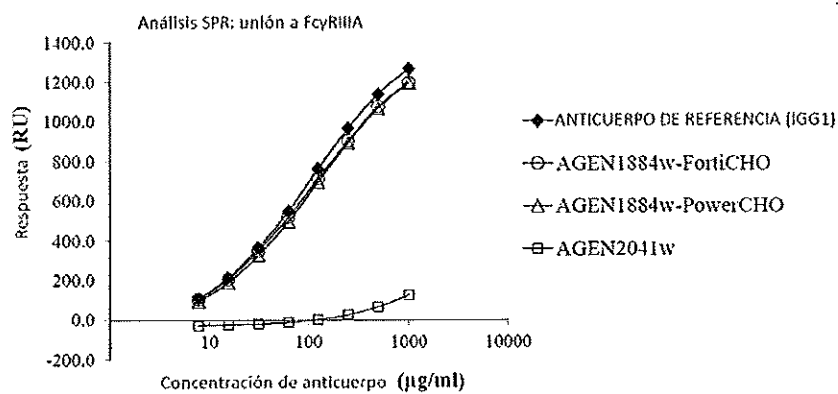


Figura 10A

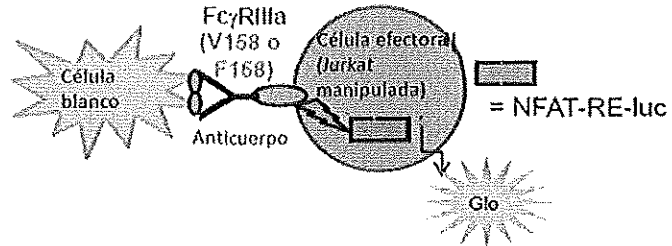


Figura 10B

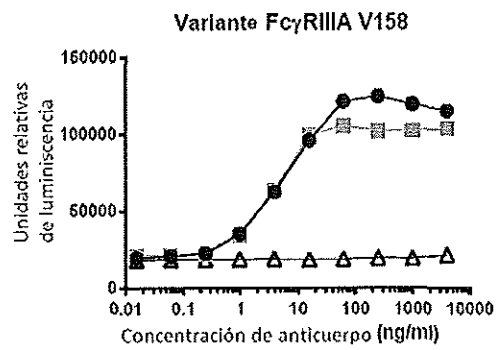
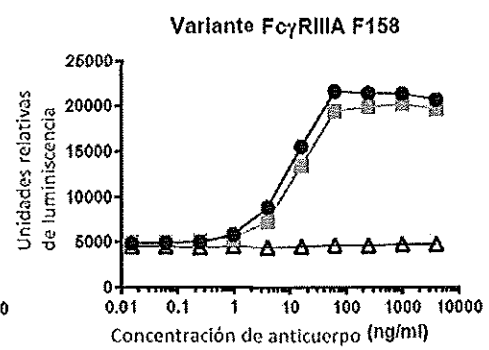


Figura 10C



- AGEN1884w-105
- ANTICUERPO DE REFERENCIA (IGG1)
- △ CONTROL DE ISOTIPO (IGG1)

Figura 10D

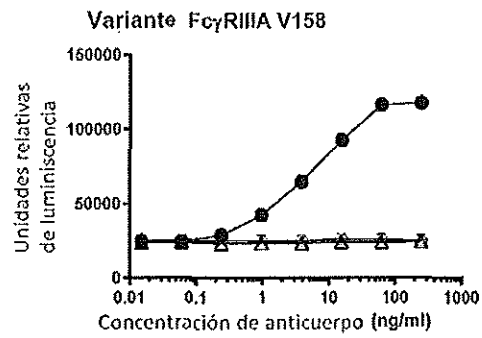
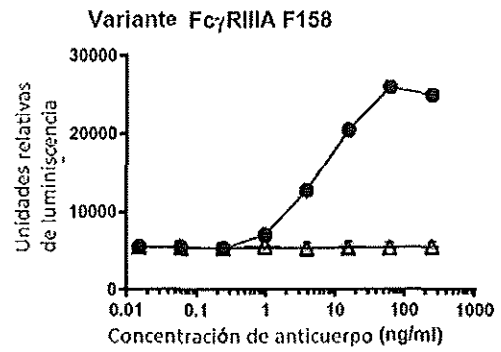


Figura 10E



- AGEN1884w
- ANTICUERPO DE REFERENCIA (IGG1)
- △ CONTROL DE ISOTIPO (IGG1)

Figura 10F

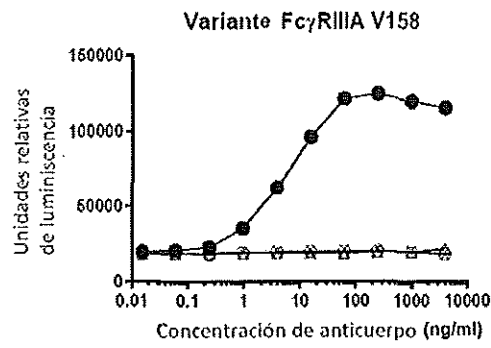
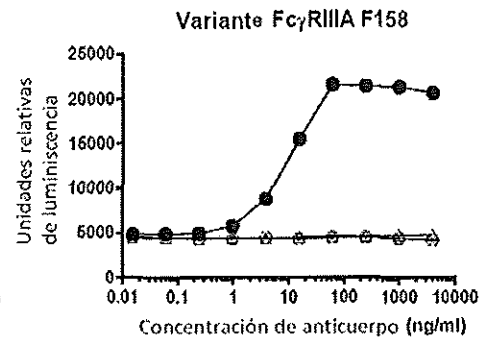


Figura 10G



- AGEN1884w-105
- ⊗ AGEN1884w-N297A
- △ CONTROL DE ISOTIPO (IGG1)

Figura 11

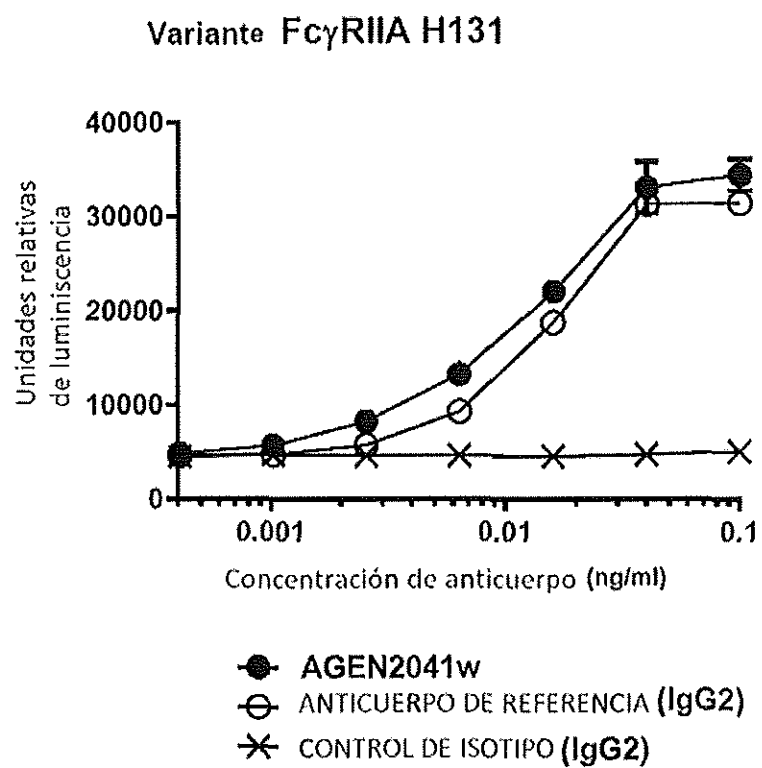


Figura 12A

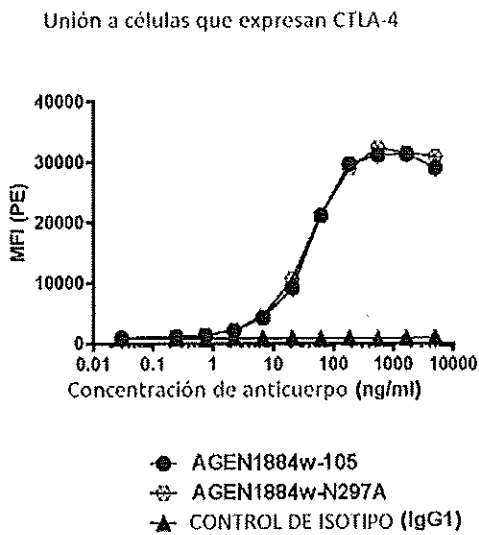


Figura 12B

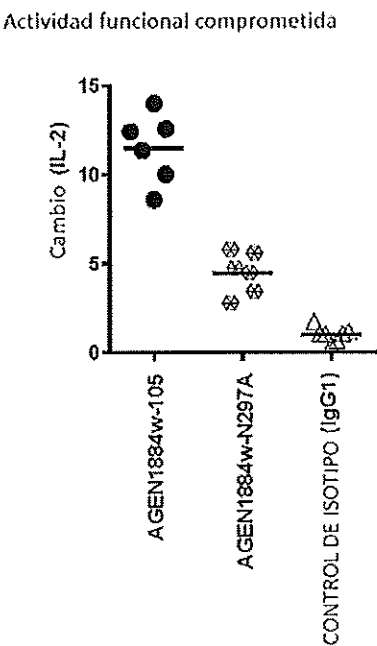


Figura 13A

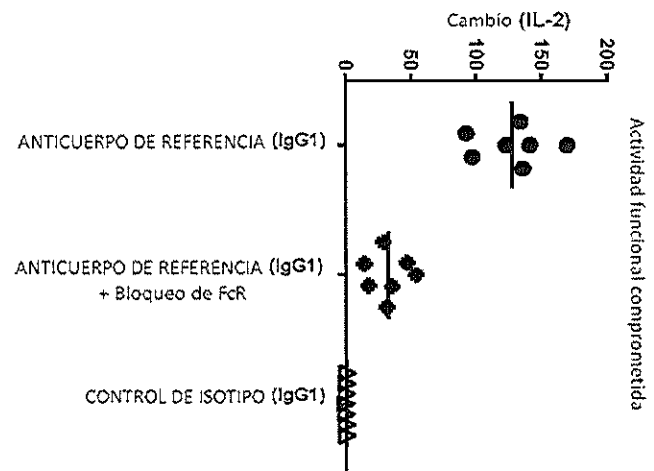


Figura 13B

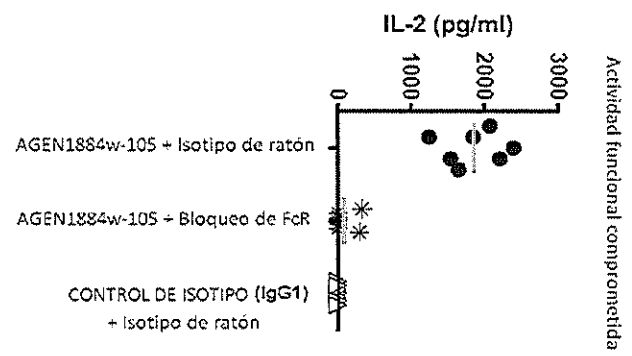


Figura 14

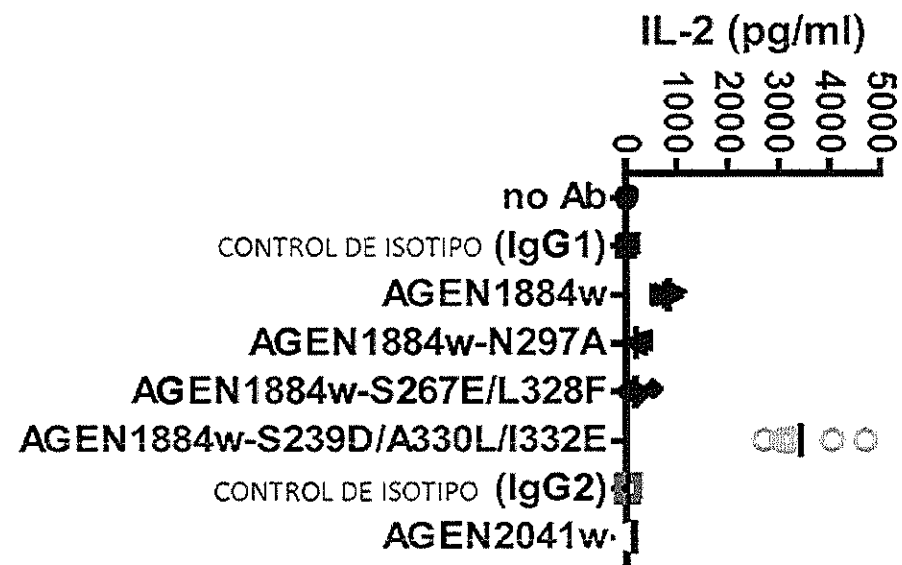


Figura 15

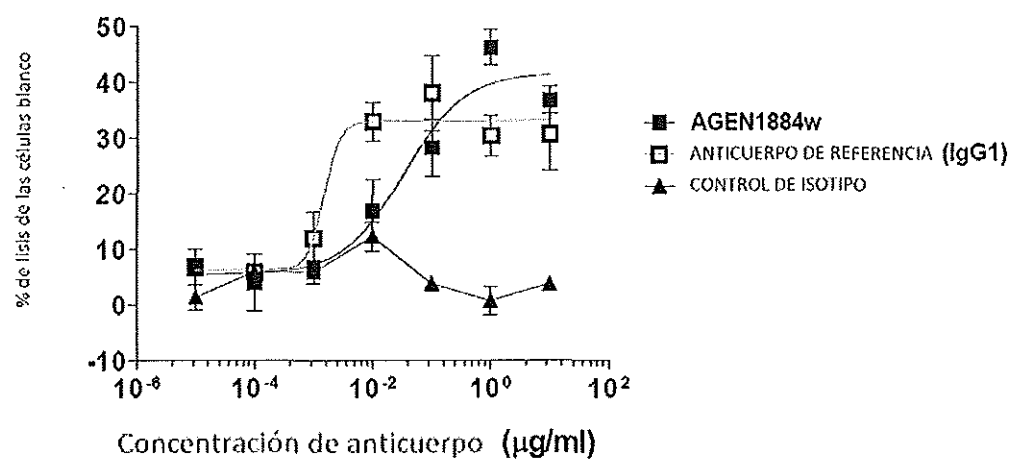
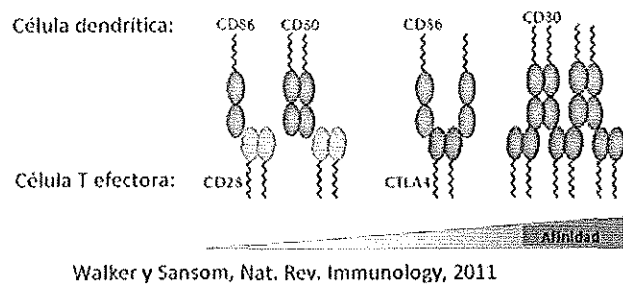


Figura 16



Walker y Sansom, Nat. Rev. Immunology, 2011

CTLA-4-Ig	K_D (nM)
CD80-Fc-His*	0.3 nM
CD86-Fc-His*	3.6 nM
ANTICUERPO DE REFERENCIA (IgG1)	0.5 nM
AGEN1884	0.6 nM

*Modified from: Xu et al., JI 2012

Figura 17A

[illegible]

Figura 17B

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SP|P16410|CTLA4_HUMAN  MACLGFRHKAQLNLATRTWPCTLLFFLLFIP--VFC-----KAMHVAQPAVVLA--- 48
TR|G7PL88|G7PL88_MACFA  MACLGFRHKAQLNLATRTRPYTLLESLLFIP--VES-----KAMHVAQPAVVLA--- 48
SP|P10747|CD28_HUMAN    -----MLRLLALN--LEPS-IQVTGNKILVKQSP-MLV--- 30
SP|Q9Y6W8|ICOS_HUMAN    -----MKSGLWY-----FF--LFCLRIKVLTGEINGSANYEMFI--- 32
SP|Q7Z6A9|BTLA_HUMAN    -----MKTLPAMLGKGLFWV---FFLIPYLDIWNHKGESCDVQLYIK 41
SP|Q15116|PDCD1_HUMAN   -----MQIFQAPWPVVWAVLQLGWRPGWELDSFD-RPWNPPTFSPALLVV--- 44
                                *
                                ;

SP|P16410|CTLA4_HUMAN  -----SSRGIASFVCEYASPKA---TEVRVTVLRQADSQVTEVCAATYMMGNE--LT 96
TR|G7PL88|G7PL88_MACFA  -----NSRGIASFVCEYASPKA---TEVRVTVLRQADSQVTEVCAATYMMGNE--LT 96
SP|P10747|CD28_HUMAN    -----AYDNAVNLSCKYSYNLES---REFRASLHKGLDSA-VEVCVVYGNYSQQLQVY 79
SP|Q9Y6W8|ICOS_HUMAN    -----FHNGGVQILCKYFD--IV---QQFKMQLLKGGQ---ILCDLTKTRGSGNTVS 76
SP|Q7Z6A9|BTLA_HUMAN    KQSEHSILAGDPFELECPVKYCANRPHVWCKLN---GT-----TCVKLEDPQ----- 86
SP|Q15116|PDCD1_HUMAN   -----TEGLNATFTCSFSNTSESEFVLNWMSPSNQTDK---LAAFPEDR----- 86
                                ; *
                                ;

SP|P16410|CTLA4_HUMAN  FLDDSICT----GTSSGNQVNLT---QGLRAMDTGLYICKVELMYPPPYLGGIG----- 144
TR|G7PL88|G7PL88_MACFA  FLDDSICT----GTSSGNQVNLT---QGLRAMDTGLYICKVELMYPPPYLGGIG----- 144
SP|P10747|CD28_HUMAN    SKTGFNCD----GKLGNESVTFFL---ONLYVNQTDIYFCKIEVMYPPPYLDNEK----- 127
SP|Q9Y6W8|ICOS_HUMAN    IKSLKFCH----SQLSNNSVSFFL---YNLDHSHANYFYCNLSIFDPPPFKVTILT----- 124
SP|Q7Z6A9|BTLA_HUMAN    -----TSWKEEKNISFFILHFEPVLENDNGSYRCSANFQSNL---IE----- 125
SP|Q15116|PDCD1_HUMAN   SQPGQDCRFRTQLPGRDFHMSV---VRARENDSGTYLCGAISLAPKAQIKESLRAELR 143
                                ... : :
                                . * *

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Figura 17C

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SP|P16410|CTLA4_HUMAN -NGTQIYVIDP-----EPCFDS-----DFLLWILAAVSSGLFFYSFLLTAVSL--S-KML 190
TR|G7PL88|G7PL88_MACFA -NGTQIYVIDP-----EPCFDS-----DFLLWILAAVSSGLFFYSFLLTAVSL--S-KML 190
SP|P10747|CD28_HUMAN SNGTIIHVKGK-----HLCFSPLPFGPSKPFVVLVVVGGVLACYSLLVTVAFI--IFWVR 180
SP|Q9Y6W8|ICOS_HUMAN --GGYLHIYES-----QLCCQL-----KFWLPIGCAA---FVVVCILGCIL--ICWLT 165
SP|Q7Z6A9|BTLA_HUMAN SHSTTLVYTDVKSASERESKDEMASP-FWLLYKLLPLGG----LPLLITTCFCLFCCLR 180
SP|Q15116|PDCD1_HUMAN VTERRAEVPTA-----HPSPSRPAG-QFQTLVVGVGGLGSLVLLVWVLAV--ICSRA 195
          :      . . .      ..      . :

SP|P16410|CTLA4_HUMAN KKRSP---LTTGVIVKMPPE-----PEC-EKQFQPYFIPIN----- 223
TR|G7PL88|G7PL88_MACFA KKRSP---LTTGVIVKMPPE-----PEC-EKQFQPYFIPIN----- 223
SP|P10747|CD28_HUMAN SKRSE---LLHSDYMMMTERR-----PGETRKHYQPYAPPED--FAAYRS 220
SP|Q9Y6W8|ICOS_HUMAN KKKYSSSVHDFNGEYMFMRVAVN-----TAKKSRLTDVTL----- 199
SP|Q7Z6A9|BTLA_HUMAN HQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYENDPDLCFPMQEGS 240
SP|Q15116|PDCD1_HUMAN ARG-----TIG-----ARR-----TGQP-LKEDPSAVFVF--SVDYGEIDFQWREKT 234
          :      .      .      .

SP|P16410|CTLA4_HUMAN -----
TR|G7PL88|G7PL88_MACFA -----
SP|P10747|CD28_HUMAN -----
SP|Q9Y6W8|ICOS_HUMAN -----
SP|Q7Z6A9|BTLA_HUMAN EVYSNPCLEENKP--GIVYASLNHSVIGPNSRLARIVKEAFTEYASI-----CVRS- 289
SP|Q15116|PDCD1_HUMAN PEPPVPCVPEQTEYATIVFF---SGMGTSSPAERGSADGFRSAQPLRPEDGHCWPL 288

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Figura 17D

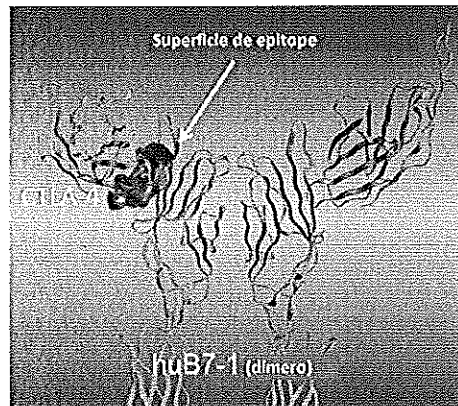


Figura 17E

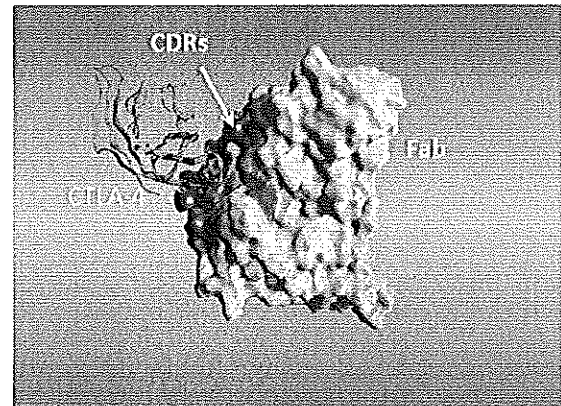


Figura 18A

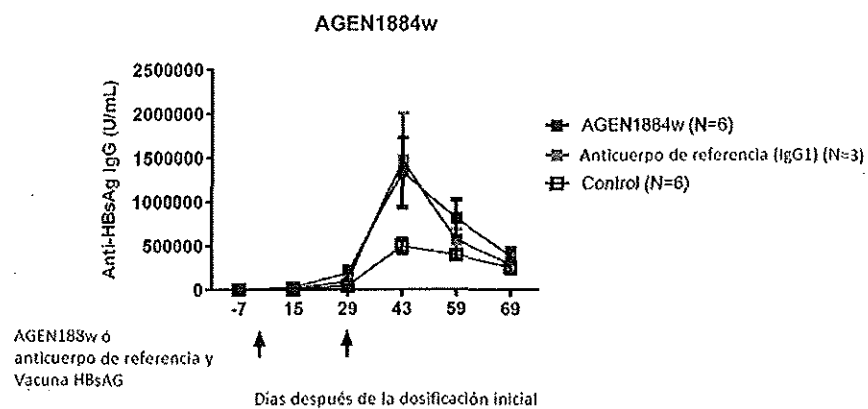


Figura 18B

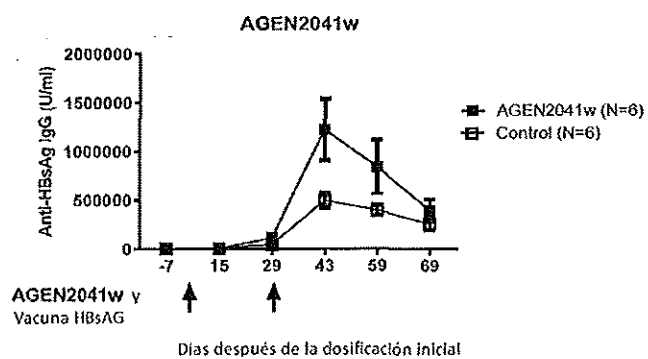


Figura 19A

		CDRH1										CDRH2																					
		10		20		30		40		50		60																					
		-----+-----+-----+-----+-----+-----																															
BADD411-2354	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	SYSMNTVRQAPGKGLEWVS										SISSSSSYIYY										60											
BADD412-2356	...L...	Q...										A.S...										V...										60	
BADD412-2357		Q.....																														60	
BADD412-2358	...L...																															60	
BADD412-2359	...L...	Q...										A.S...										V...										60	
BADD412-2360		Q...										T...																				60	

		CDRH3													
		70		80		90		100		110					
		-----+-----+-----+-----+-----													
BADD411-2354	ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAE	VGLMGFFDIWGQ										GTMTVSS		118	
BADD412-2356														118	
BADD412-2357		T.....												118	
BADD412-2358														118	
BADD412-2359												N.....		118	
BADD412-2360														118	

Figura 19B

		CDRL1				CDRL2					
		10	20	30	40	50	60				
		-----+-----+-----+-----+-----+-----+-----+-----+									
BADD412-2376	EIVLTQSPGTLSLSPGERATLSC	RASQSVSKYLGH	YQQKFGQAPRLLIY	GASTRATG	IPD			60			
BADD412-2367	A.....	GT..A...	..H.V.....	...R.....				60			
BADD412-2382	A.....	S..A...	S.....	AT.S.....				60			
BADD412-2384	A...F.....	S..A...	S.....	...S.....				60			
BADD412-2390	A...V.....	S..A...	A.....	...A.....				60			
BADD412-2393	A.....	S..A...	N.....	...A.....				60			

		CDRL3					
		70	80	90	100		
		-----+-----+-----+-----+					
BADD412-2376	RFSGSGSGTDFTLTITRLEPEDEFAVYYC	QYGSSEFWTE	FGQGTKVEIK			107	
BADD412-2367	S.....					107	
BADD412-2382	V.....S.....	T.....				107	
BADD412-2384	F..S.....	F..P...D..				107	
BADD412-2390	A.....S.....					107	
BADD412-2393	SS.....	F..P...D..				107	

Figura 20A

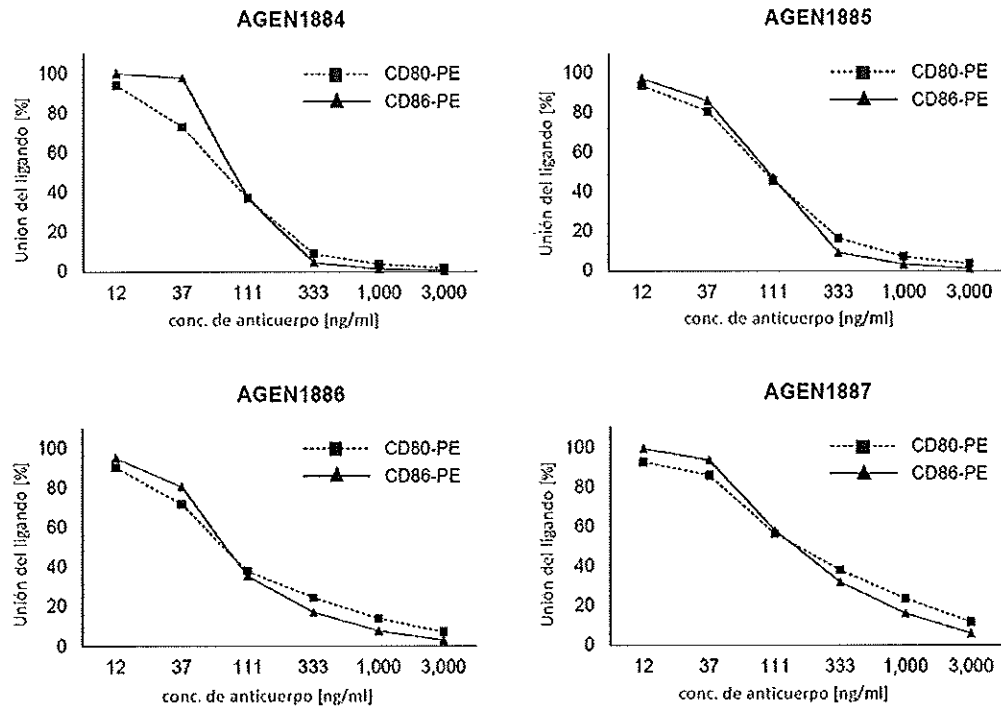


Figura 20B

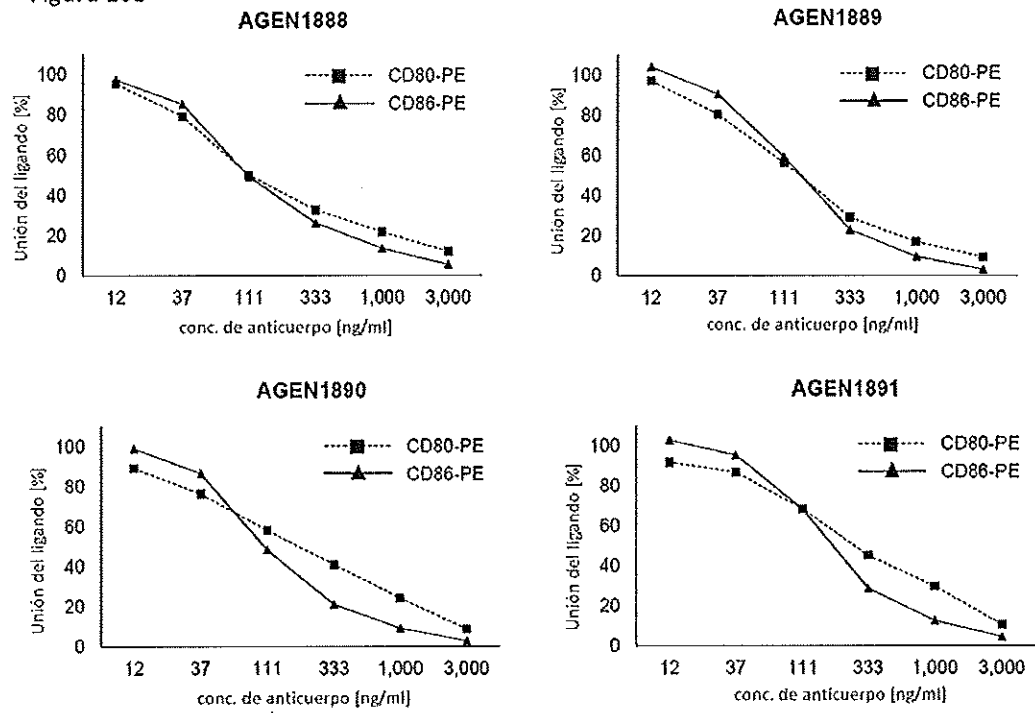


Figura 20C

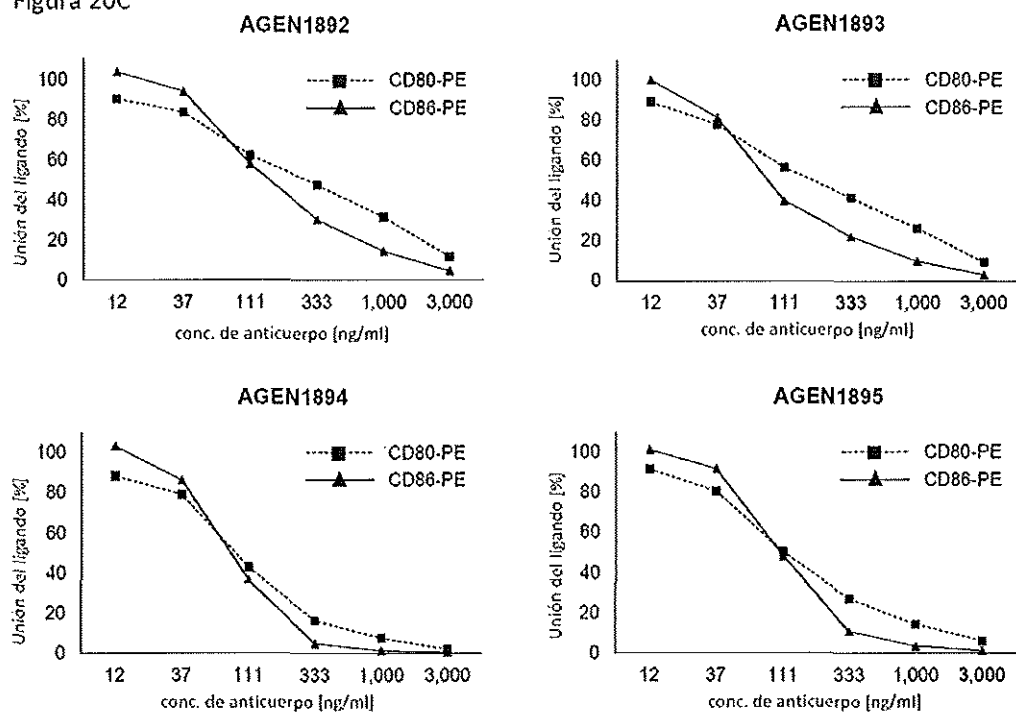


Figura 20D

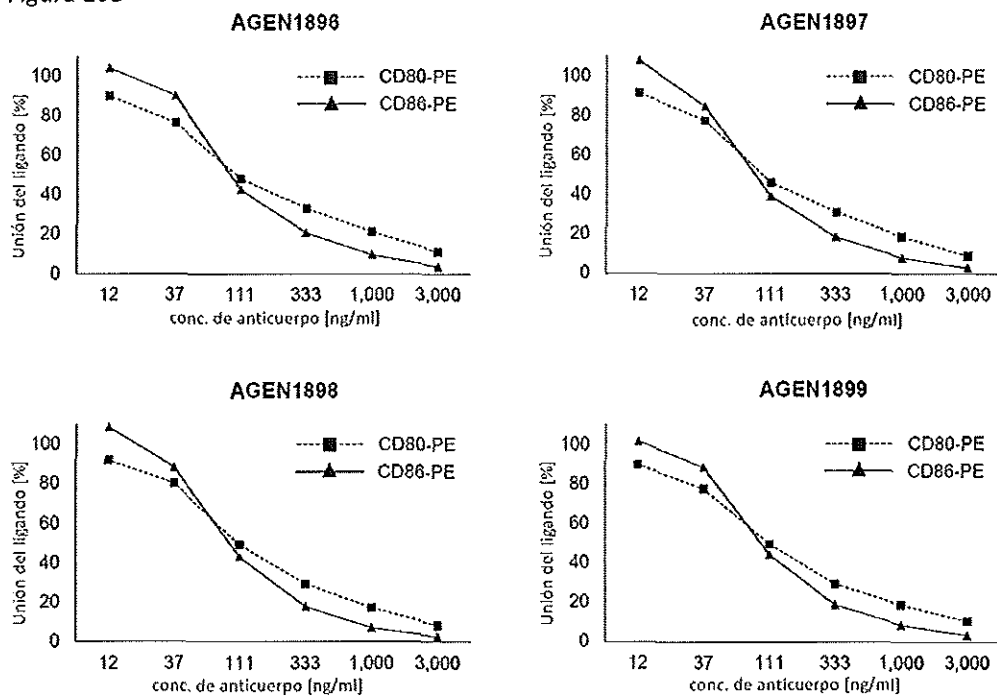


Figura 20E

