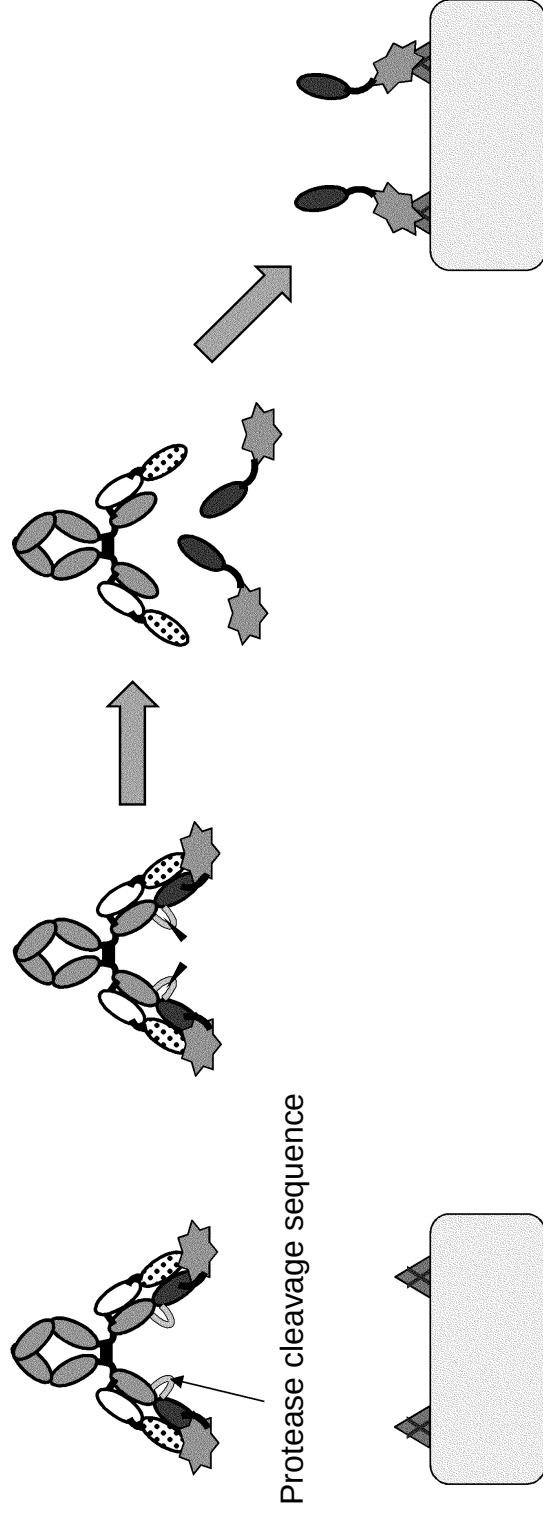


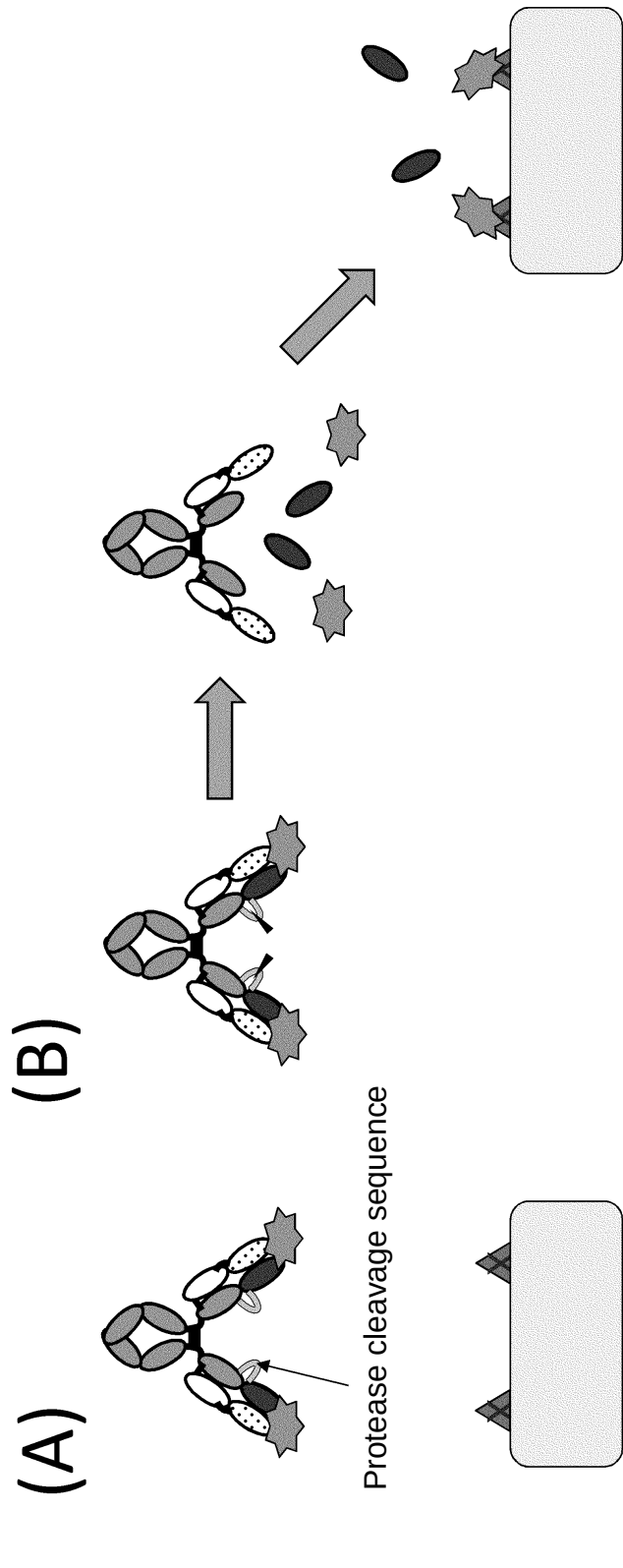
(A) (B)



Insert protease cleavage sequence into a molecule of ligand and anti-ligand antibody that fused via linker

(A molecule of Ligand-linker-anti-ligand antibody VH) is released by protease cleavage, and the released (molecule of ligand-linker-anti-ligand antibody VH) binds to receptor

FIG. 1



Insert protease cleavage sequence
into an anti-ligand antibody

Ligand and VH molecule are released from ligand-anti-
ligand antibody complex by protease cleavage, and the
released ligand binds to receptor

FIG. 2

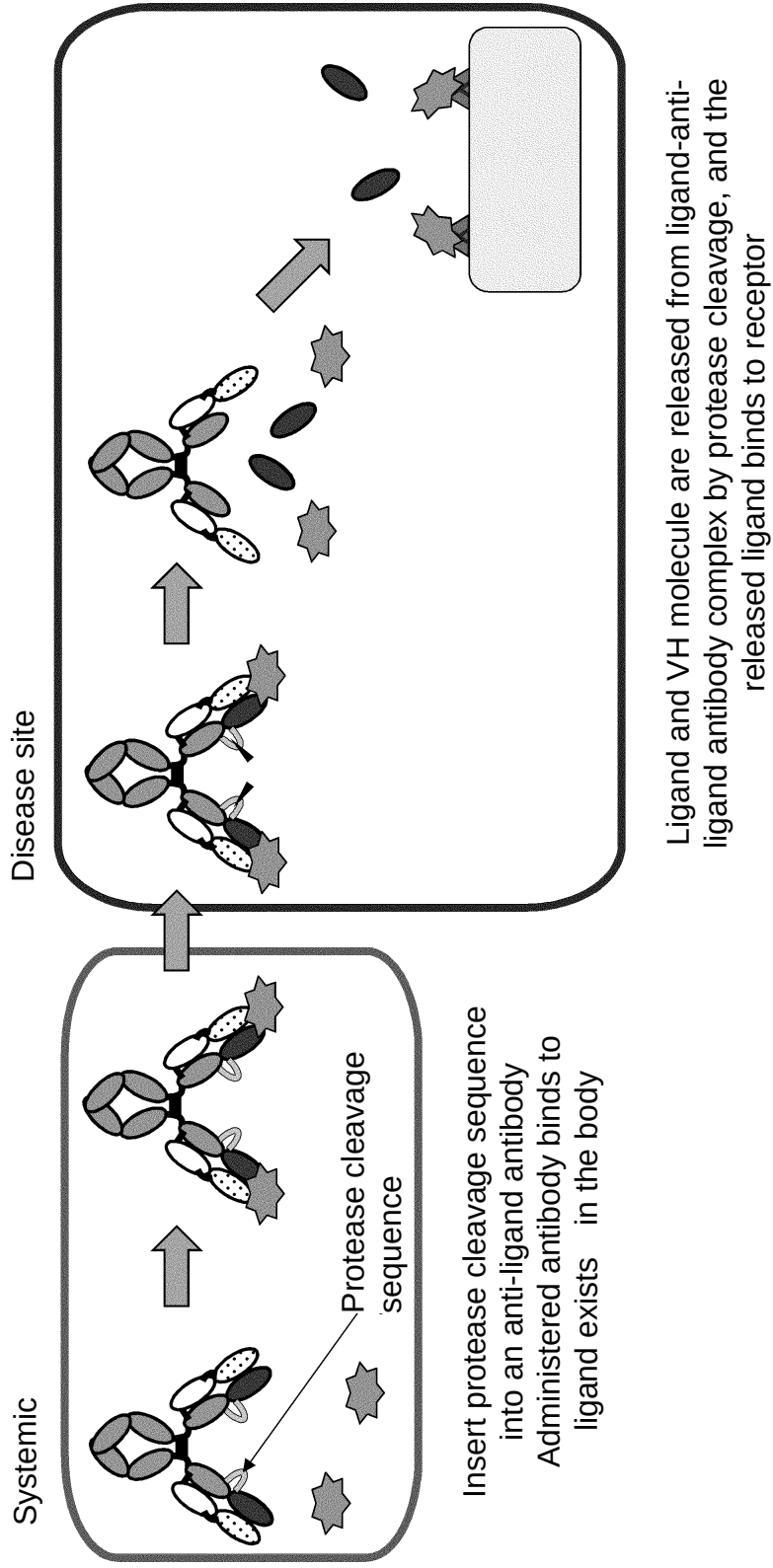


FIG. 3

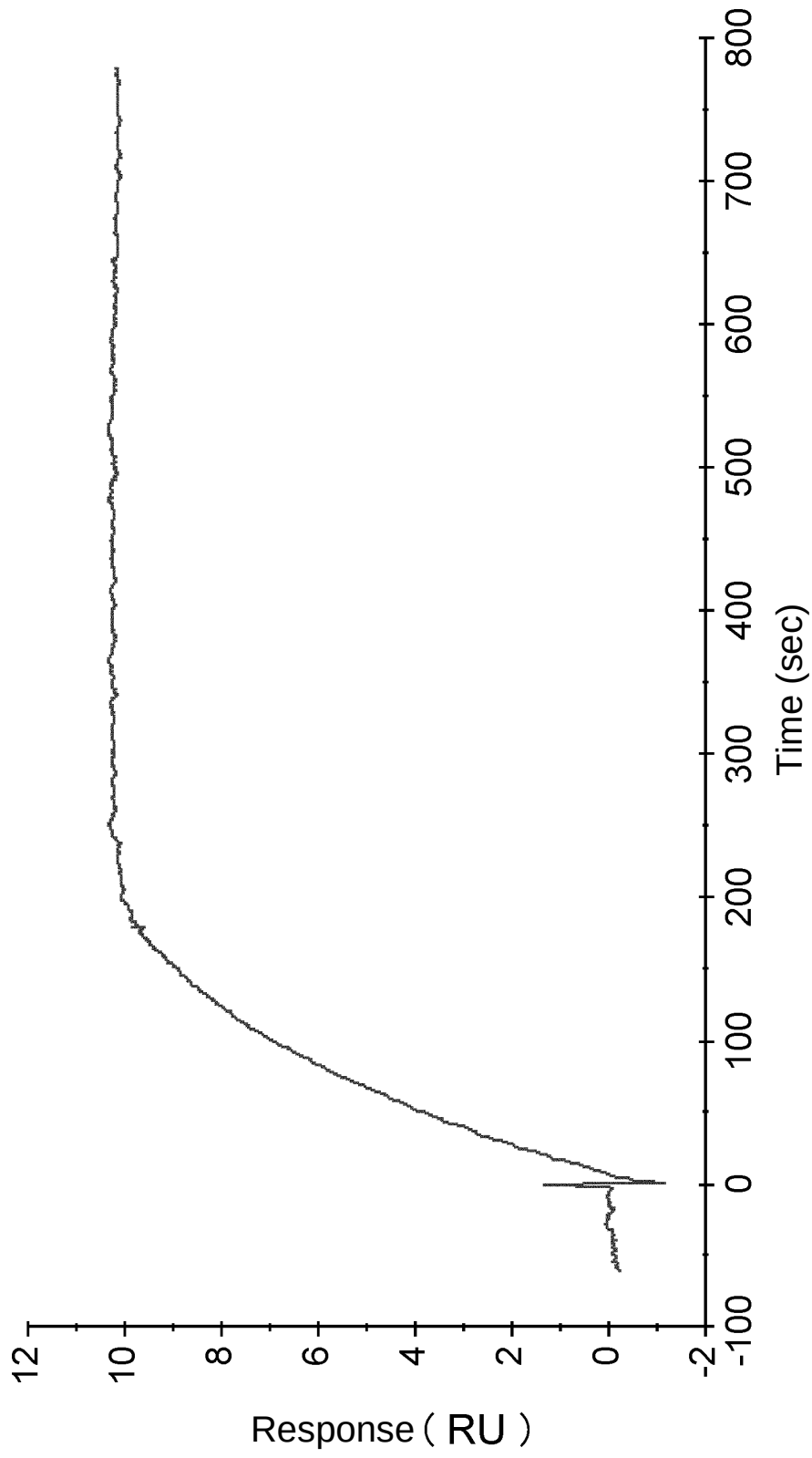
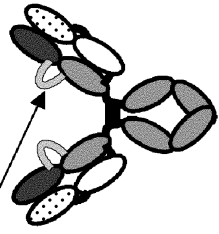


FIG. 4

(A)

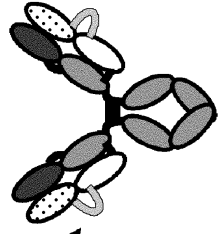
Protease cleavage sequence

Heavy chain



Protease cleavage sequence

Light chain



Insertion position name: CDAEBFG
Sequence: TVSSASTKG

CDAEBFG
VDIKRTVAA

Protease cleavage sequence was inserted at each position from insertion position names A to G in variable and constant regions

FIG. 5A

(B) Heavy chain

Heavy chain variant name	Protease cleavage sequence insertion position	Inserted sequence
EEIVHA	TVSS [insert] ASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHB	TVSSAS [insert] TKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHC	TV [insert] SSASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHD	TVS [insert] SASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHE	TVSSA [insert] STKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHF	TVSSAST [insert] KGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHG	TVSSASTK [insert] GP	LSGRSDNH (SEQ ID NO: 3)
EEIVHBG	TVSSAS [insert] GKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHCG	TV [insert] GSASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHDG	TVS [insert] GASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHEG	TVSSA [insert] GTKGP	LSGRSDNH (SEQ ID NO: 3)

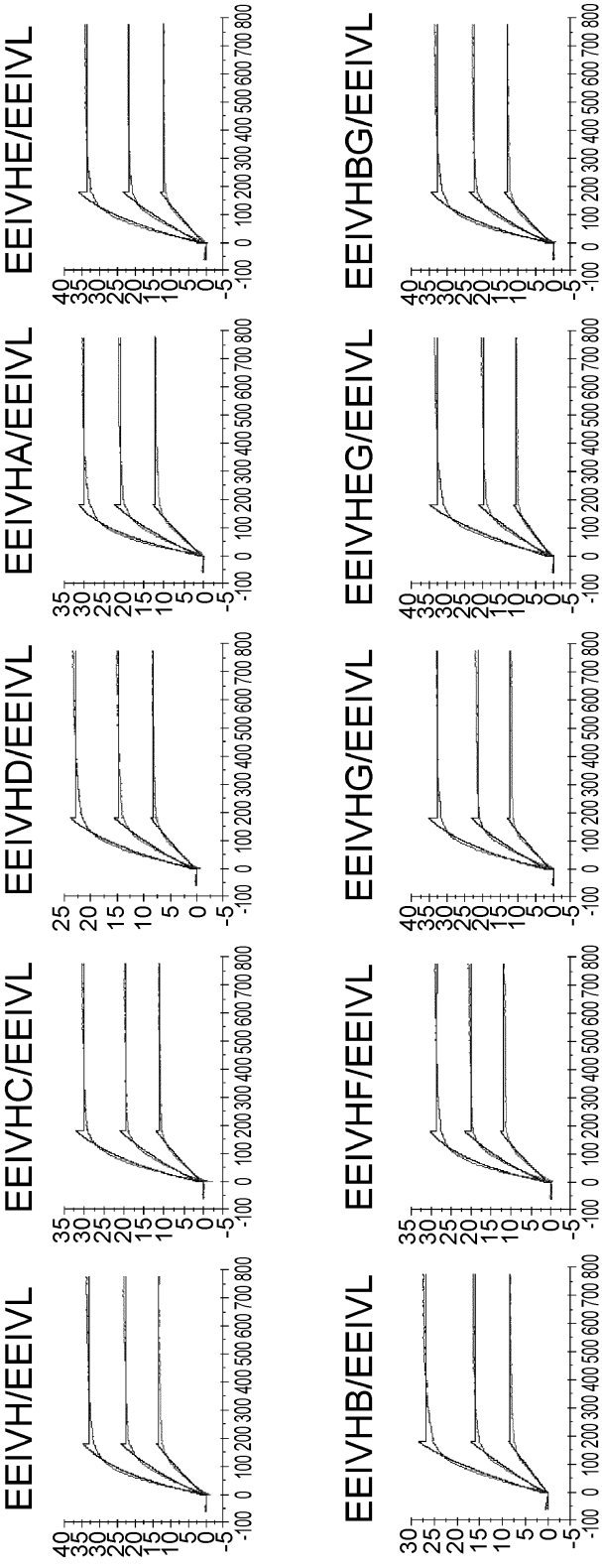
FIG. 5B

(C) Light chain

Light chain variant name	Protease cleavage sequence	insertion position	Inserted sequence
EEIVLA	VDIK [insert]	RTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLB	VDIKRT [insert]	VAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLC	VD [insert]	IKRTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLD	VDI [insert]	KRTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLE	VDIKR [insert]	TVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLF	VDIKRTV [insert]	AAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLG	VDIKRTVA [insert]	AP	LSGRSDNH (SEQ ID NO: 3)
EEIVLEG	VDIKR [insert]	GVAAP	LSGRSDNH (SEQ ID NO: 3)

FIG. 5C

(A)



Ordinate: Response (RU)
Abscissa: Time (sec)

FIG. 6A

(B)

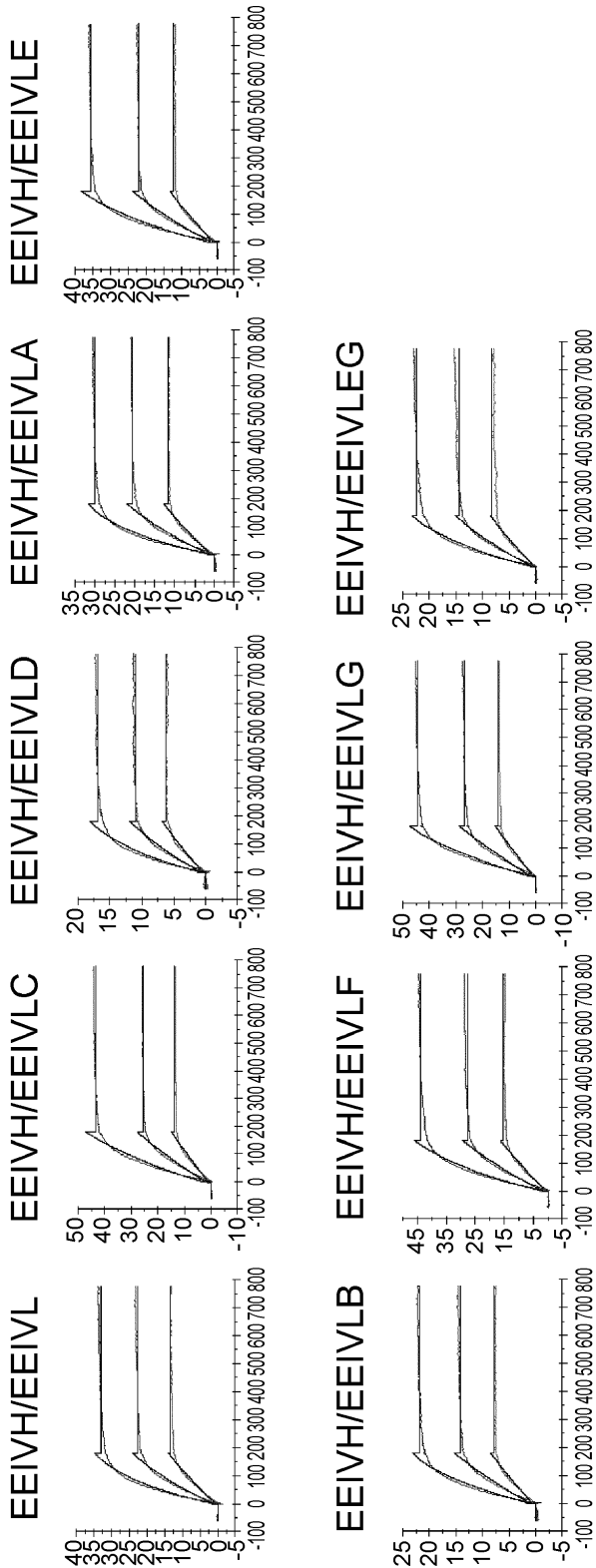


FIG. 6B

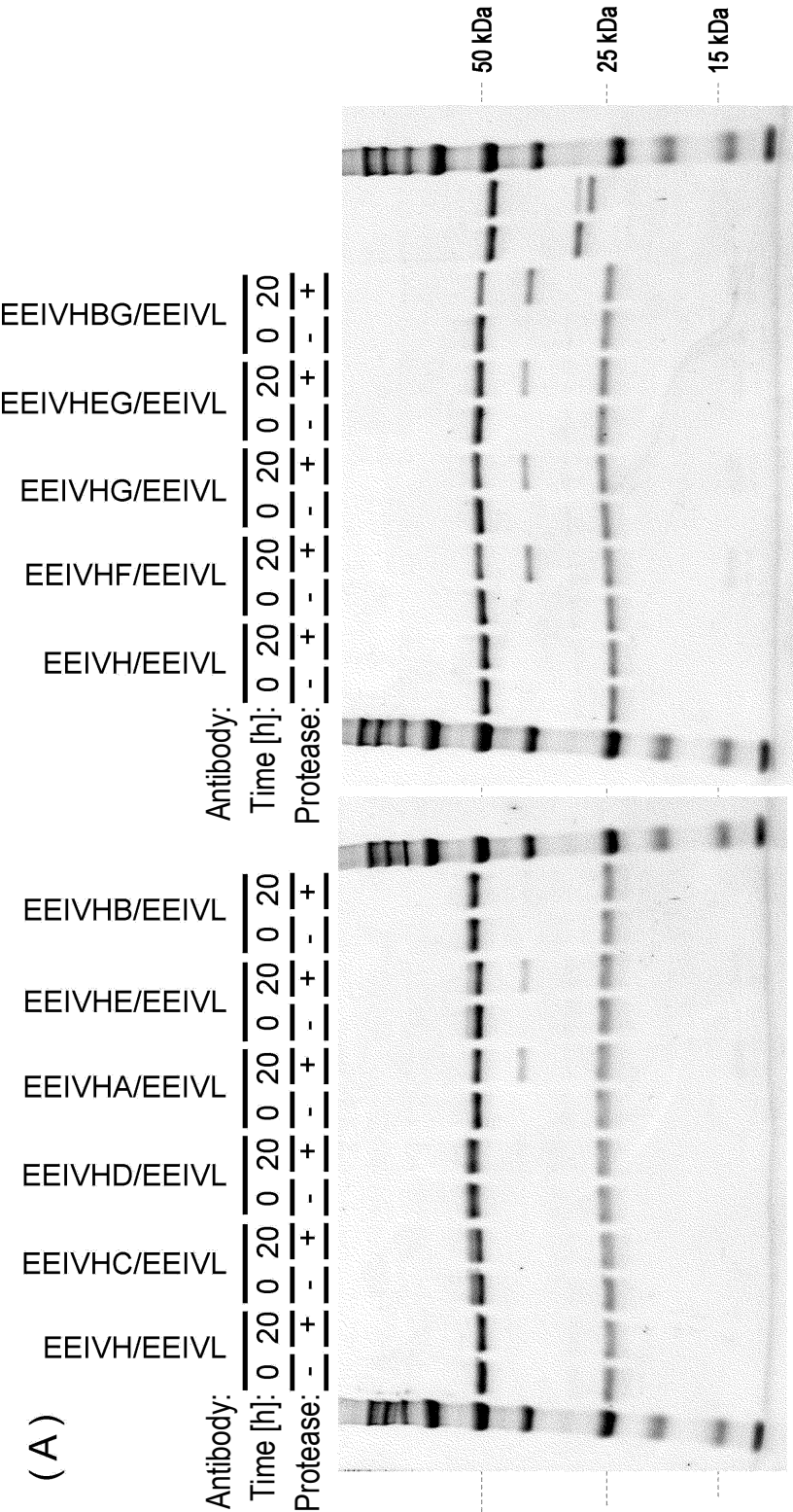


FIG. 7-1

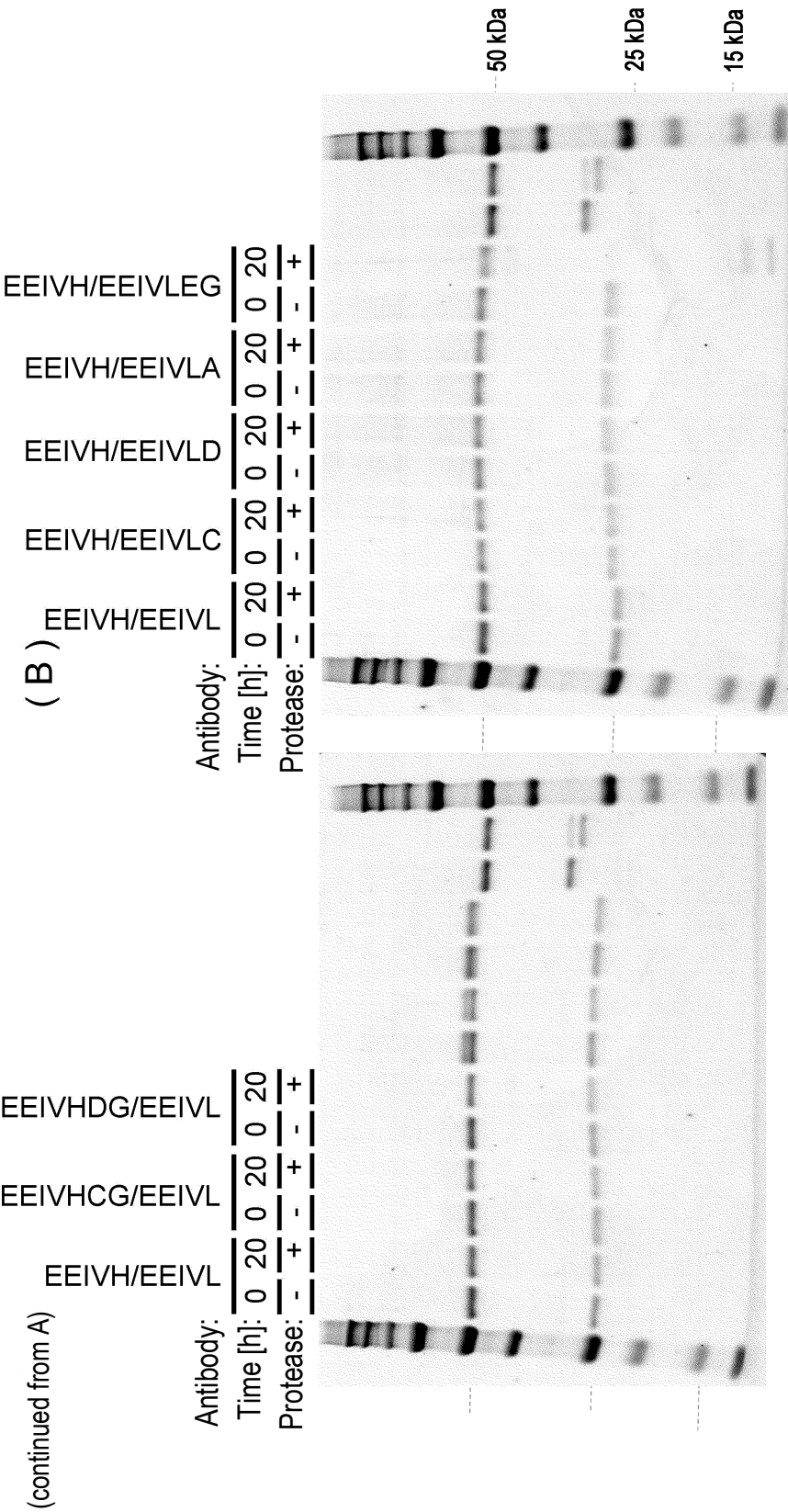


FIG. 7-2

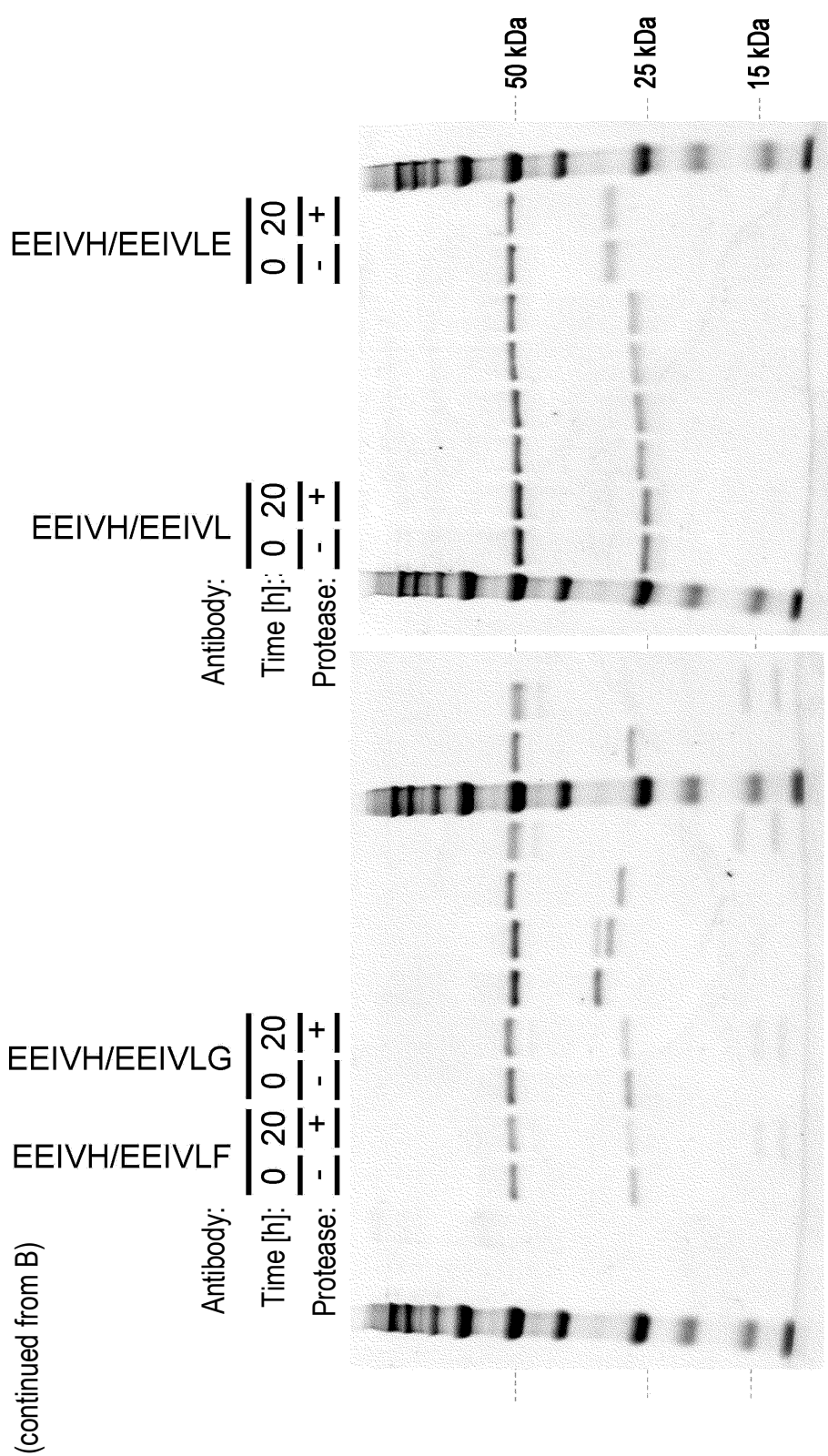


FIG. 7-3

Heavy chain variant name	Protease cleavage sequence	insertion position	Inserted sequence
EEIVHC	TV [insert]	SSASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHC002	TV [insert]	SSASTKGP	GLSGRSDNHG (SEQ ID NO: 28)
EEIVHC003	TV [insert]	SSASTKGP	SGLSGRSDNHGS (SEQ ID NO: 29)
EEIVHC004	TV [insert]	SSASTKGP	GSGLSGRSDNHGSS (SEQ ID NO: 30)
EEIVHC005	TV [insert]	SSASTKGP	GGSLSGRSDNHGSSG (SEQ ID NO: 31)
EEIVHC006	TV [insert]	SSASTKGP	SGGSLSGRSDNHGSSGT (SEQ ID NO: 32)

FIG. 8

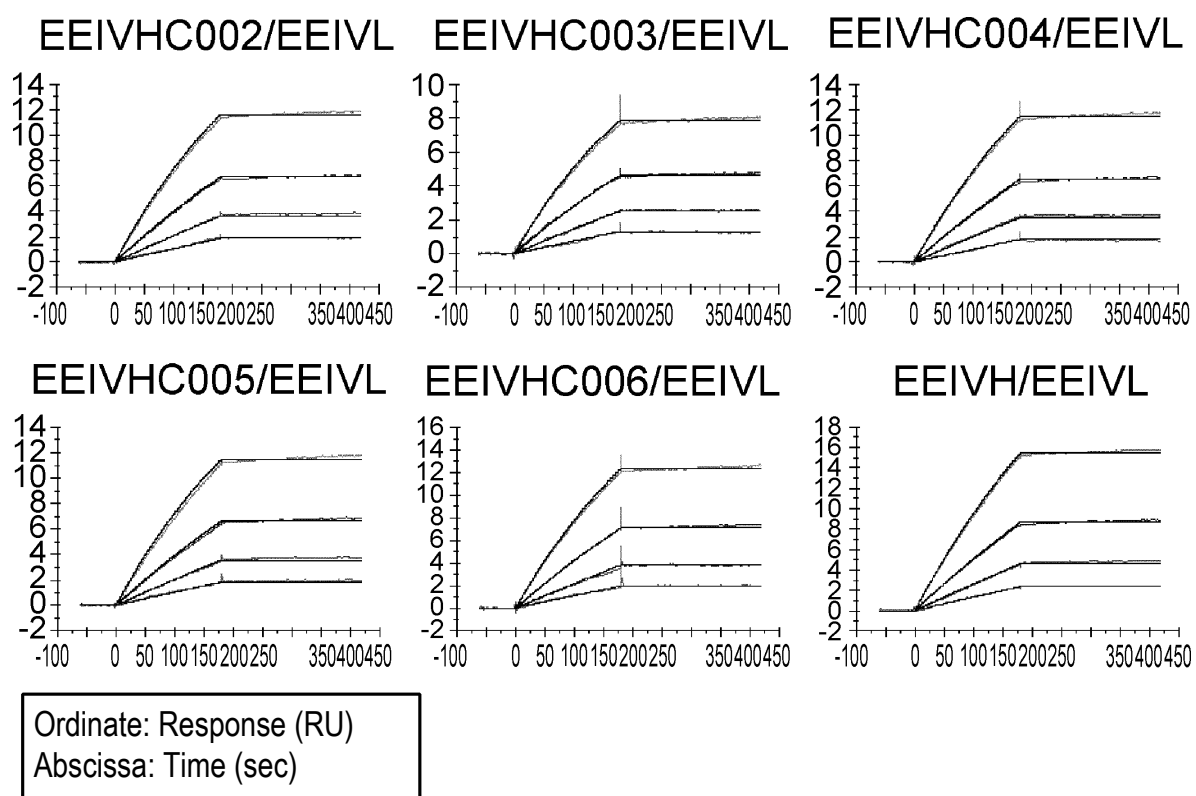


FIG. 9

(A)

Antibody:	EEIVHC/EEIVL	Antibody:	EEIVHC002/EEIVL	Antibody:	EEIVHC003/EEIVL
Protease:	- uPA - MT-SP1	Protease:	- uPA - MT-SP1	Protease:	- uPA - MT-SP1
Time [h]:	0 2 20 0 2 20	Time [h]:	0 2 20 0 2 20	Time [h]:	0 2 20 0 2 20

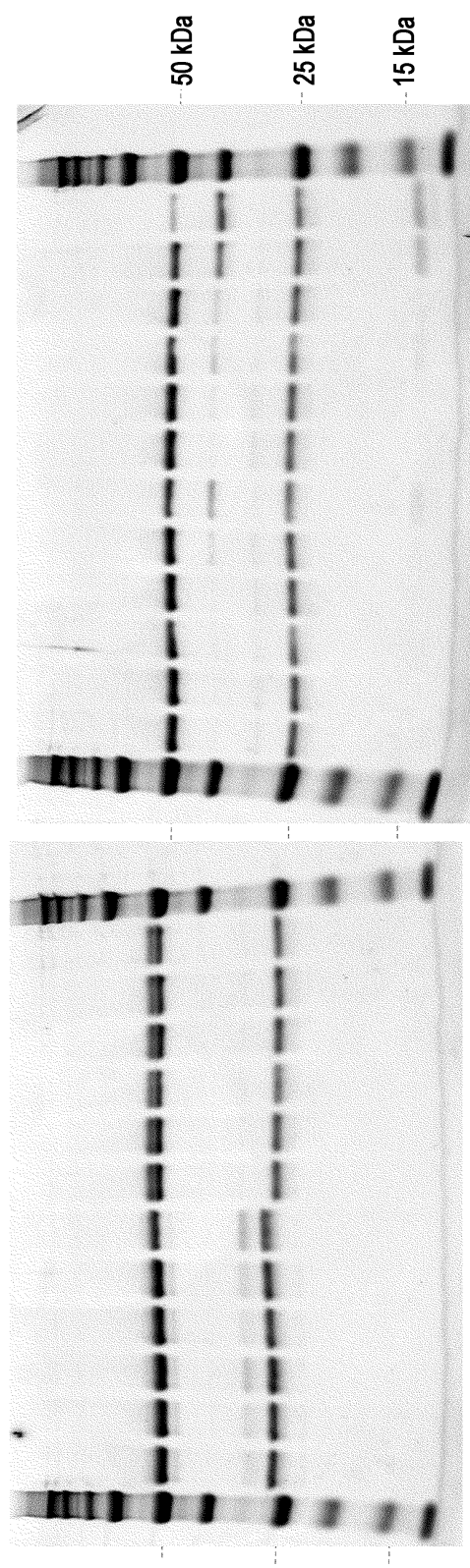


FIG. 10A

(B)

Antibody:	EEIVHC004/EEIVL				EEIVHC005/EEIVL				Antibody: EEIVHC006/EEIVL				EEIVH/EEIVL					
Protease:	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1		
Time [h]:	0	2	20	0	2	20	0	2	20	0	2	20	0	2	20	0	2	20

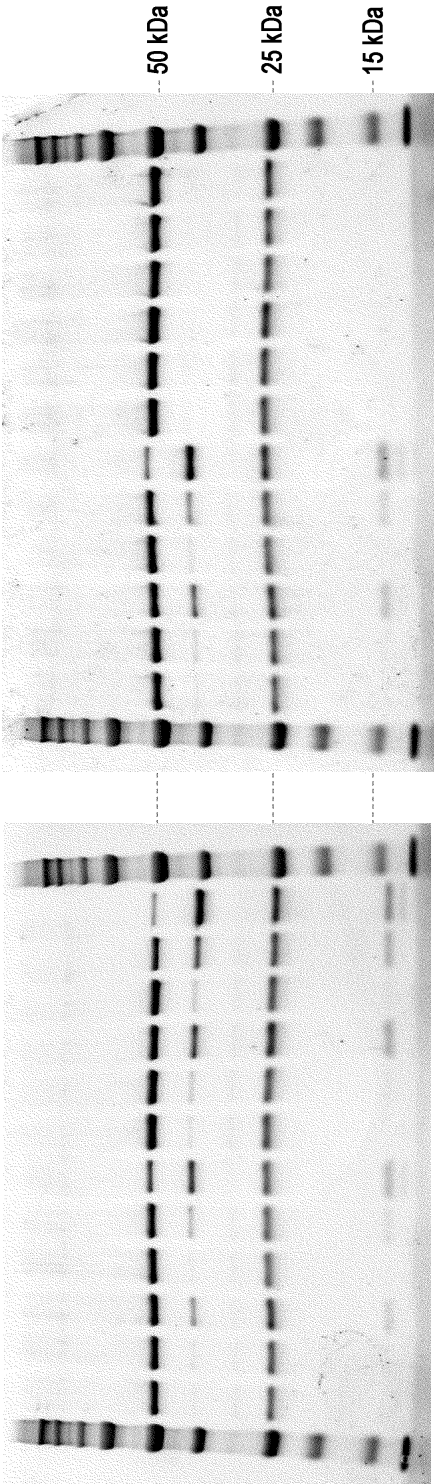


FIG. 10B

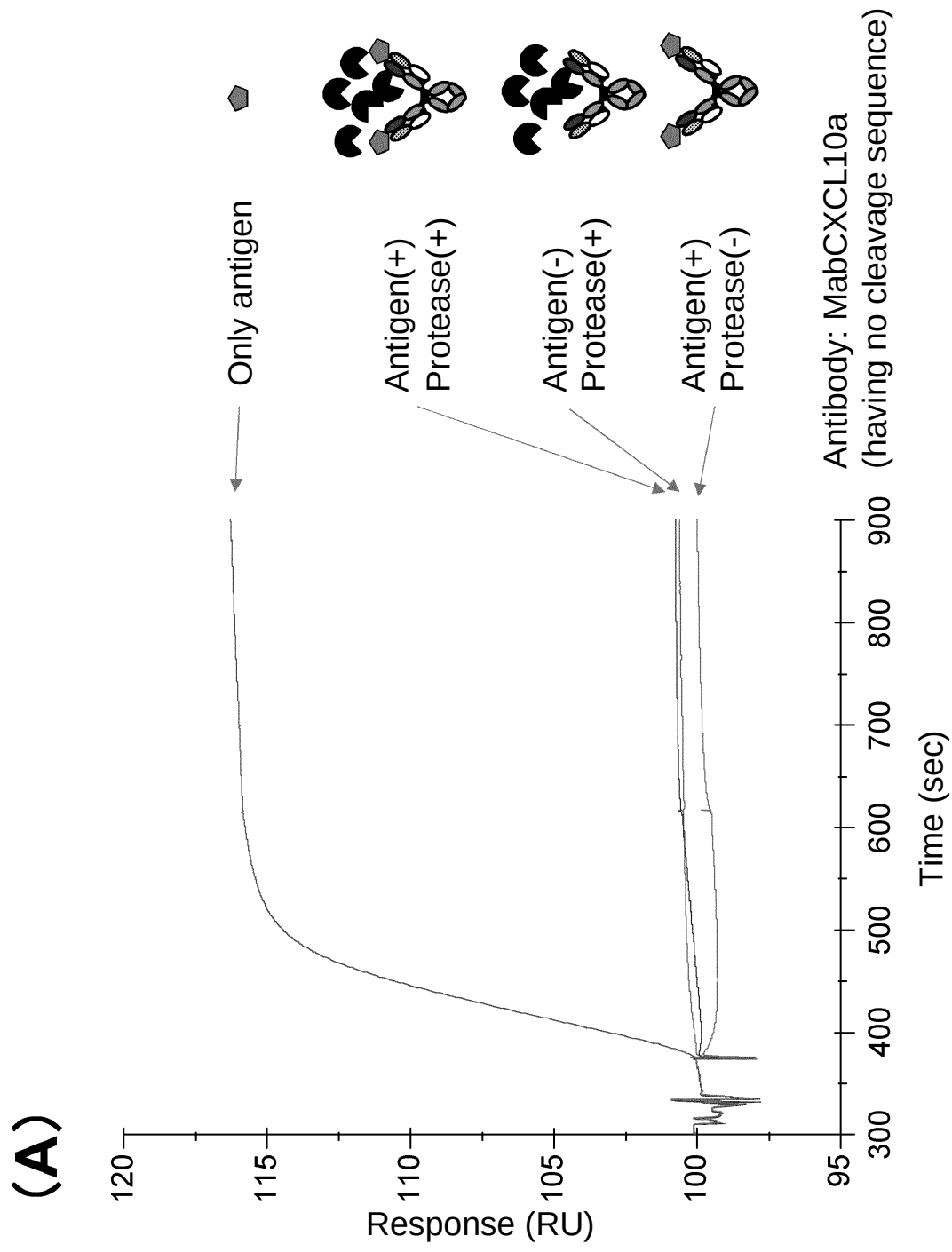


FIG. 11A

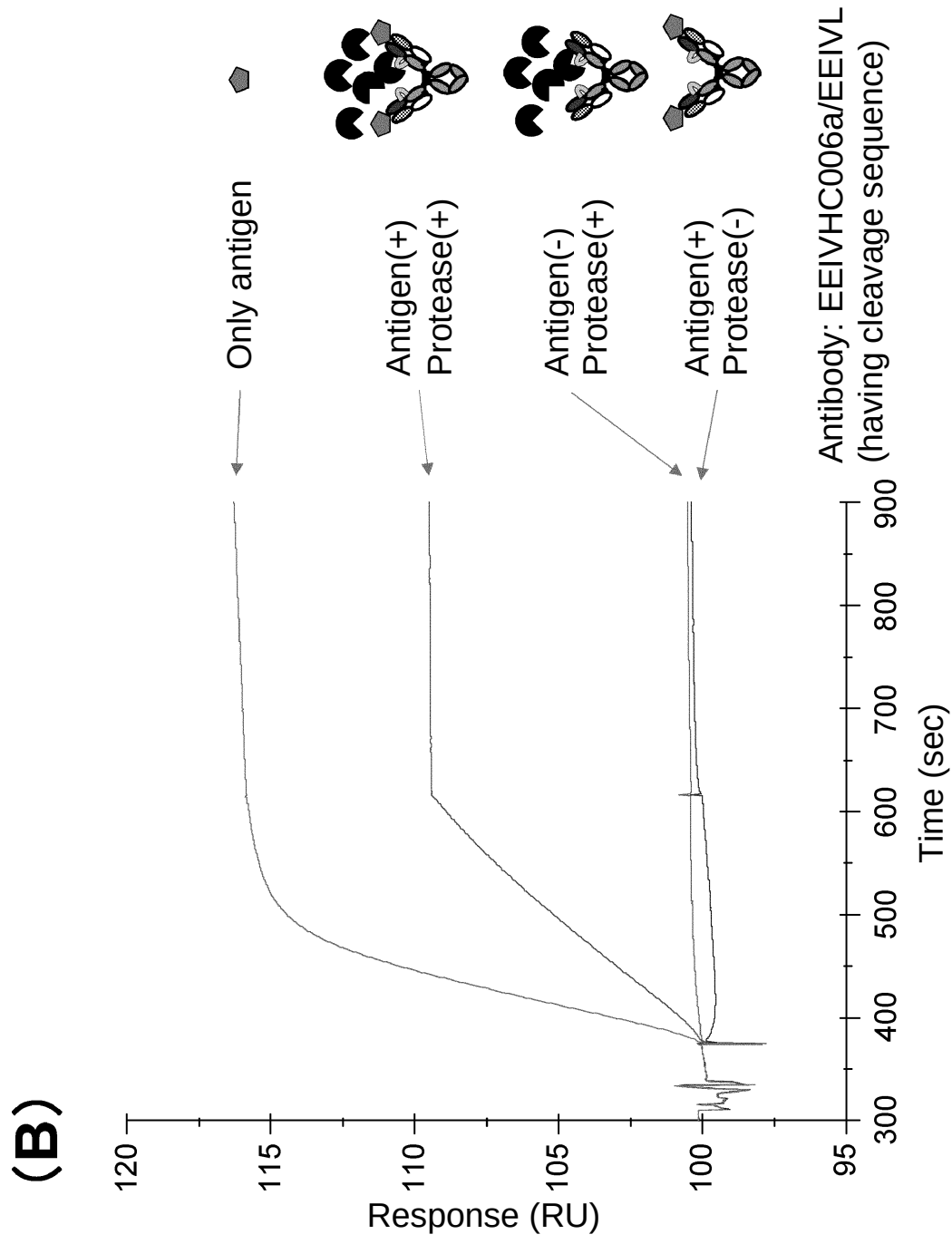


FIG. 11B

Heavy chain variant name	Insertion position and alteration position	Inserted sequence	Sequence after insertion and alteration
EESVHA009	TVSS [insert] A-STKGP	GLSGRSDNHGS (SEQ ID NO: 61)	TVSSGLSGRSDNHGS STKGP (SEQ ID NO: 62)
EESVHA012	TVSS [insert] A-STKGP	GGSLSGRSDNHGSSGT (SEQ ID NO: 63)	TVSSGGSLSGRSDNHGSS GTSTKGP (SEQ ID NO: 64)

FIG. 12

	EESVHA009/EEIVL			EESVHA012/EEIVL		
Antibody:						
Time [h]:	0	20	0	0	20	0
Protease:	-	U	M	-	U	M

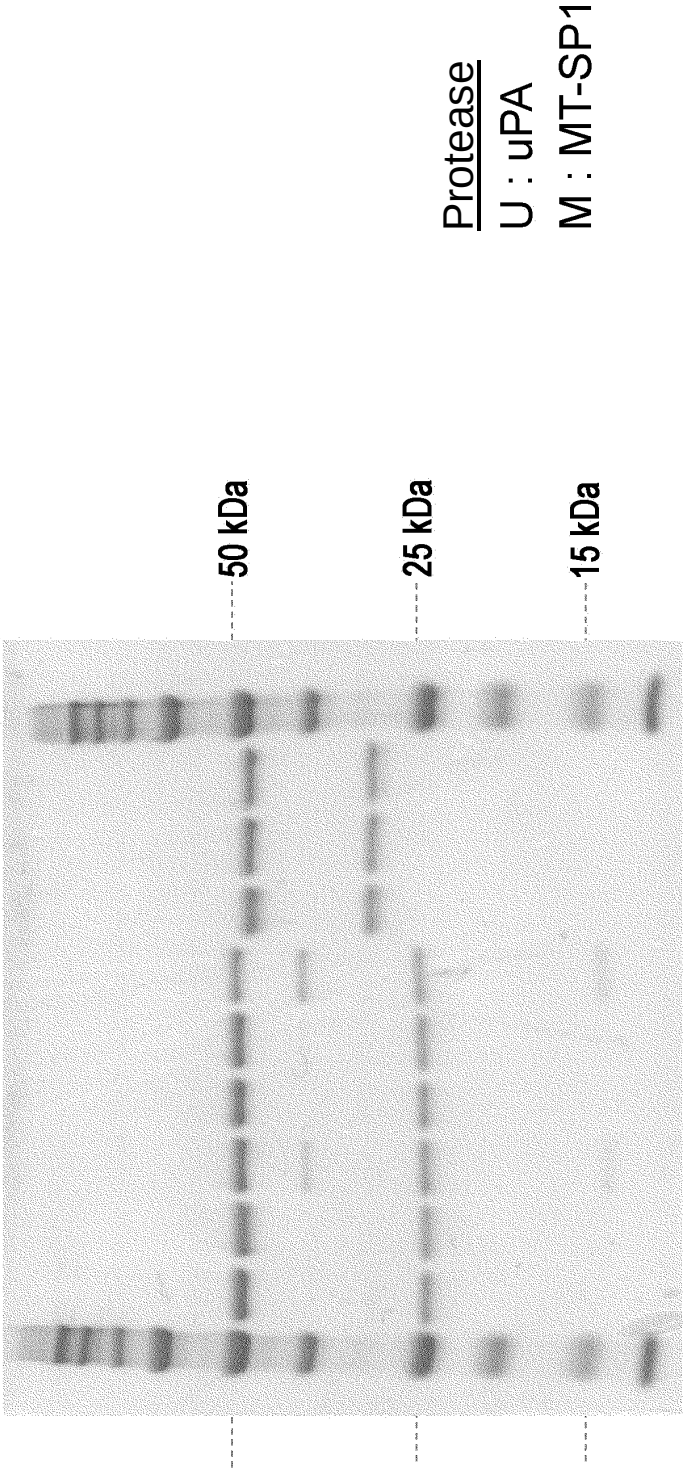


FIG. 13

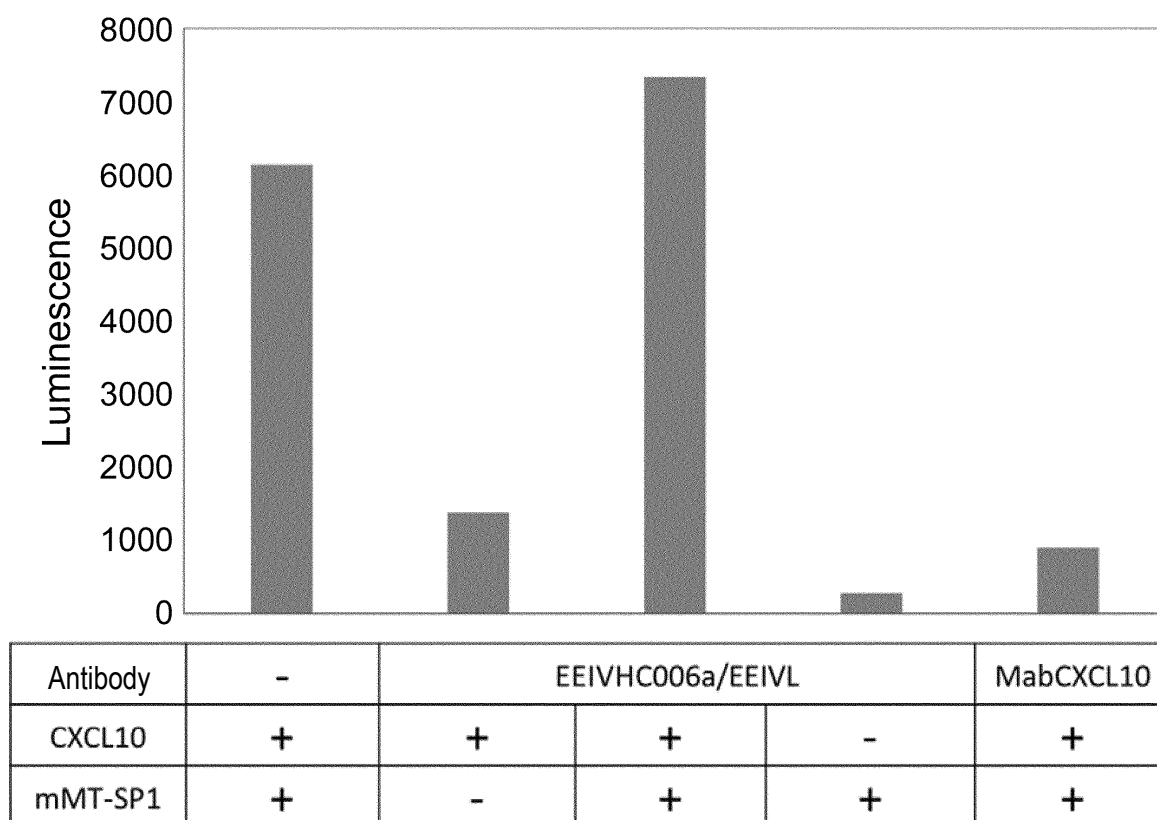


FIG. 14

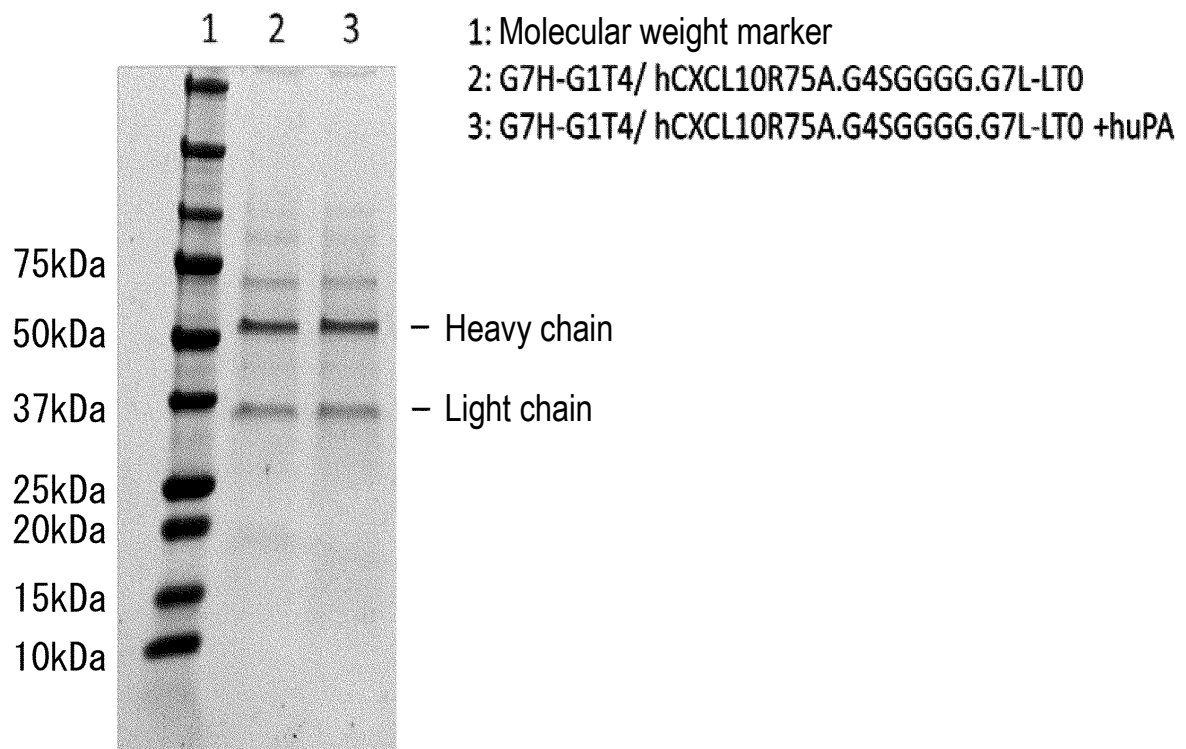


FIG. 15

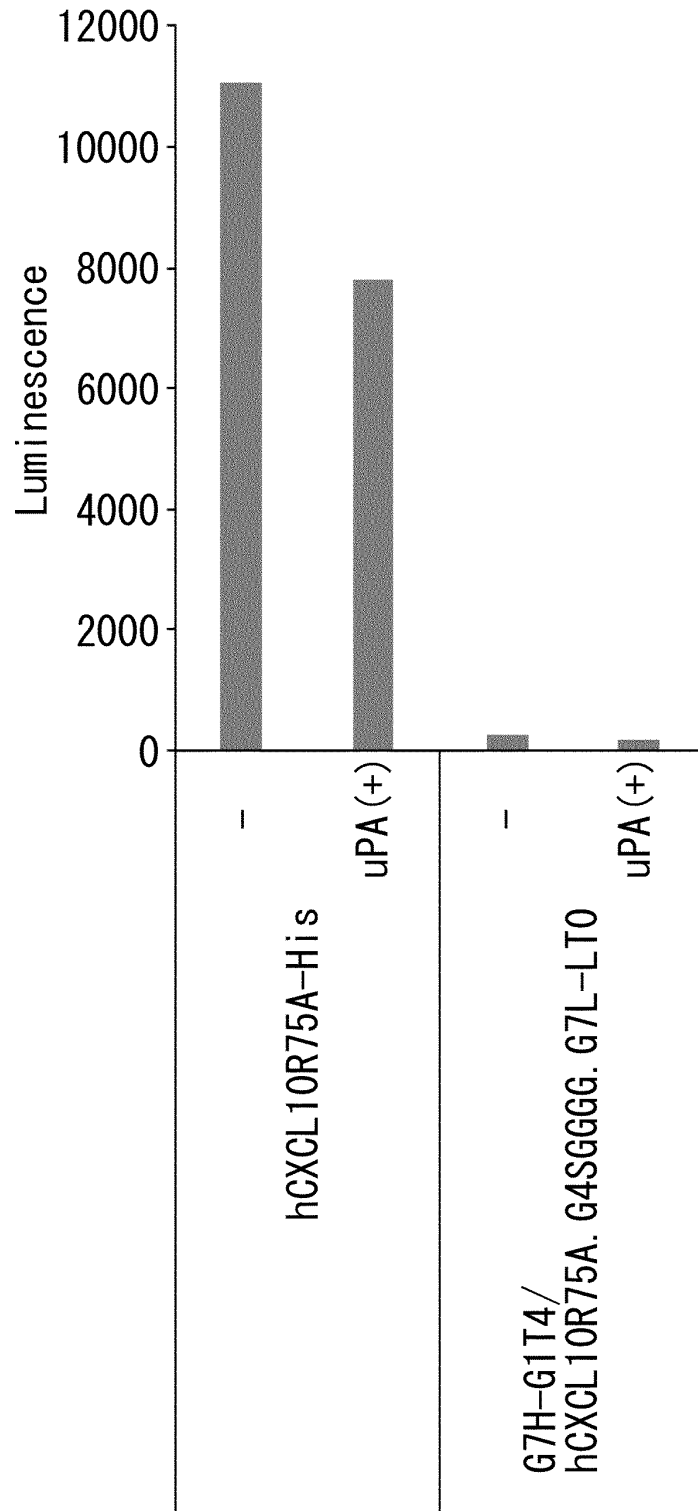


FIG. 16

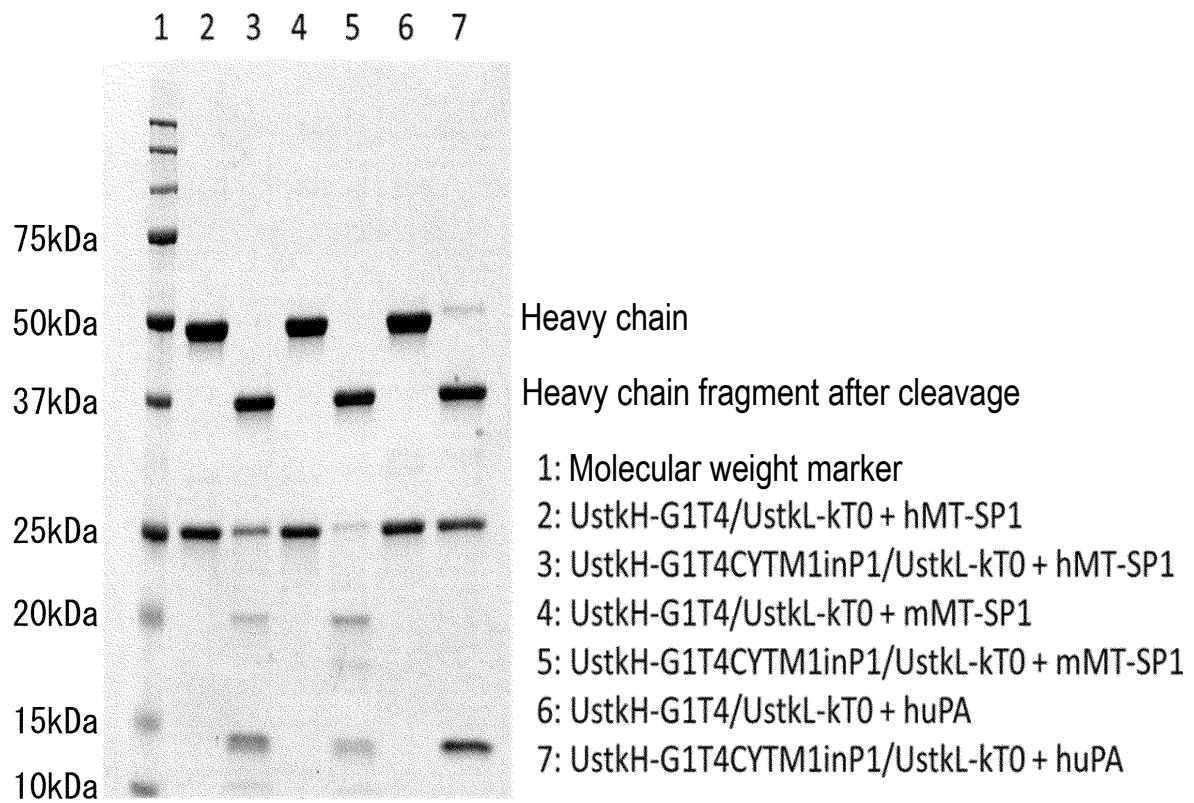


FIG. 17

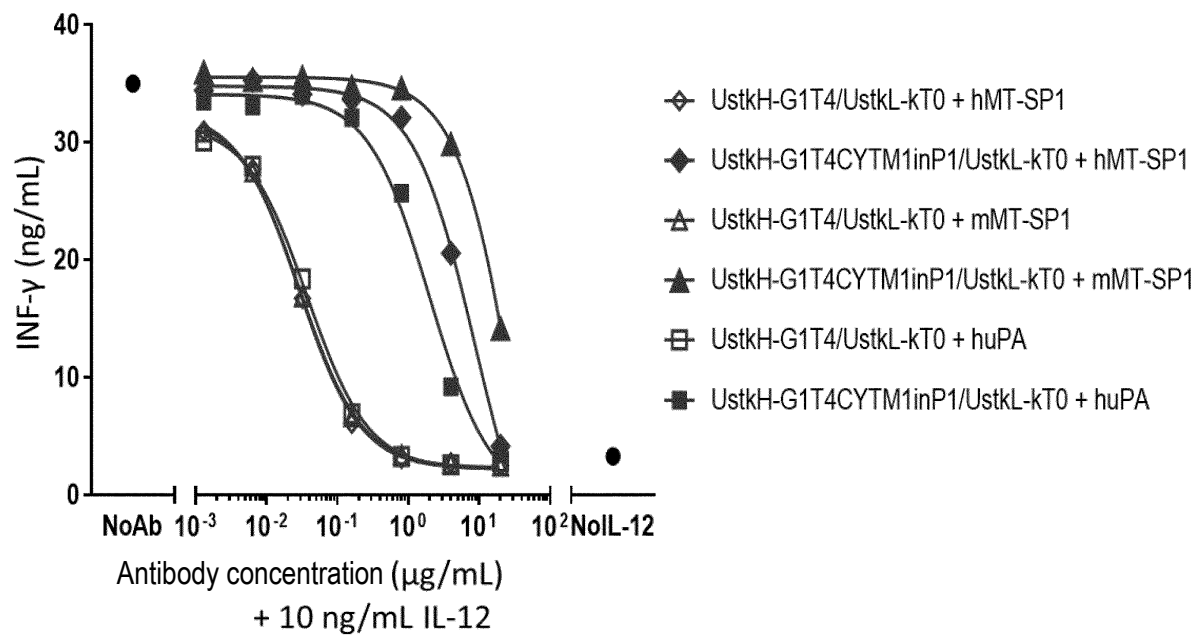


FIG. 18

Antibody EldHA0003

Protease MT-SP1

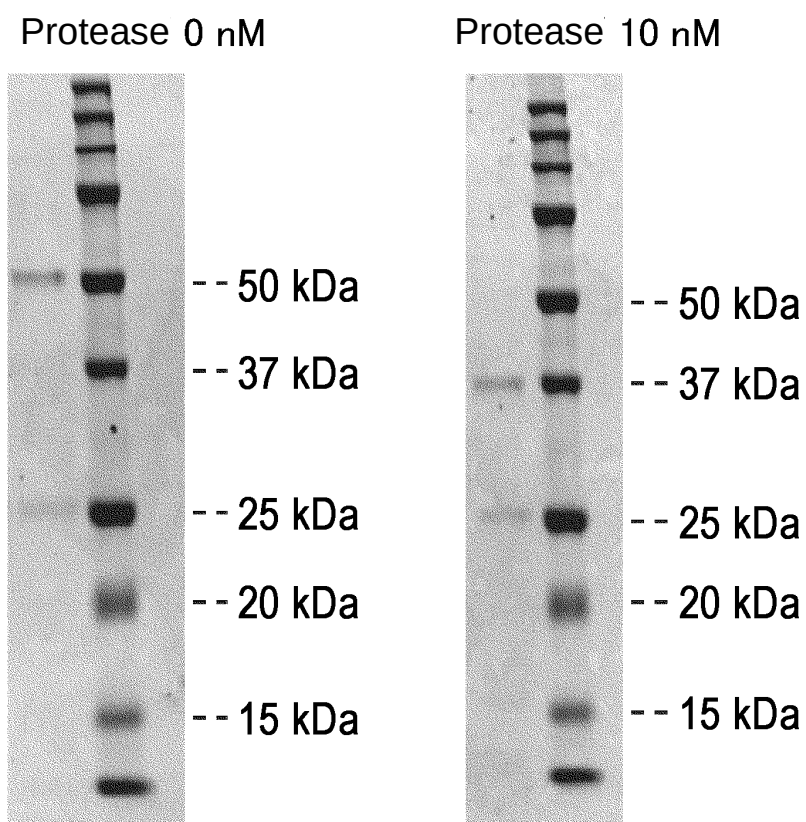


FIG. 19A

Antibody G7H.12aa

Protease MT-SP1

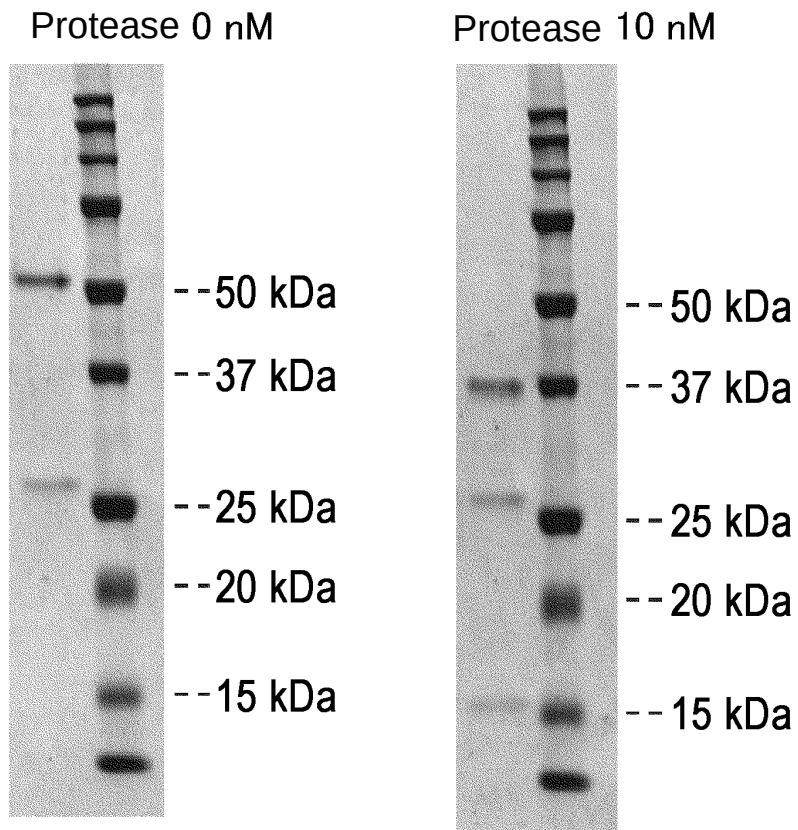


FIG. 19B

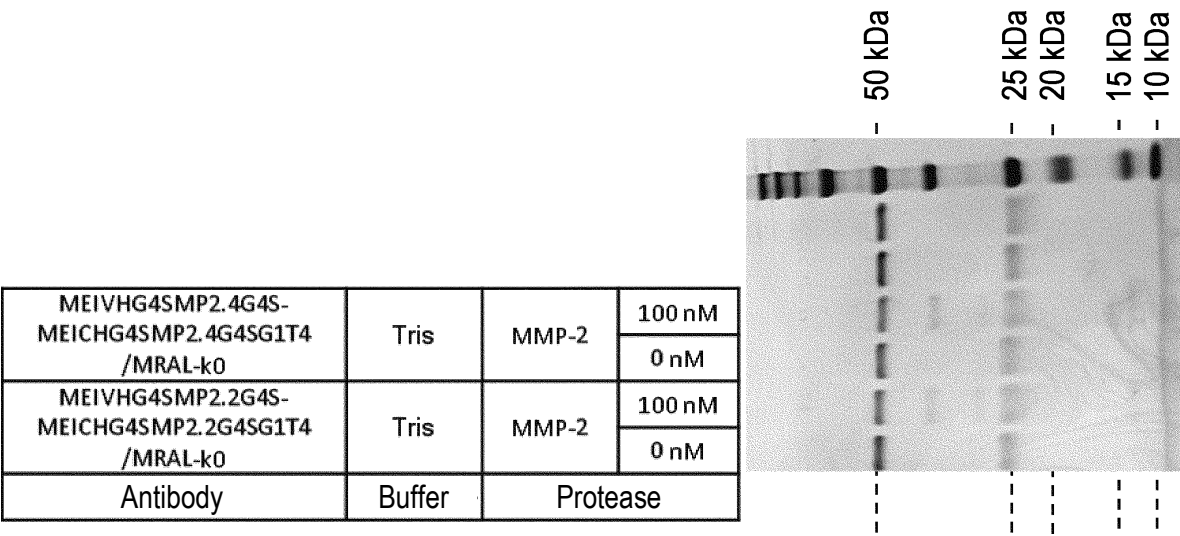


FIG. 20A

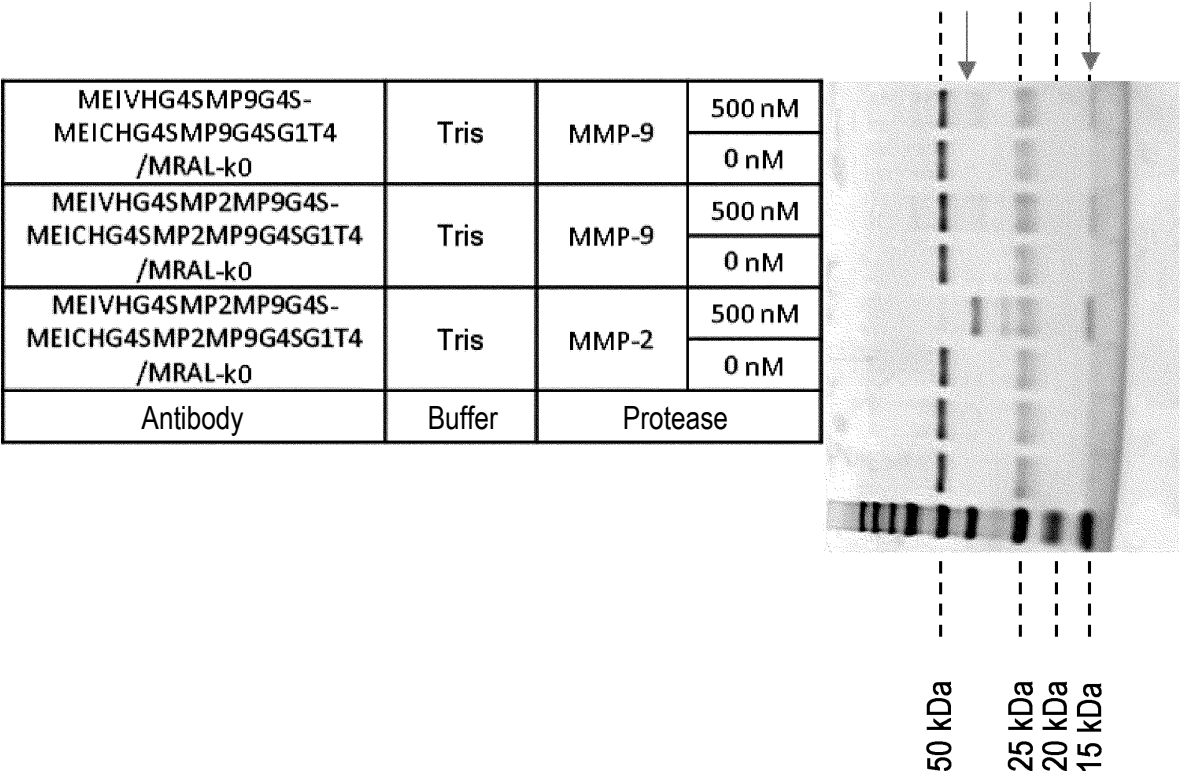


FIG. 20B

MEIVHMP7.2- MEIVHMP7.2G1T4/MRAL-k0	Assay buffer	MMP-7	50 nM
			0 nM
Sample	Buffer	Protease	
MEIVHMP2.3- MEIVHMP2.3G1T4/MRAL-k0	assay buffer	MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	

MEIVHMP2.1- MEIVHMP2.1G1T4/MRAL-k0	Assay buffer	MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	

MRAH-G1T4/MRAL-k0	assay buffer	MMP-9	50 nM
		MMP-7	50 nM
		MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	



FIG. 21

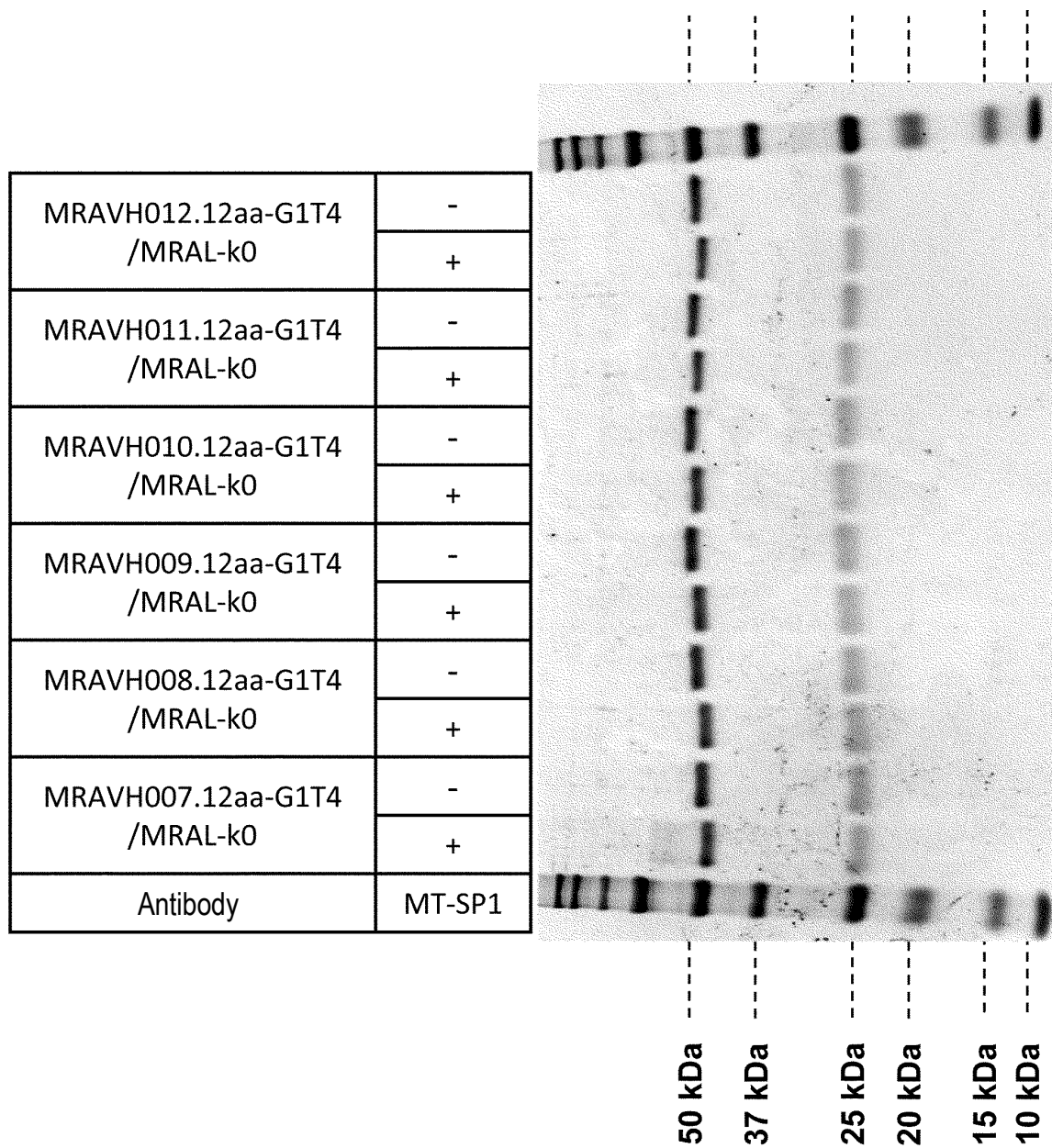


FIG. 22A

MRAVH043.12aa-G1T4 /MRAL-k0	-
	+
MRAVH042.12aa-G1T4 /MRAL-k0	-
	+
MRAVH041.12aa-G1T4 /MRAL-k0	-
	+
MRAVH015.12aa-G1T4 /MRAL-k0	-
	+
MRAVH014.12aa-G1T4 /MRAL-k0	-
	+
MRAVH013.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

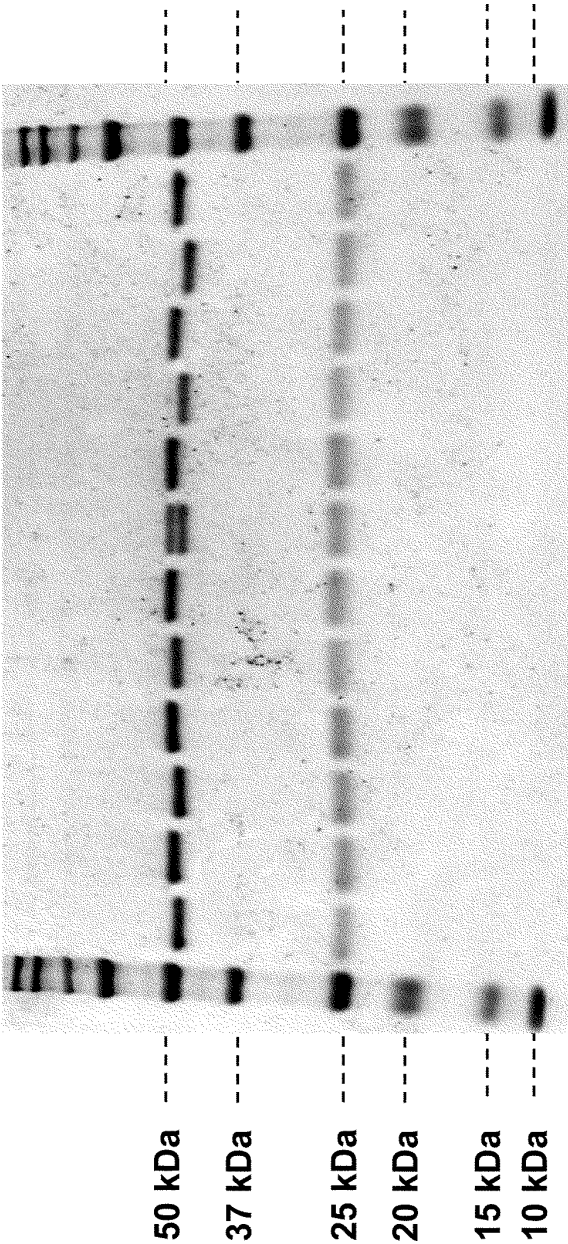


FIG. 22B

MRAVH058.12aa-G1T4 /MRAL-k0	-
	+
MRAVH057.12aa-G1T4 /MRAL-k0	-
	+
MRAVH056.12aa-G1T4 /MRAL-k0	-
	+
MRAVH046.12aa-G1T4 /MRAL-k0	-
	+
MRAVH045.12aa-G1T4 /MRAL-k0	-
	+
MRAVH044.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

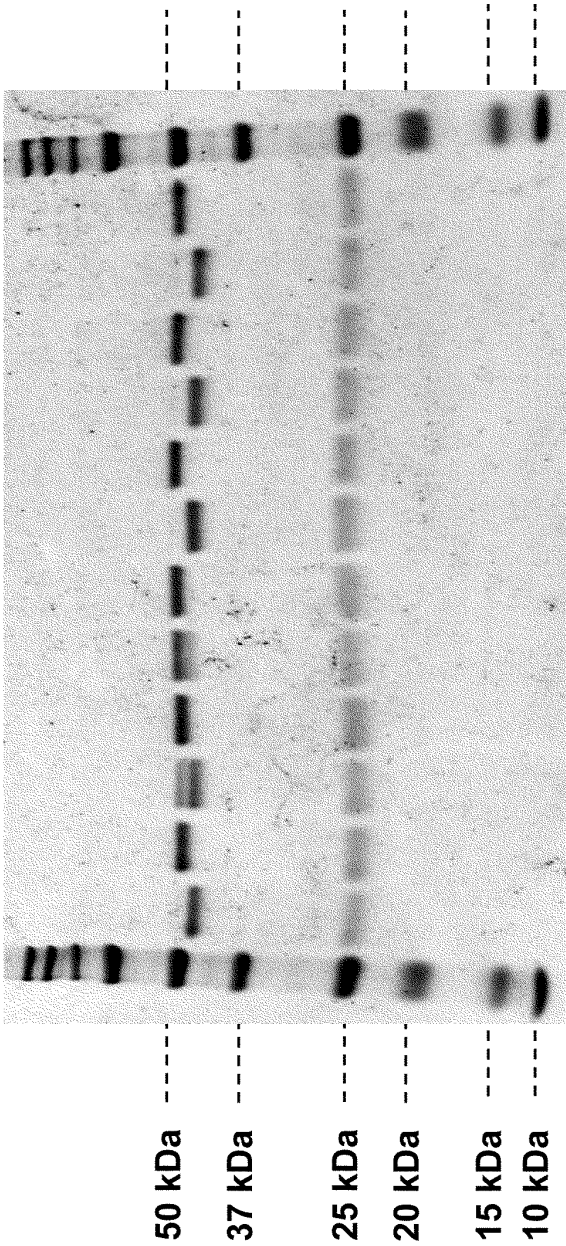


FIG. 22C

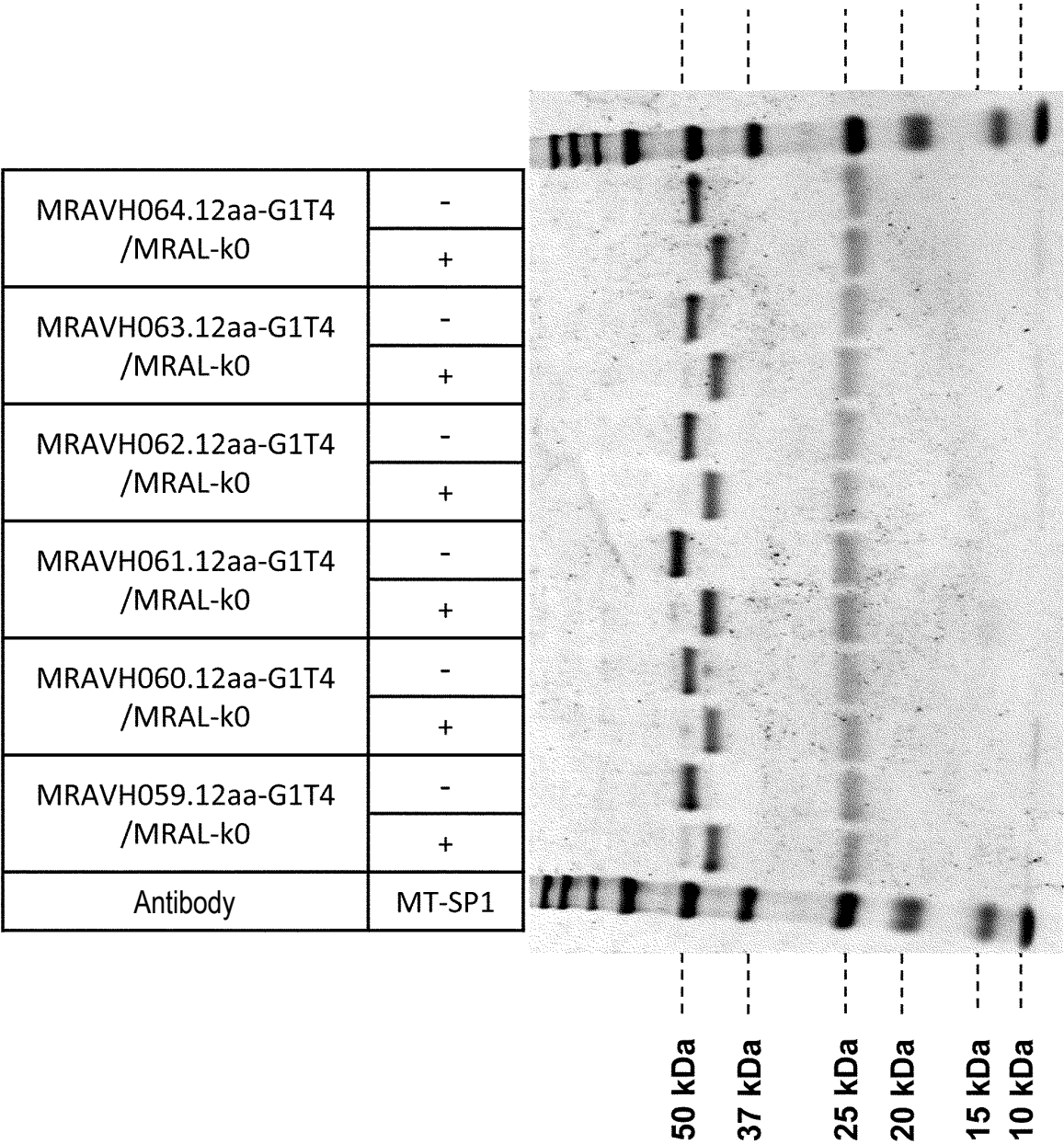


FIG. 22D

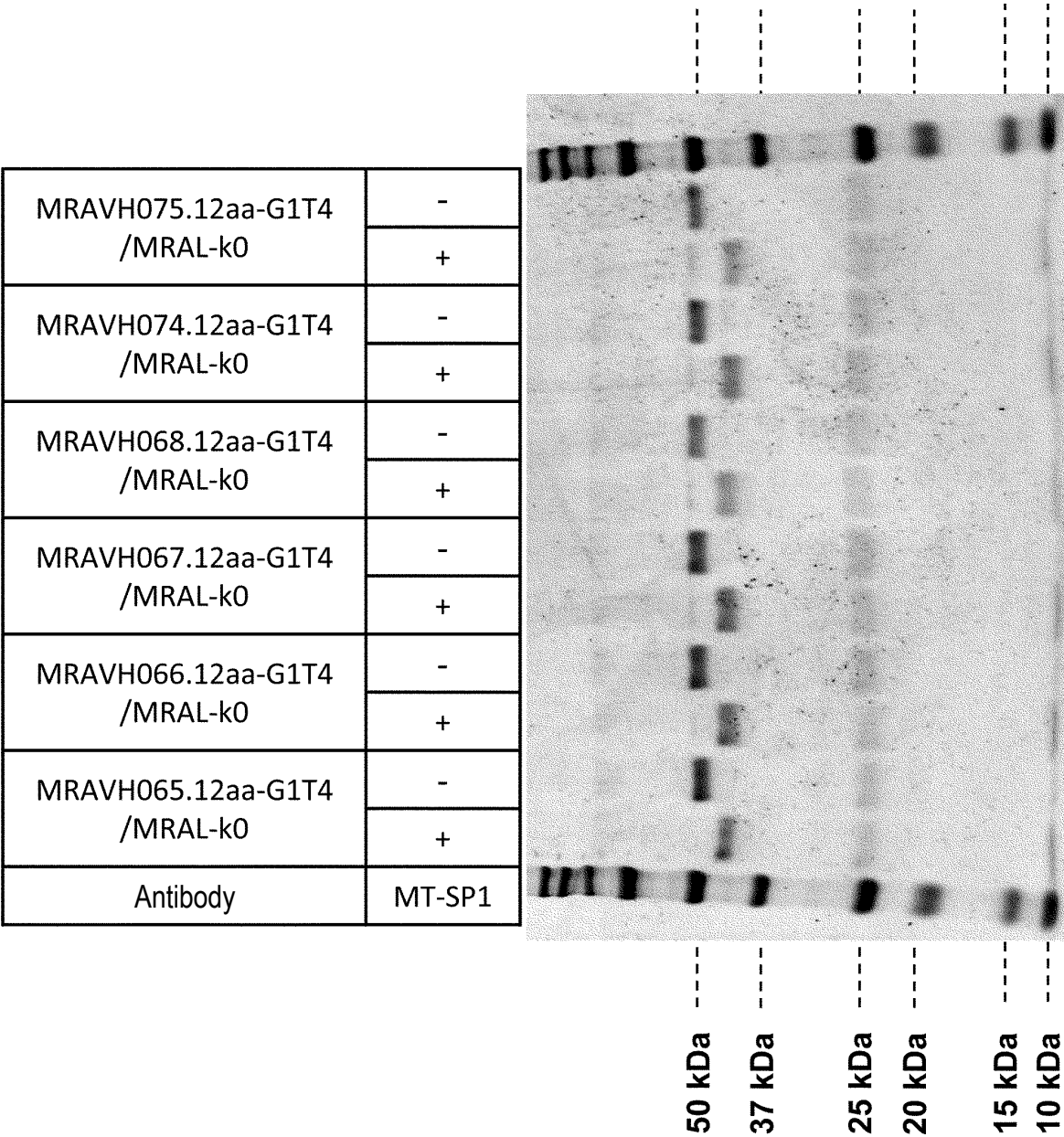


FIG. 22E

MRAVH101.12aa-G1T4 /MRAL-k0	-
	+
MRAVH088.12aa-G1T4 /MRAL-k0	-
	+
MRAVH087.12aa-G1T4 /MRAL-k0	-
	+
MRAVH078.12aa-G1T4 /MRAL-k0	-
	+
MRAVH077.12aa-G1T4 /MRAL-k0	-
	+
MRAVH076.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

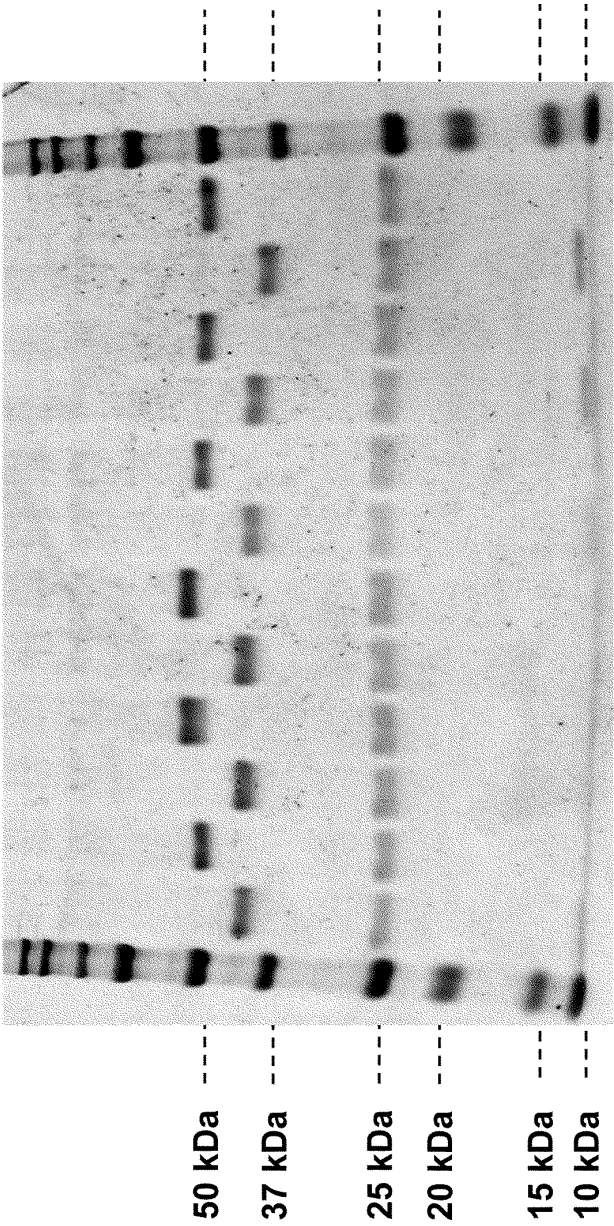


FIG. 22F

MRAVH113.12aa-G1T4 /MRAL-k0	-
	+
MRAVH112.12aa-G1T4 /MRAL-k0	-
	+
MRAVH111.12aa-G1T4 /MRAL-k0	-
	+
MRAVH110.12aa-G1T4 /MRAL-k0	-
	+
MRAVH109.12aa-G1T4 /MRAL-k0	-
	+
MRAVH102.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

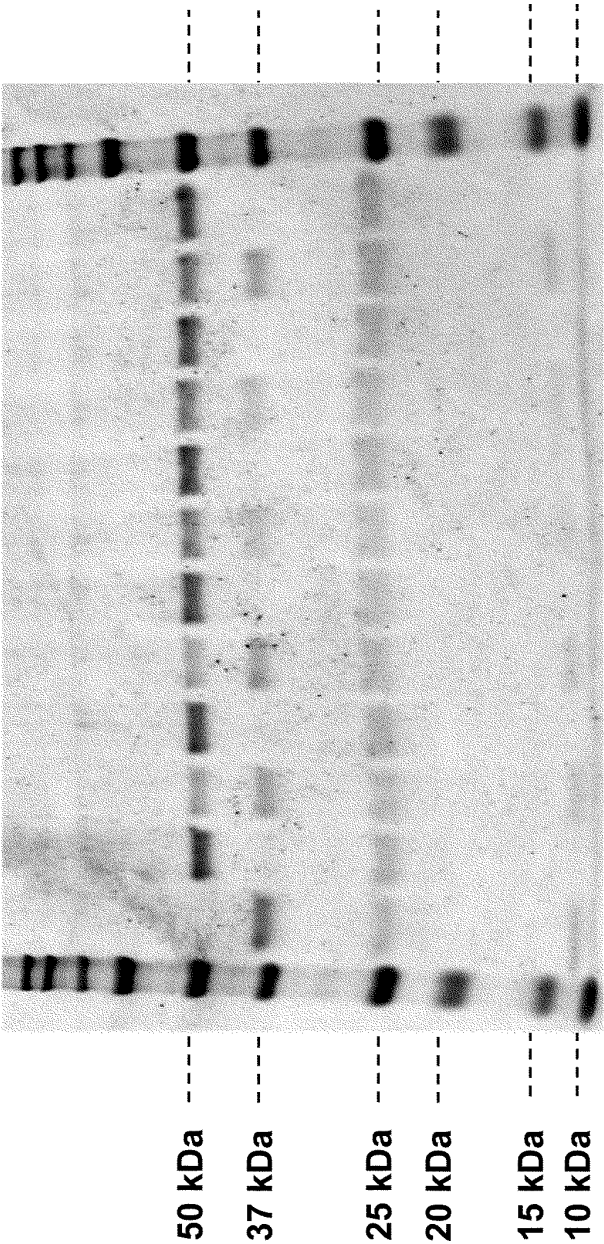


FIG. 22G

MRAVH115.12aa-G1T4 /MRAL-k0	-
	+
MRAVH114.12aa-G1T4 /MRAL-k0	-
	+
MRAVH100.12aa-G1T4 /MRAL-k0	-
	+
MRAVH099.12aa-G1T4 /MRAL-k0	-
	+
MRAVH089.12aa-G1T4 /MRAL-k0	-
	+
MRAVH069.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

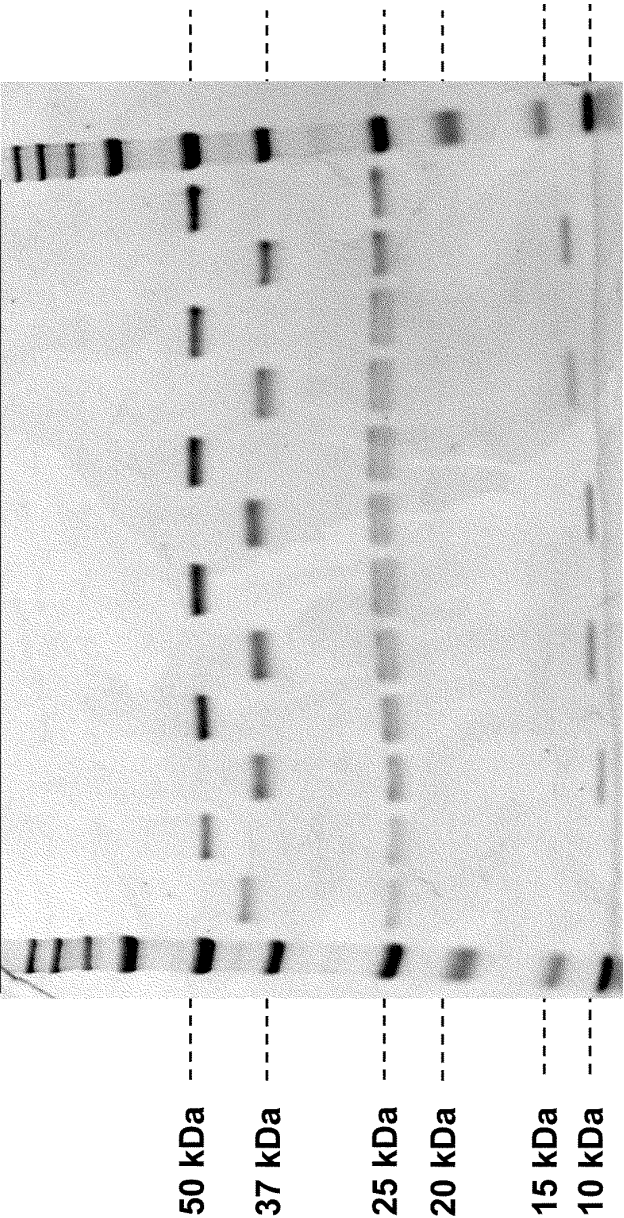


FIG. 22H

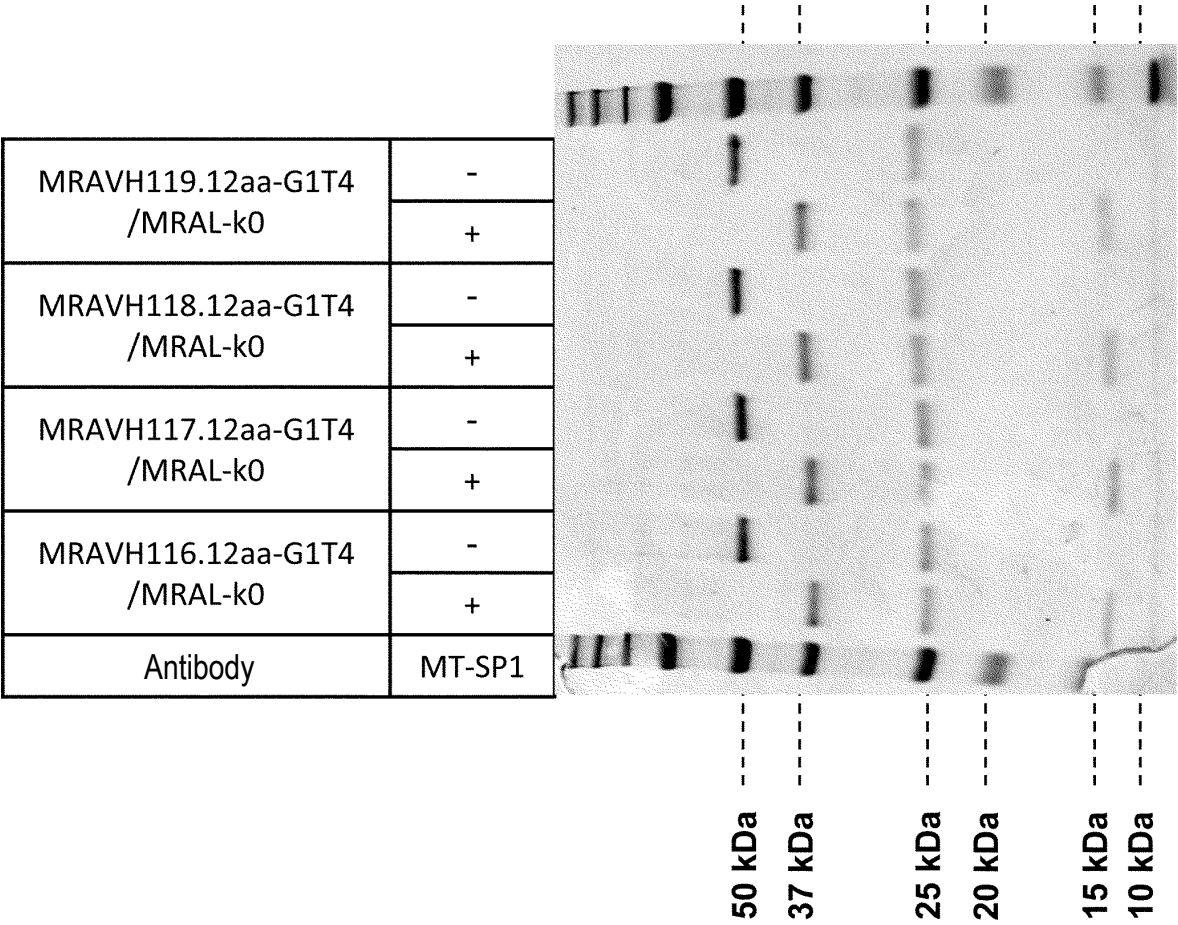


FIG. 22I

MRAH-G1T4.123.12aa /MRAL-k0	-
	+
MRAH-G1T4.122.12aa /MRAL-k0	-
	+
MRAH-G1T4.121.12aa /MRAL-k0	-
	+
MRAH-G1T4.120.12aa /MRAL-k0	-
	+
MRAH-G1T4.119.12aa /MRAL-k0	-
	+
MRAH-G1T4.118.12aa /MRAL-k0	-
	+
Antibody	MT-SP1

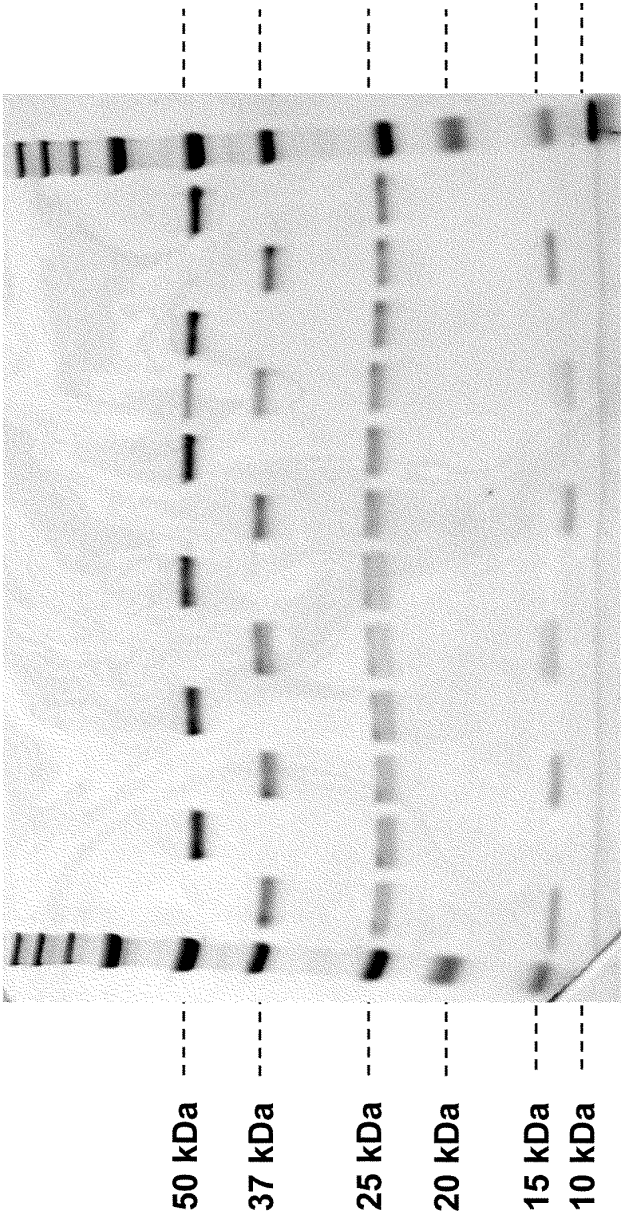


FIG. 23A

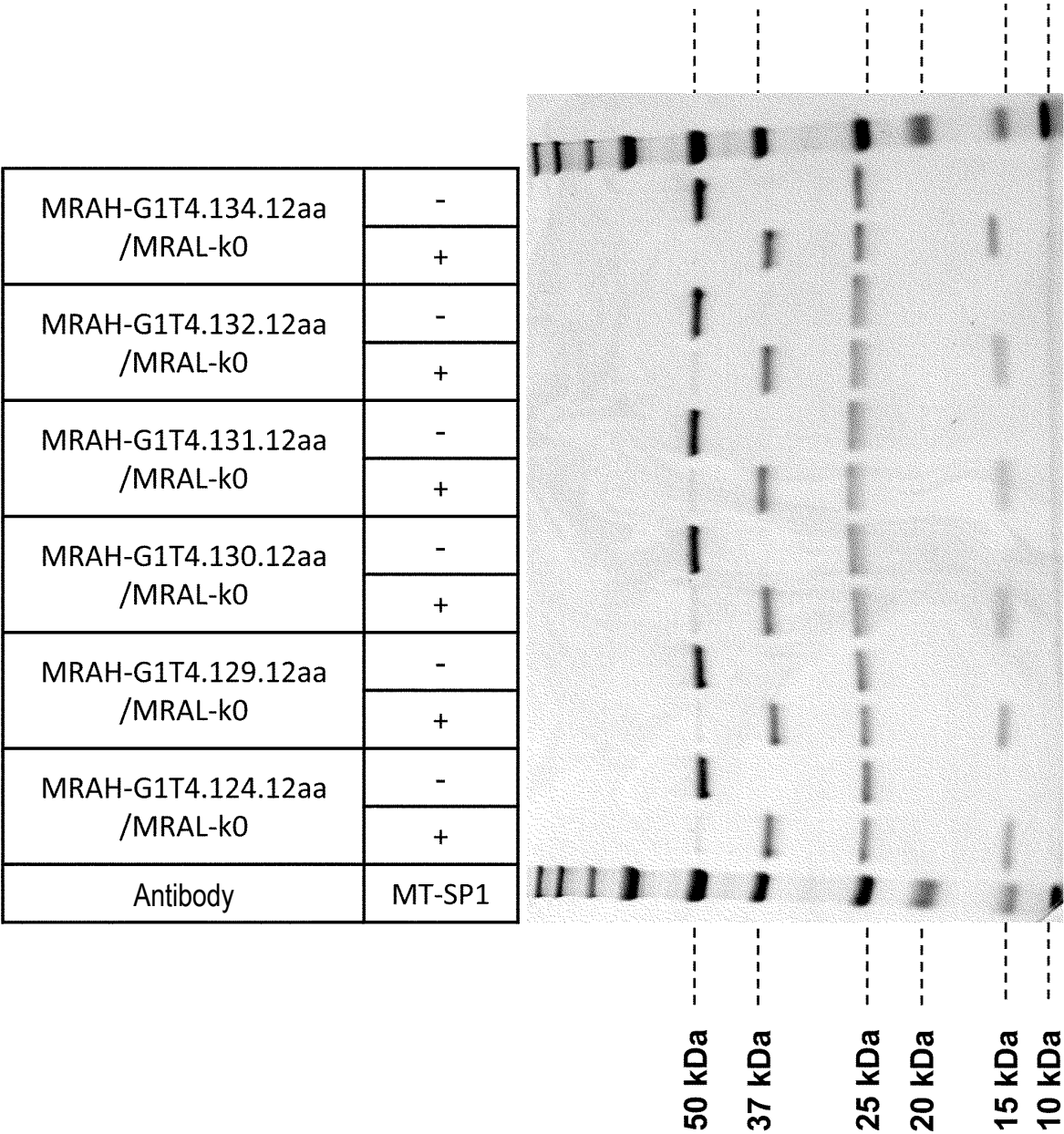


FIG. 23B

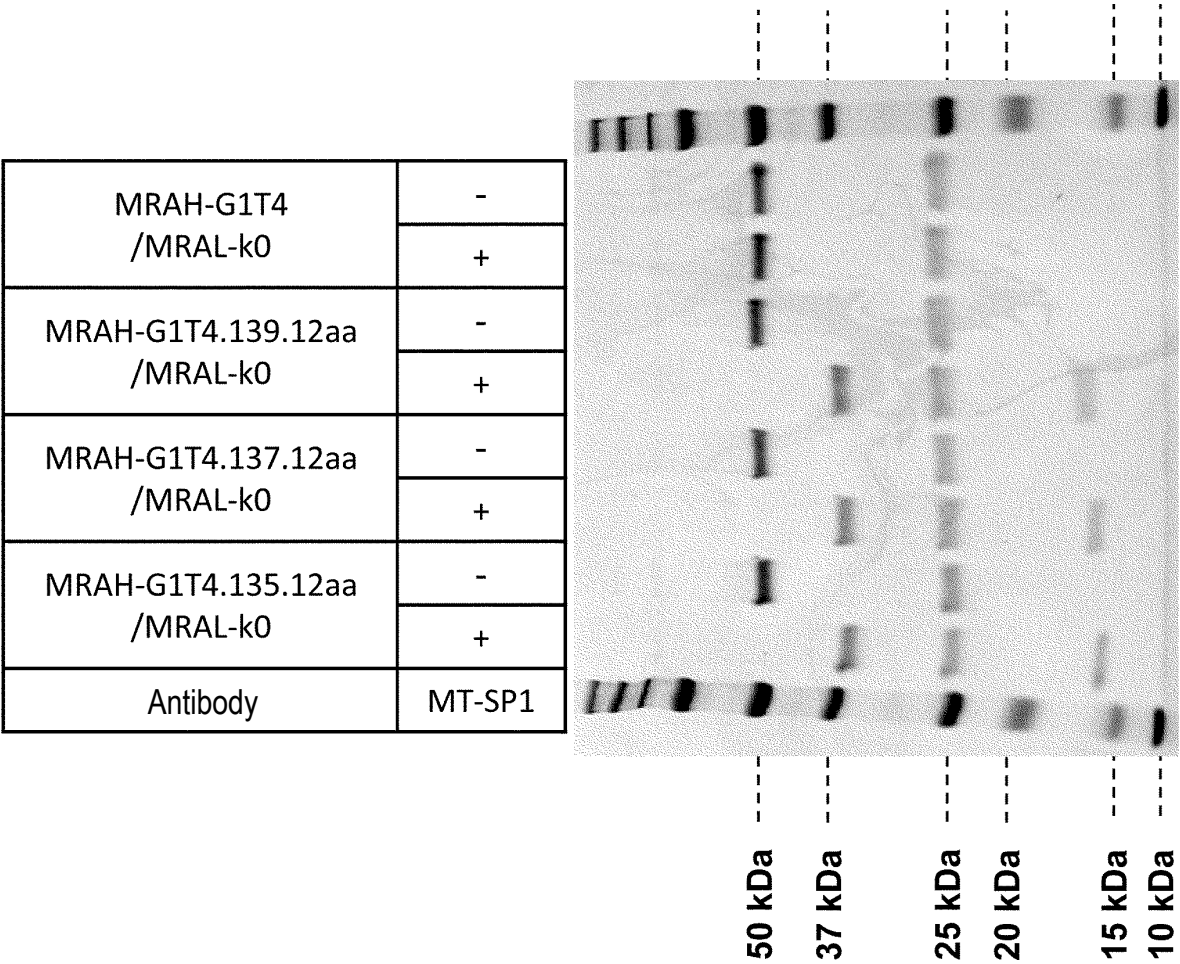
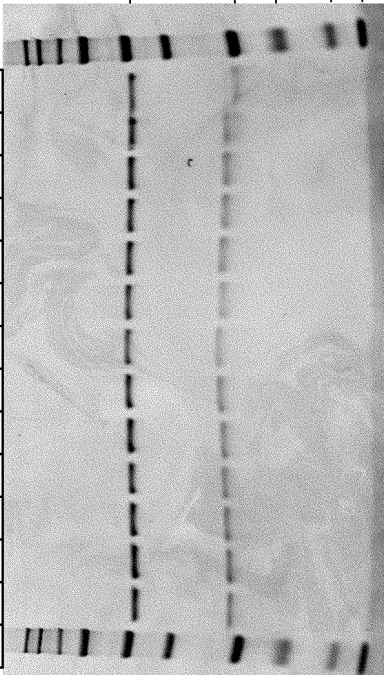
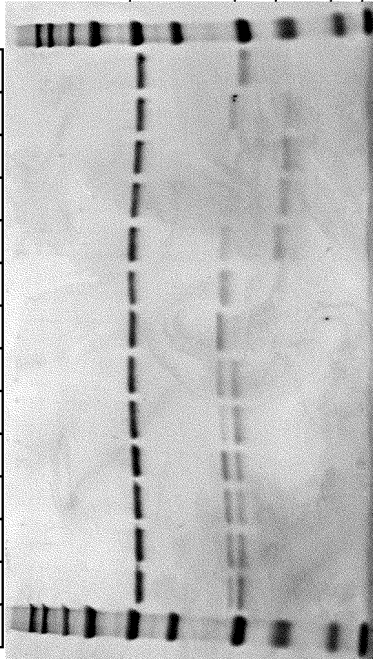


FIG. 23C

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL052.12aa-k0	-
MRAH-G1T4/MRAVL051.12aa-k0	-
MRAH-G1T4/MRAVL050.12aa-k0	-
MRAH-G1T4/MRAVL049.12aa-k0	-
MRAH-G1T4/MRAVL041.12aa-k0	-
MRAH-G1T4/MRAVL013.12aa-k0	-
MRAH-G1T4/MRAVL012.12aa-k0	-
MRAH-G1T4/MRAVL011.12aa-k0	-
MRAH-G1T4/MRAVL010.12aa-k0	-
MRAH-G1T4/MRAVL009.12aa-k0	-
MRAH-G1T4/MRAVL008.12aa-k0	-
MRAH-G1T4/MRAVL007.12aa-k0	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL052.12aa-k0	+
MRAH-G1T4/MRAVL051.12aa-k0	+
MRAH-G1T4/MRAVL050.12aa-k0	+
MRAH-G1T4/MRAVL049.12aa-k0	+
MRAH-G1T4/MRAVL041.12aa-k0	+
MRAH-G1T4/MRAVL013.12aa-k0	+
MRAH-G1T4/MRAVL012.12aa-k0	+
MRAH-G1T4/MRAVL011.12aa-k0	+
MRAH-G1T4/MRAVL010.12aa-k0	+
MRAH-G1T4/MRAVL009.12aa-k0	+
MRAH-G1T4/MRAVL008.12aa-k0	+
MRAH-G1T4/MRAVL007.12aa-k0	+
Antibody	MT-SP1



50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 24A

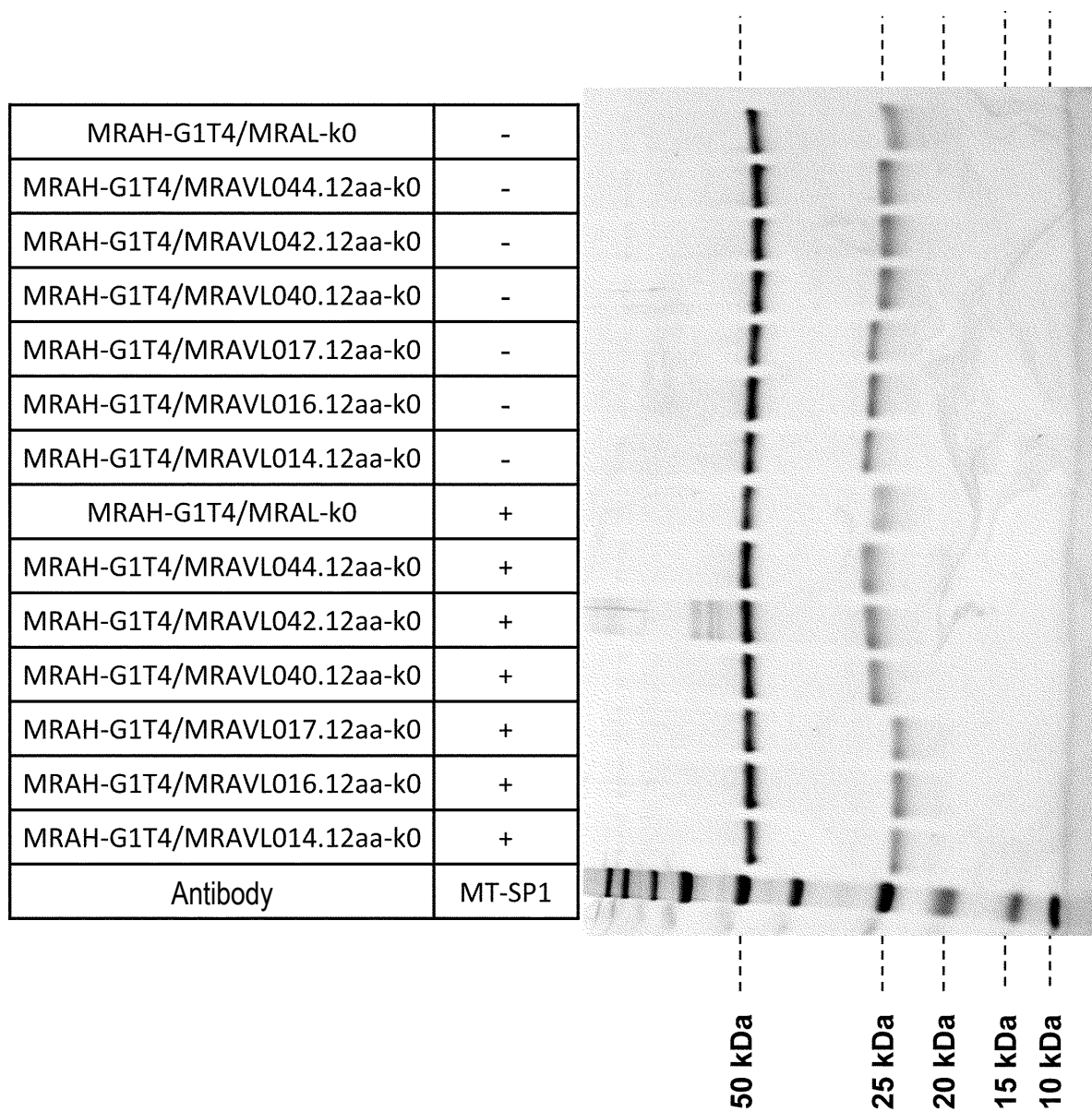
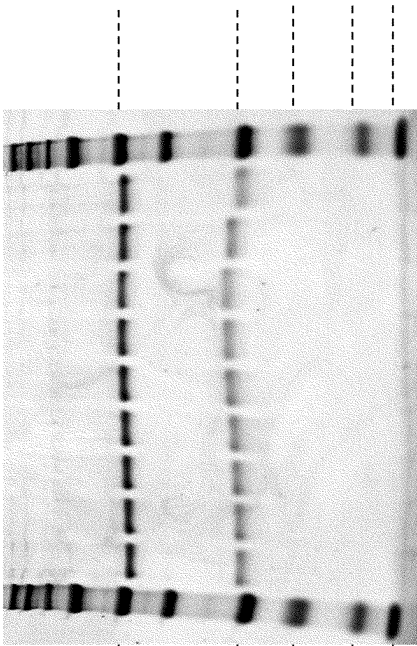


FIG. 24B

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL098.12aa-k0	-
MRAH-G1T4/MRAVL097.12aa-k0	-
MRAH-G1T4/MRAVL096.12aa-k0	-
MRAH-G1T4/MRAVL059.12aa-k0	-
MRAH-G1T4/MRAVL058.12aa-k0	-
MRAH-G1T4/MRAVL057.12aa-k0	-
MRAH-G1T4/MRAVL056.12aa-k0	-
MRAH-G1T4/MRAVL054.12aa-k0	-
Antibody	MT-SP1



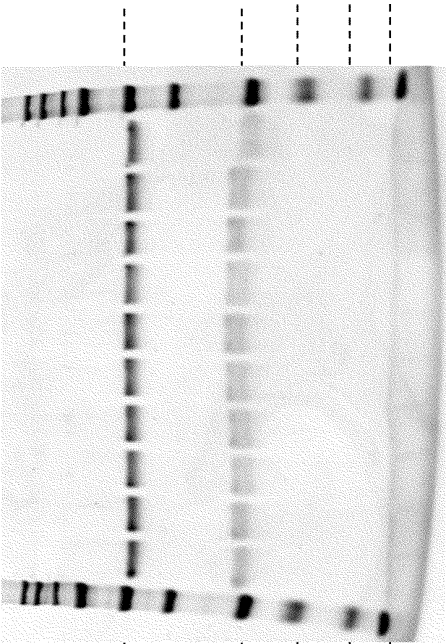
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL098.12aa-k0	+
MRAH-G1T4/MRAVL097.12aa-k0	+
MRAH-G1T4/MRAVL096.12aa-k0	+
MRAH-G1T4/MRAVL059.12aa-k0	+
MRAH-G1T4/MRAVL058.12aa-k0	+
MRAH-G1T4/MRAVL057.12aa-k0	+
MRAH-G1T4/MRAVL056.12aa-k0	+
MRAH-G1T4/MRAVL054.12aa-k0	+
Antibody	MT-SP1



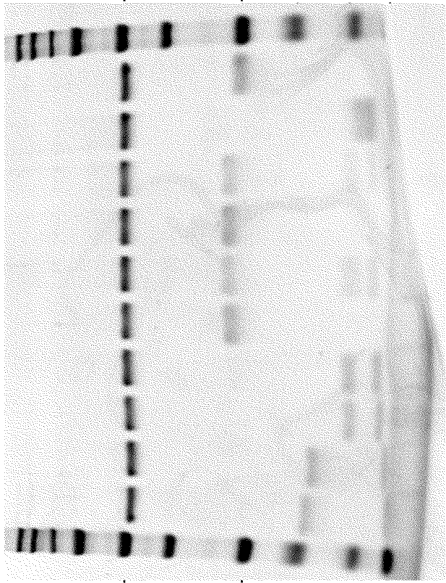
50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 24C

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL106.12aa-k0	-
MRAH-G1T4/MRAVL104.12aa-k0	-
MRAH-G1T4/MRAVL103.12aa-k0	-
MRAH-G1T4/MRAVL102.12aa-k0	-
MRAH-G1T4/MRAVL101.12aa-k0	-
MRAH-G1T4/MRAVL100.12aa-k0	-
MRAH-G1T4/MRAVL099.12aa-k0	-
MRAH-G1T4/MRAVL060.12aa-k0	-
MRAH-G1T4/MRAVL055.12aa-k0	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL106.12aa-k0	+
MRAH-G1T4/MRAVL104.12aa-k0	+
MRAH-G1T4/MRAVL103.12aa-k0	+
MRAH-G1T4/MRAVL102.12aa-k0	+
MRAH-G1T4/MRAVL101.12aa-k0	+
MRAH-G1T4/MRAVL100.12aa-k0	+
MRAH-G1T4/MRAVL099.12aa-k0	+
MRAH-G1T4/MRAVL060.12aa-k0	+
MRAH-G1T4/MRAVL055.12aa-k0	+
Antibody	MT-SP1



50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 24D

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL107.12aa-k0	-
MRAH-G1T4/MRAVL105.12aa-k0	-
MRAH-G1T4/MRAVL053.12aa-k0	-
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL107.12aa-k0	+
MRAH-G1T4/MRAVL105.12aa-k0	+
MRAH-G1T4/MRAVL053.12aa-k0	+
Antibody	MT-SP1

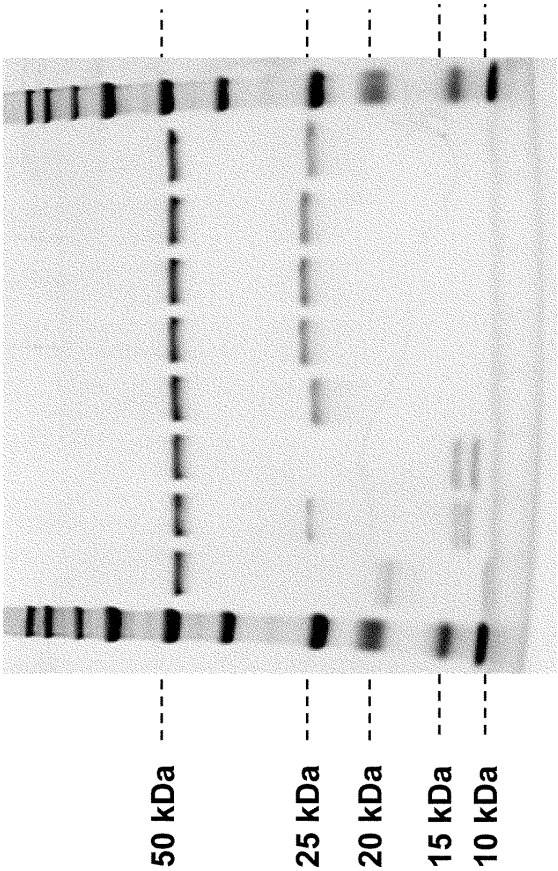
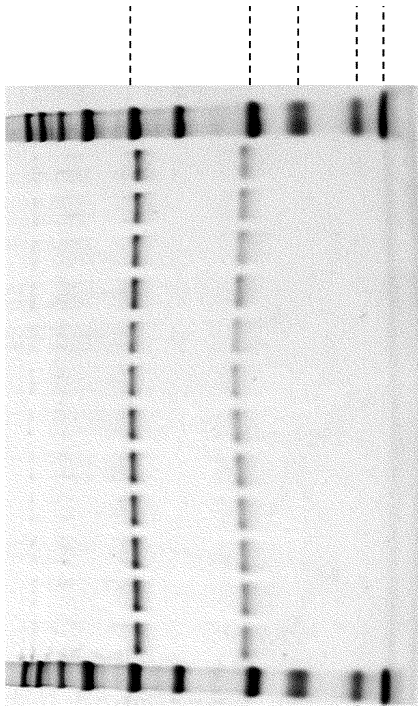


FIG. 24E

MRAH-G1T4/MRAL-k0.120.12aa	-
MRAH-G1T4/MRAL-k0.119.12aa	-
MRAH-G1T4/MRAL-k0.118.12aa	-
MRAH-G1T4/MRAL-k0.117.12aa	-
MRAH-G1T4/MRAL-k0.116.12aa	-
MRAH-G1T4/MRAL-k0.115.12aa	-
MRAH-G1T4/MRAL-k0.113.12aa	-
MRAH-G1T4/MRAL-k0.112.12aa	-
MRAH-G1T4/MRAL-k0.111.12aa	-
MRAH-G1T4/MRAL-k0.110.12aa	-
MRAH-G1T4/MRAL-k0.109.12aa	-
MRAH-G1T4/MRAL-k0.108.12aa	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0.120.12aa	+
MRAH-G1T4/MRAL-k0.119.12aa	+
MRAH-G1T4/MRAL-k0.118.12aa	+
MRAH-G1T4/MRAL-k0.117.12aa	+
MRAH-G1T4/MRAL-k0.116.12aa	+
MRAH-G1T4/MRAL-k0.115.12aa	+
MRAH-G1T4/MRAL-k0.113.12aa	+
MRAH-G1T4/MRAL-k0.112.12aa	+
MRAH-G1T4/MRAL-k0.111.12aa	+
MRAH-G1T4/MRAL-k0.110.12aa	+
MRAH-G1T4/MRAL-k0.109.12aa	+
MRAH-G1T4/MRAL-k0.108.12aa	+
Antibody	MT-SP1

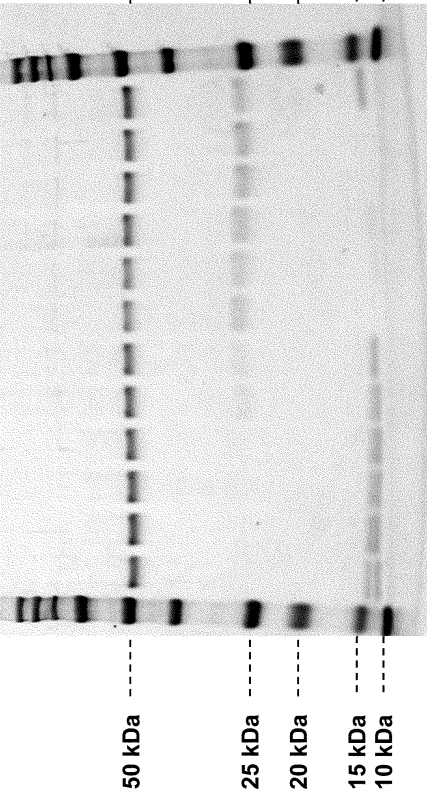
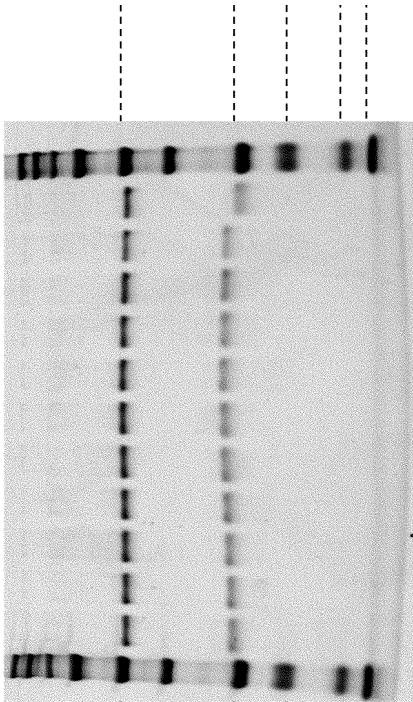
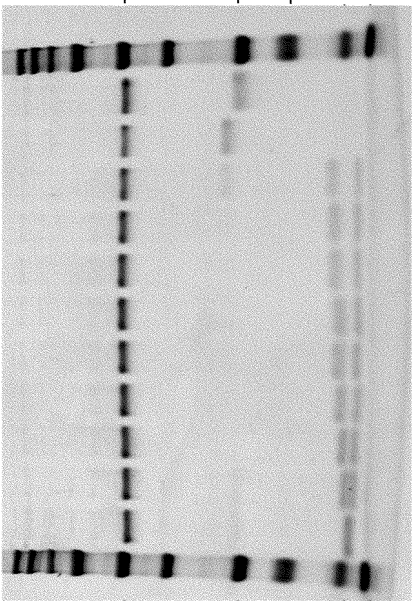


FIG. 25A

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAL-k0.130.12aa	-
MRAH-G1T4/MRAL-k0.129.12aa	-
MRAH-G1T4/MRAL-k0.128.12aa	-
MRAH-G1T4/MRAL-k0.127.12aa	-
MRAH-G1T4/MRAL-k0.126.12aa	-
MRAH-G1T4/MRAL-k0.125.12aa	-
MRAH-G1T4/MRAL-k0.124.12aa	-
MRAH-G1T4/MRAL-k0.123.12aa	-
MRAH-G1T4/MRAL-k0.122.12aa	-
MRAH-G1T4/MRAL-k0.121.12aa	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAL-k0.130.12aa	+
MRAH-G1T4/MRAL-k0.129.12aa	+
MRAH-G1T4/MRAL-k0.128.12aa	+
MRAH-G1T4/MRAL-k0.127.12aa	+
MRAH-G1T4/MRAL-k0.126.12aa	+
MRAH-G1T4/MRAL-k0.125.12aa	+
MRAH-G1T4/MRAL-k0.124.12aa	+
MRAH-G1T4/MRAL-k0.123.12aa	+
MRAH-G1T4/MRAL-k0.122.12aa	+
MRAH-G1T4/MRAL-k0.121.12aa	+
Antibody	MT-SP1



50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 25B

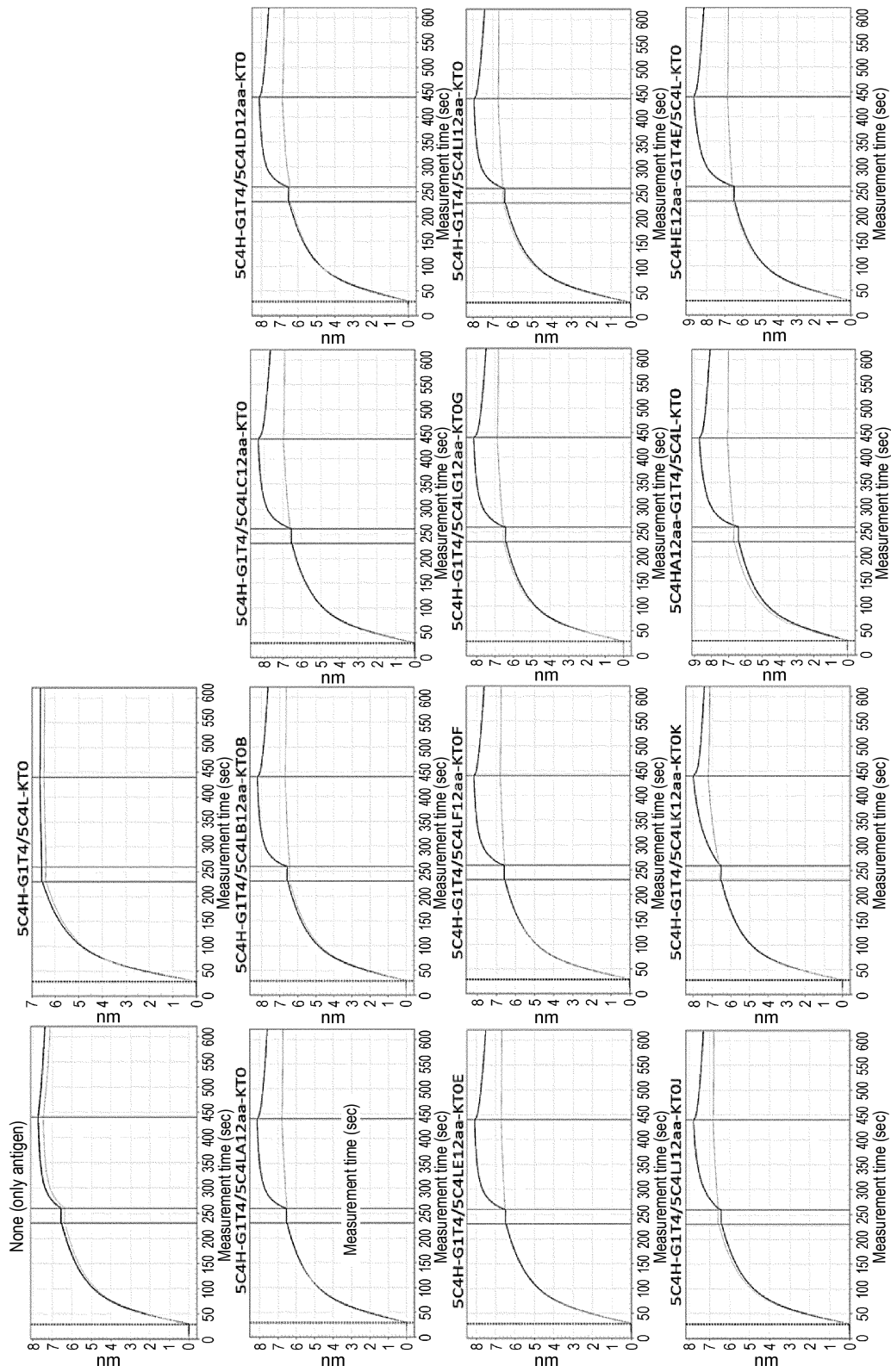


FIG. 26

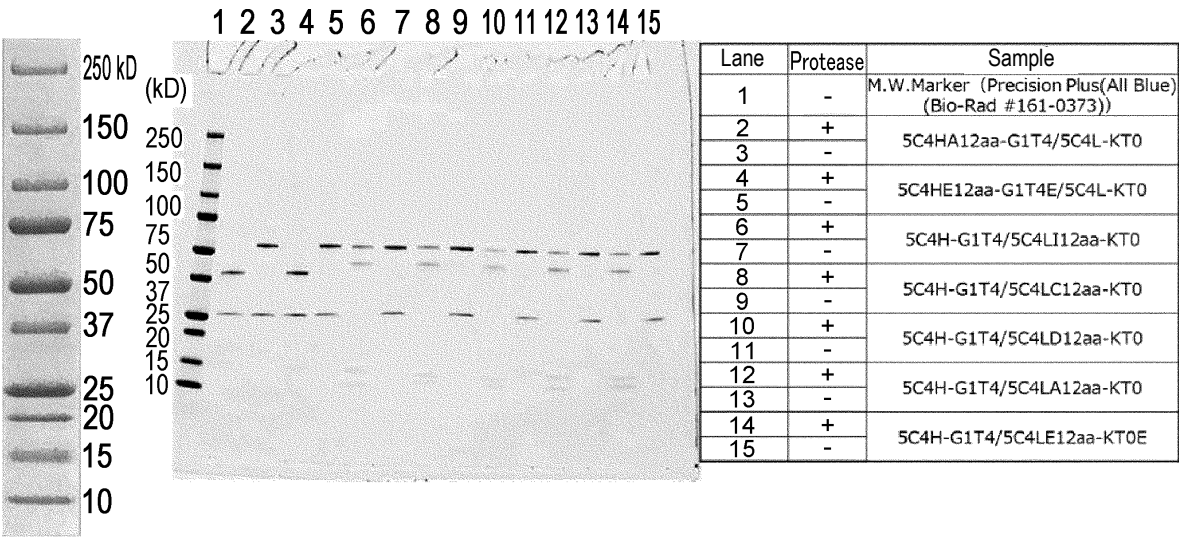
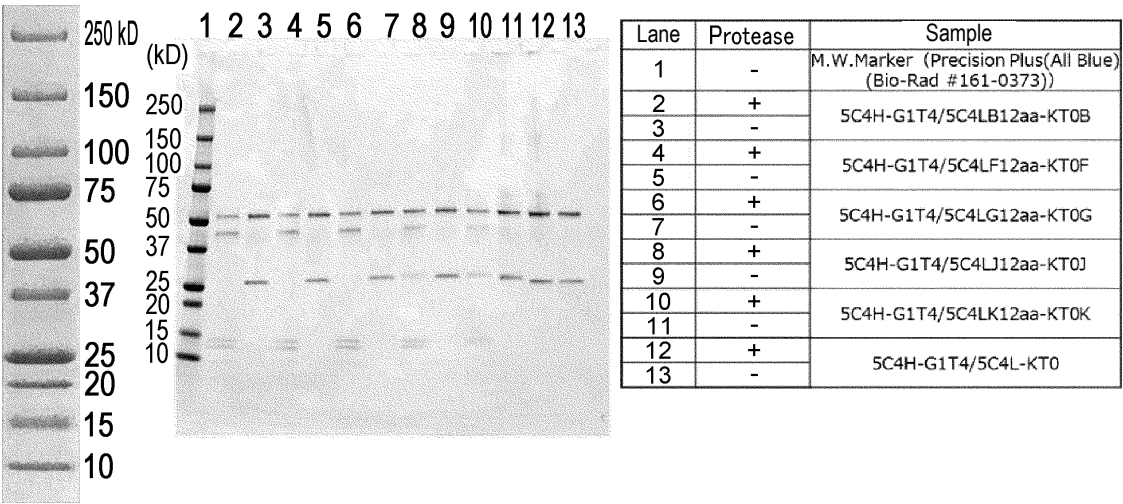


FIG. 27

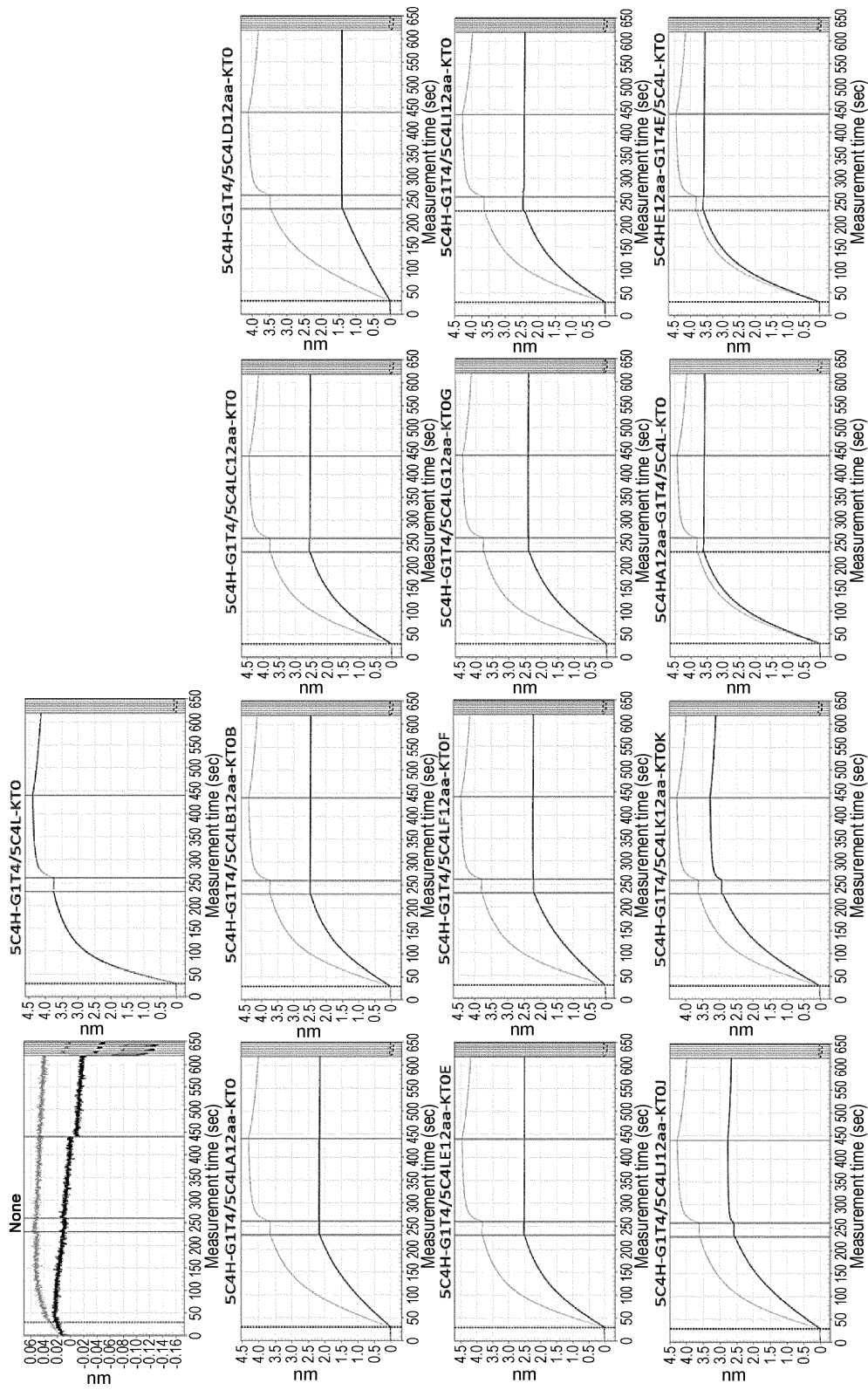


FIG. 28

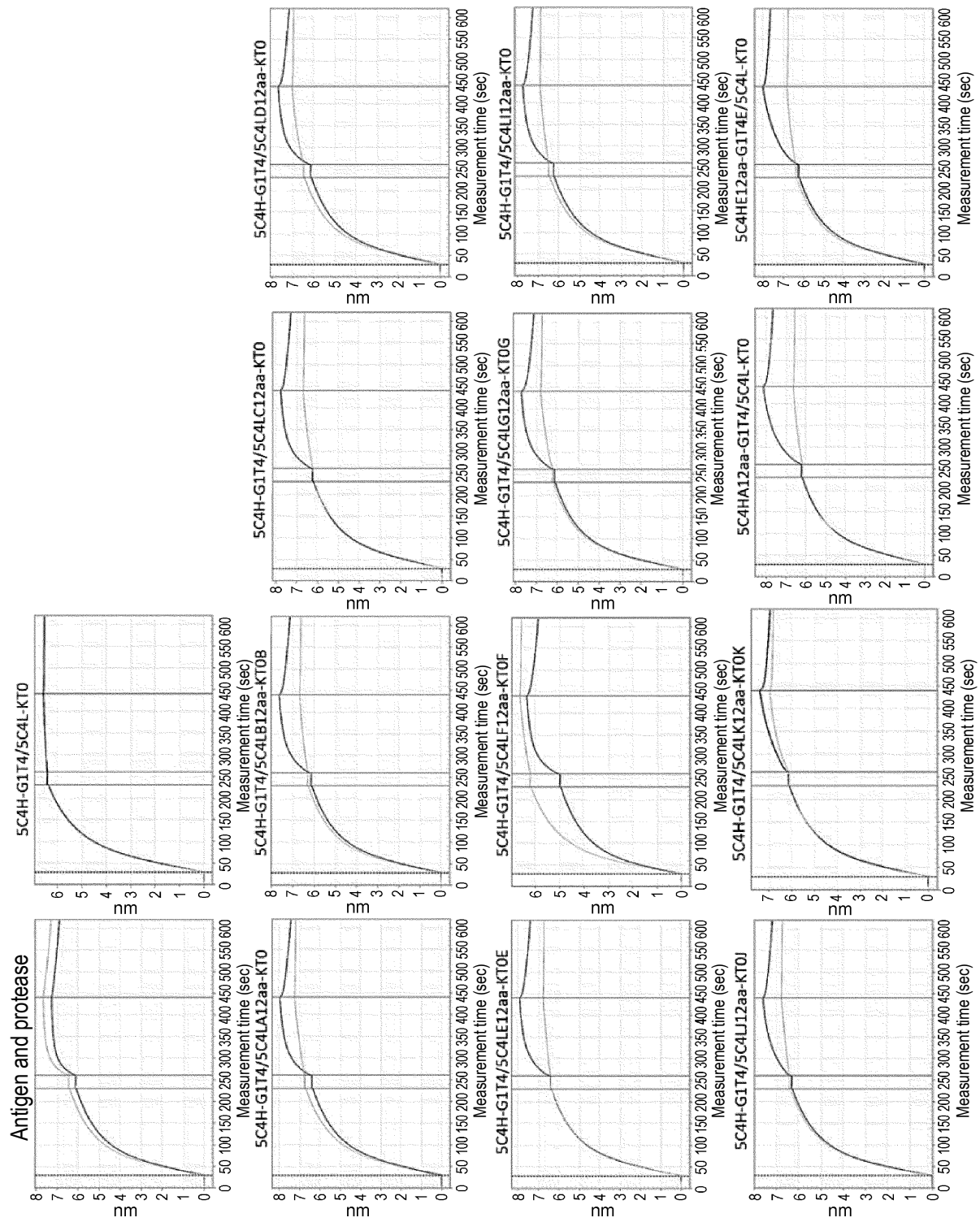


FIG. 29

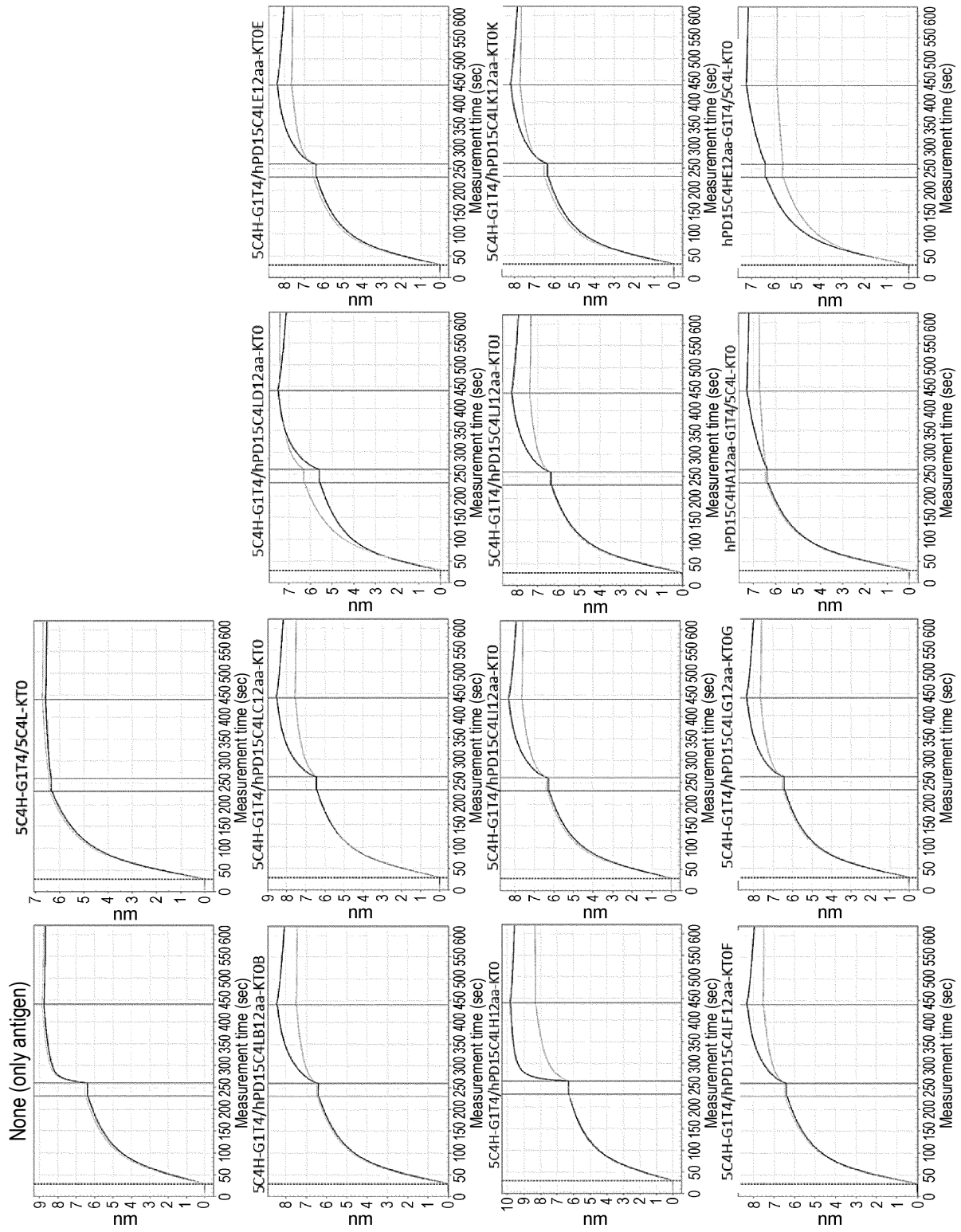


FIG. 30

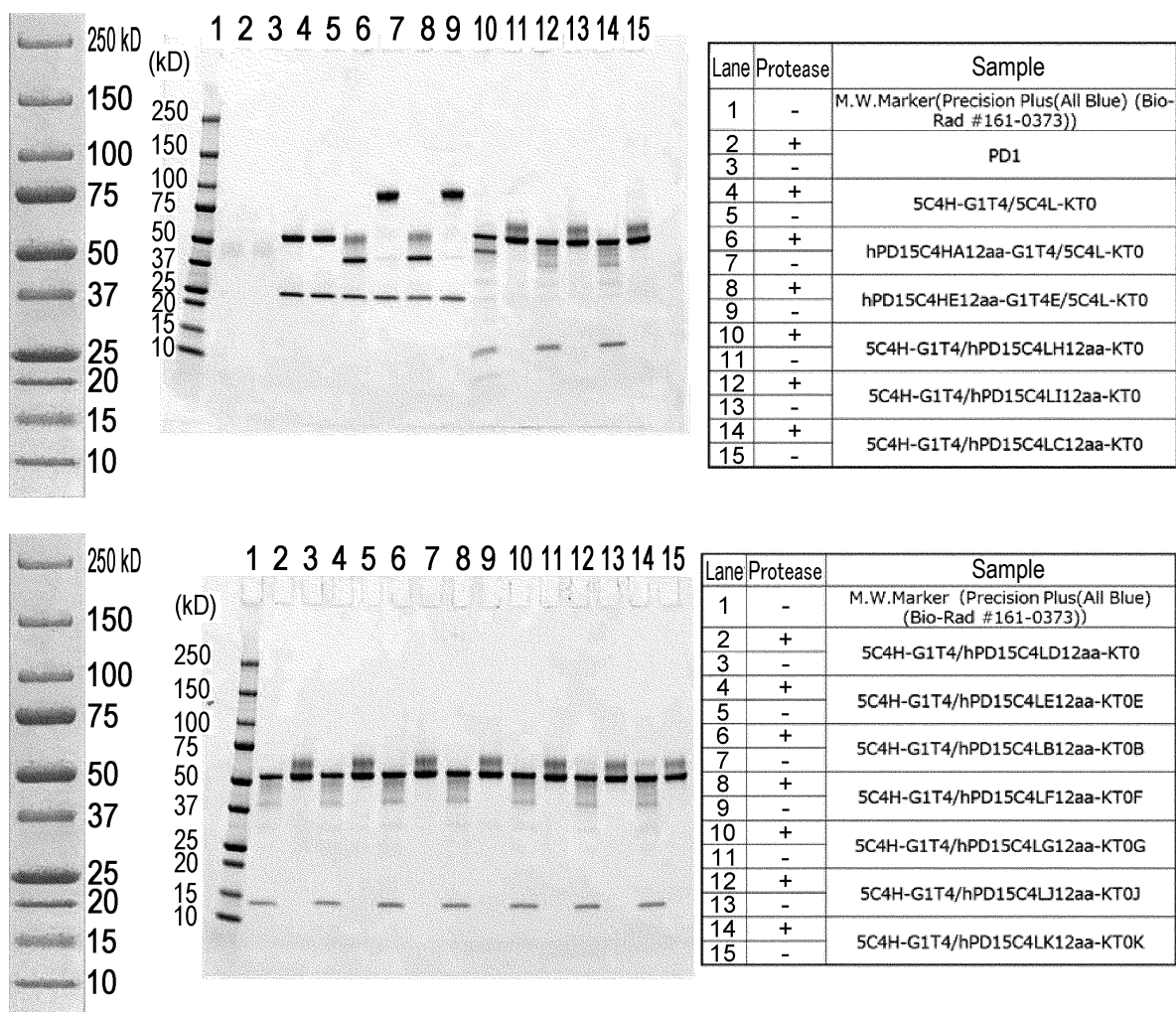


FIG. 31