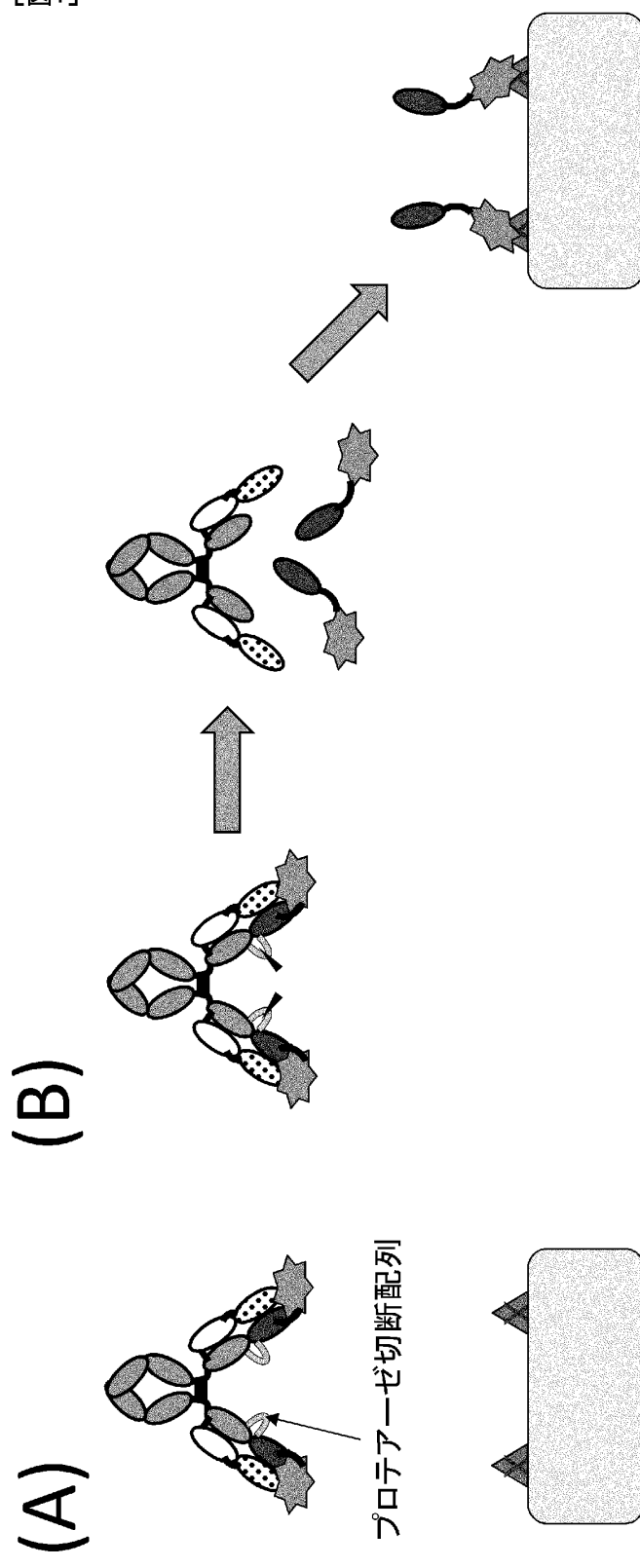
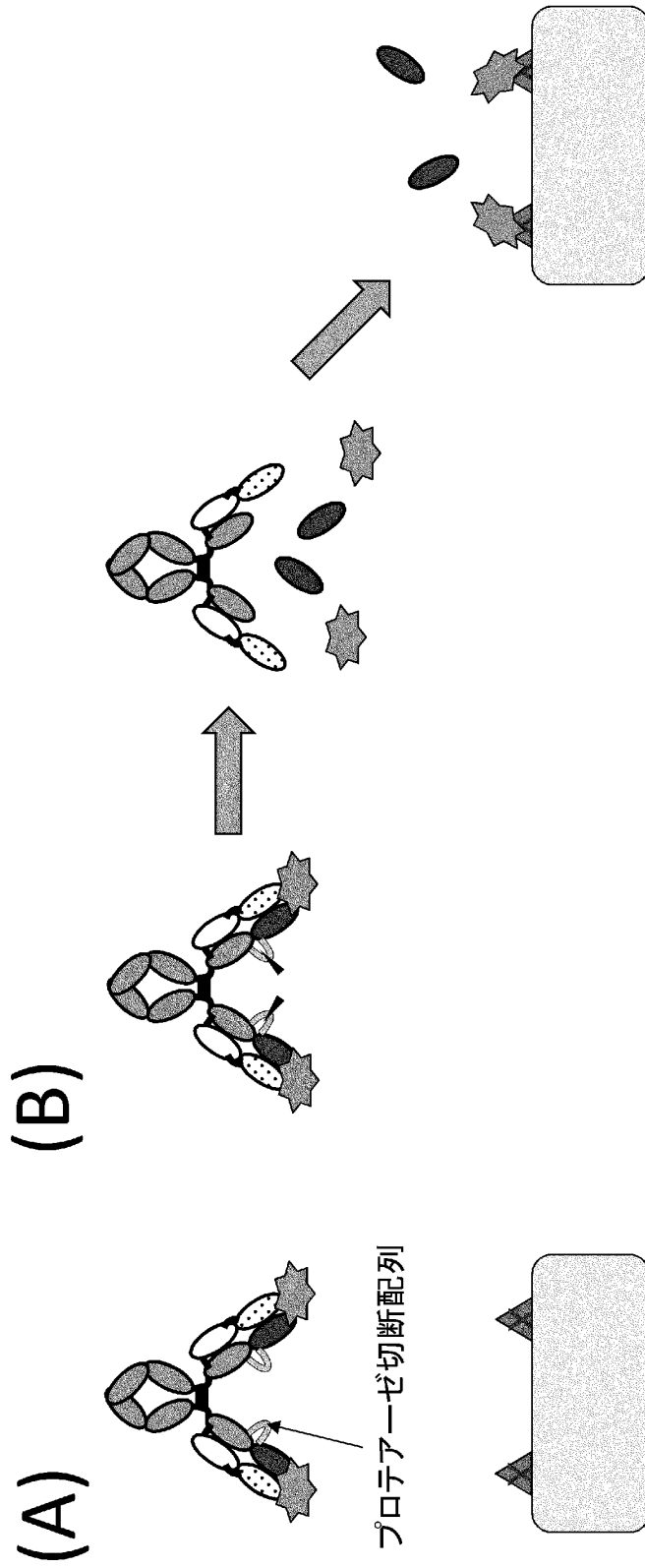


【図1】



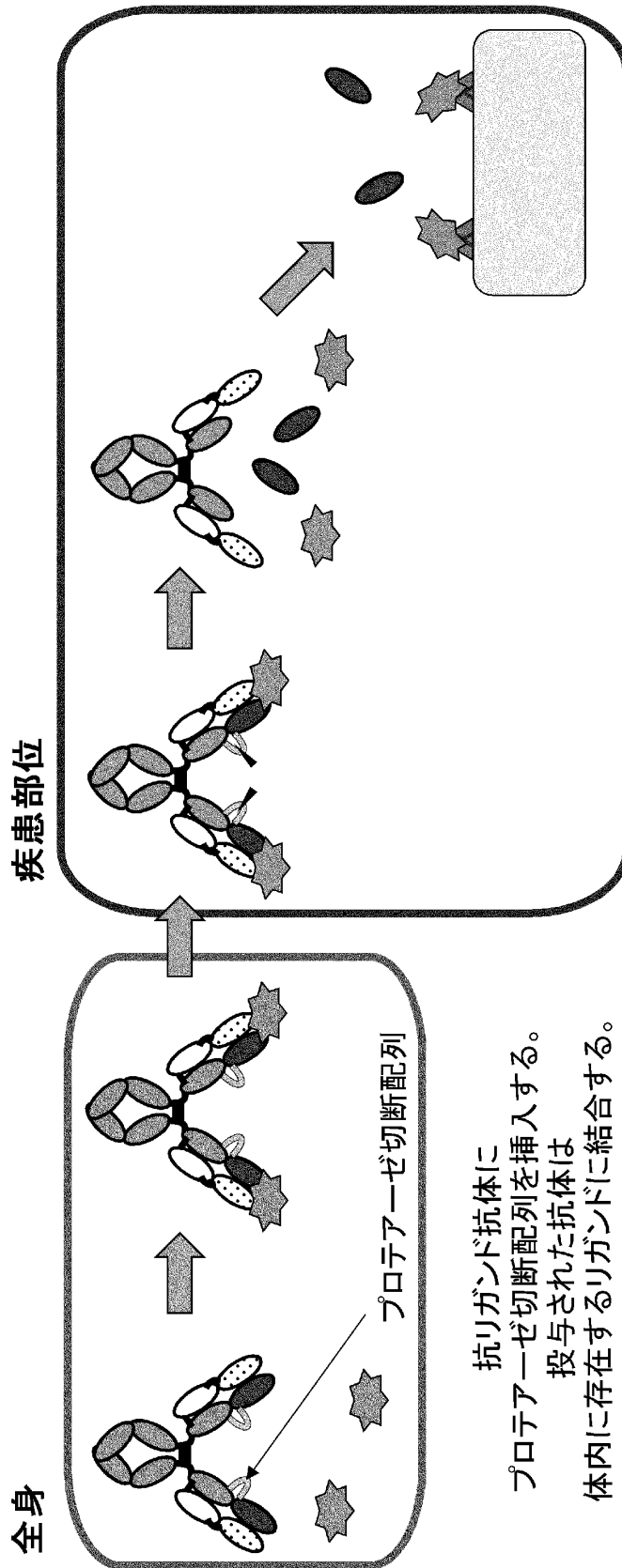
[図2]



プロテアーゼによって切断されることで
リガンド-抗リガンド抗体複合体のリガンドとVH分子が遊離し、
遊離したリガンドが受容体に結合する

抗リガンド抗体に
プロテアーゼ切断配列を挿入する

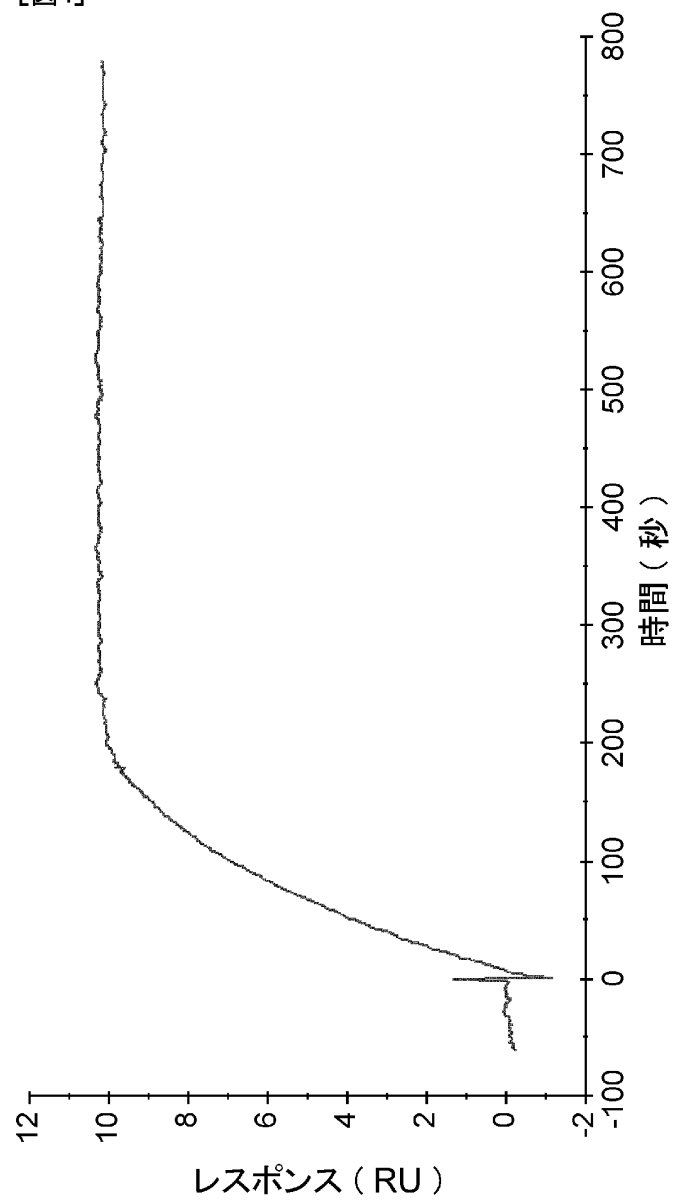
[図3]



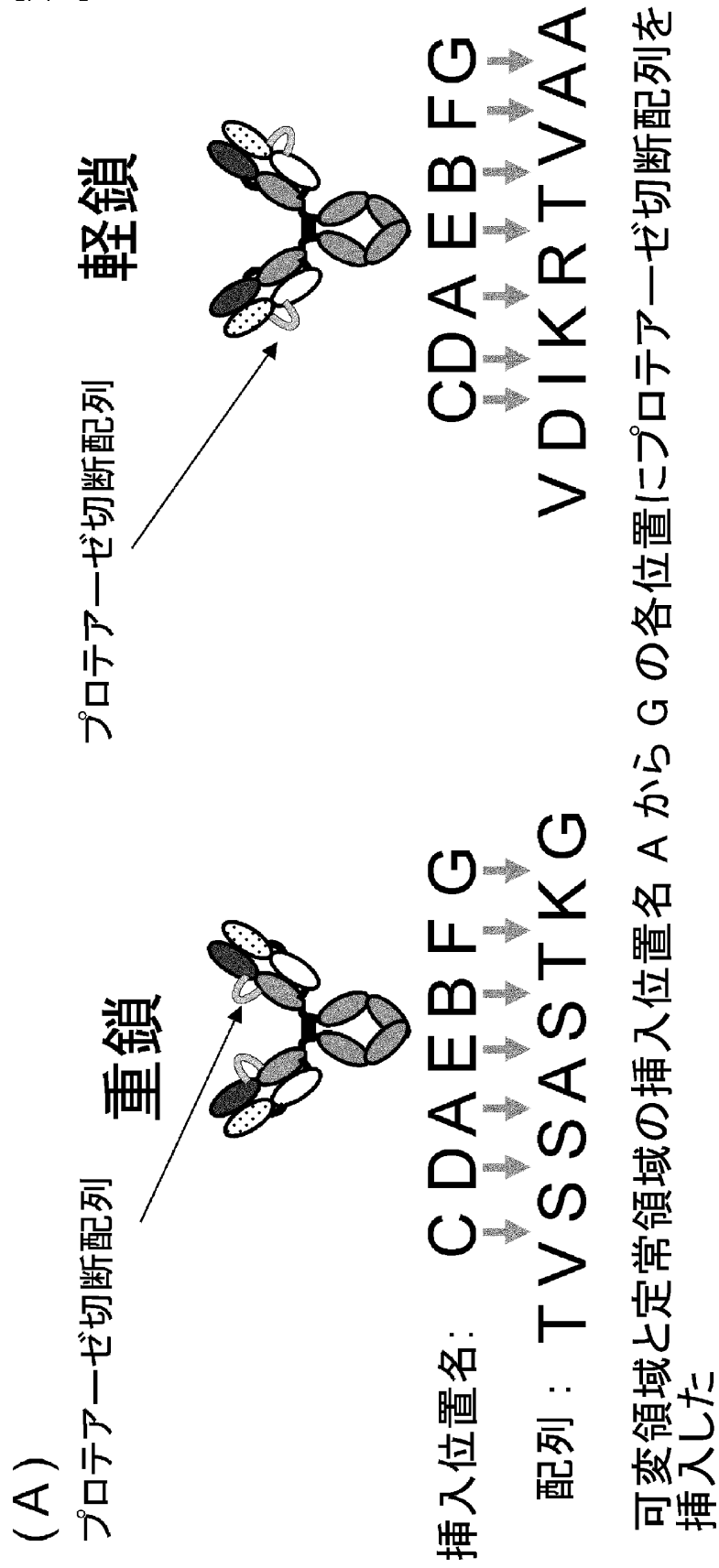
抗リガンド抗体に
プロテアーゼ切断配列を挿入する。
投与された抗体は
体内に存在するリガンドに結合する。

プロテアーゼによって切断されることで
リガンド-抗リガンド抗体複合体のリガンドとVH分子が遊離し、
遊離したリガンドが受容体に結合する

[図4]



[図5A]



[図5B]

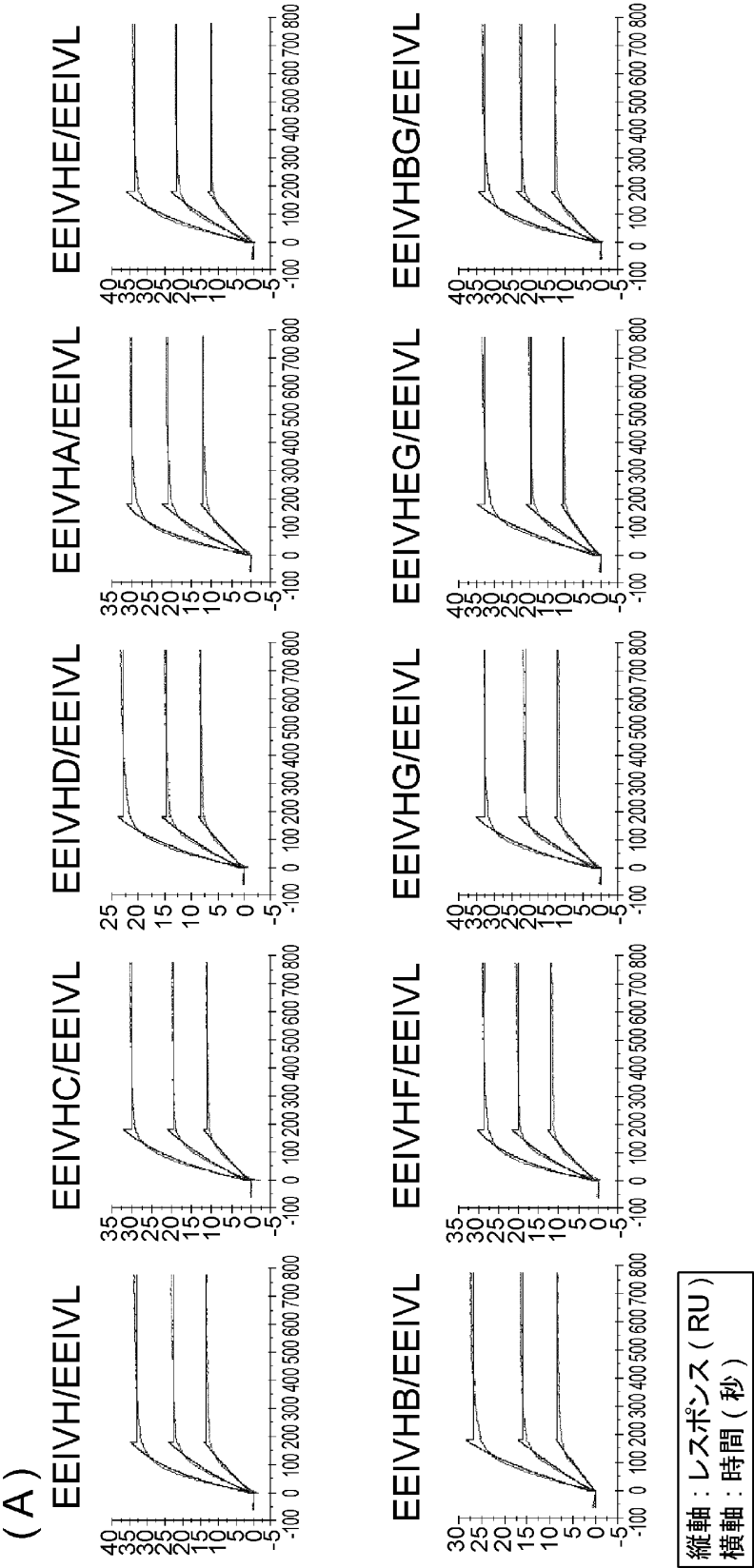
(B) 重鎖

重鎖改変体名	プロテアーゼ切断配列挿入位置	挿入配列
EEIVHA	TVSS [insert] ASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHB	TVSSAS [insert] TKGP	LSGRSDNH (配列番号 : 3)
EEIVHC	TV [insert] SSASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHD	TVS [insert] SASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHE	TVSSA [insert] STKGP	LSGRSDNH (配列番号 : 3)
EEIVHF	TVSSAST [insert] KGP	LSGRSDNH (配列番号 : 3)
EEIVHG	TVSSASTK [insert] GP	LSGRSDNH (配列番号 : 3)
EEIVHBG	TVSSAS [insert] GKGP	LSGRSDNH (配列番号 : 3)
EEIVHCG	TV [insert] GSASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHDG	TVS [insert] GASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHEG	TVSSA [insert] GTKGP	LSGRSDNH (配列番号 : 3)

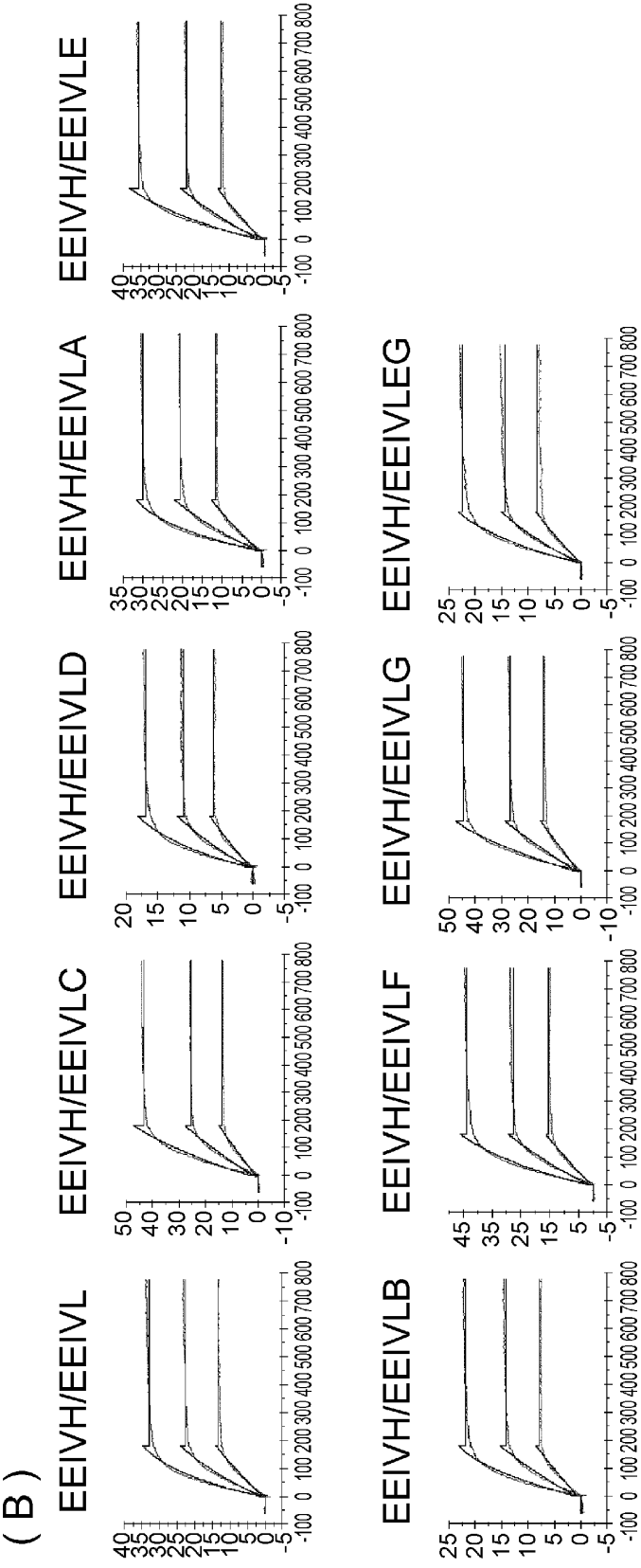
[図5C]

(C) 軽鎖		
軽鎖改変体名	プロテアーゼ切断配列挿入位置	挿入配列
EEIVLA	VDIK [insert] RTVAAP	LSGRSDNH (配列番号 : 3)
EEIVLB	VDIKRT [insert] VAAP	LSGRSDNH (配列番号 : 3)
EEIVLC	VD [insert] IKRTVAAP	LSGRSDNH (配列番号 : 3)
EEIVLD	VDI [insert] KRTVAAP	LSGRSDNH (配列番号 : 3)
EEIVLE	VDIKR [insert] TVAAP	LSGRSDNH (配列番号 : 3)
EEIVLF	VDIKRTV [insert] AAP	LSGRSDNH (配列番号 : 3)
EEIVLG	VDIKRTVA [insert] AP	LSGRSDNH (配列番号 : 3)
EEIVLEG	VDIKR [insert] GVAAP	LSGRSDNH (配列番号 : 3)

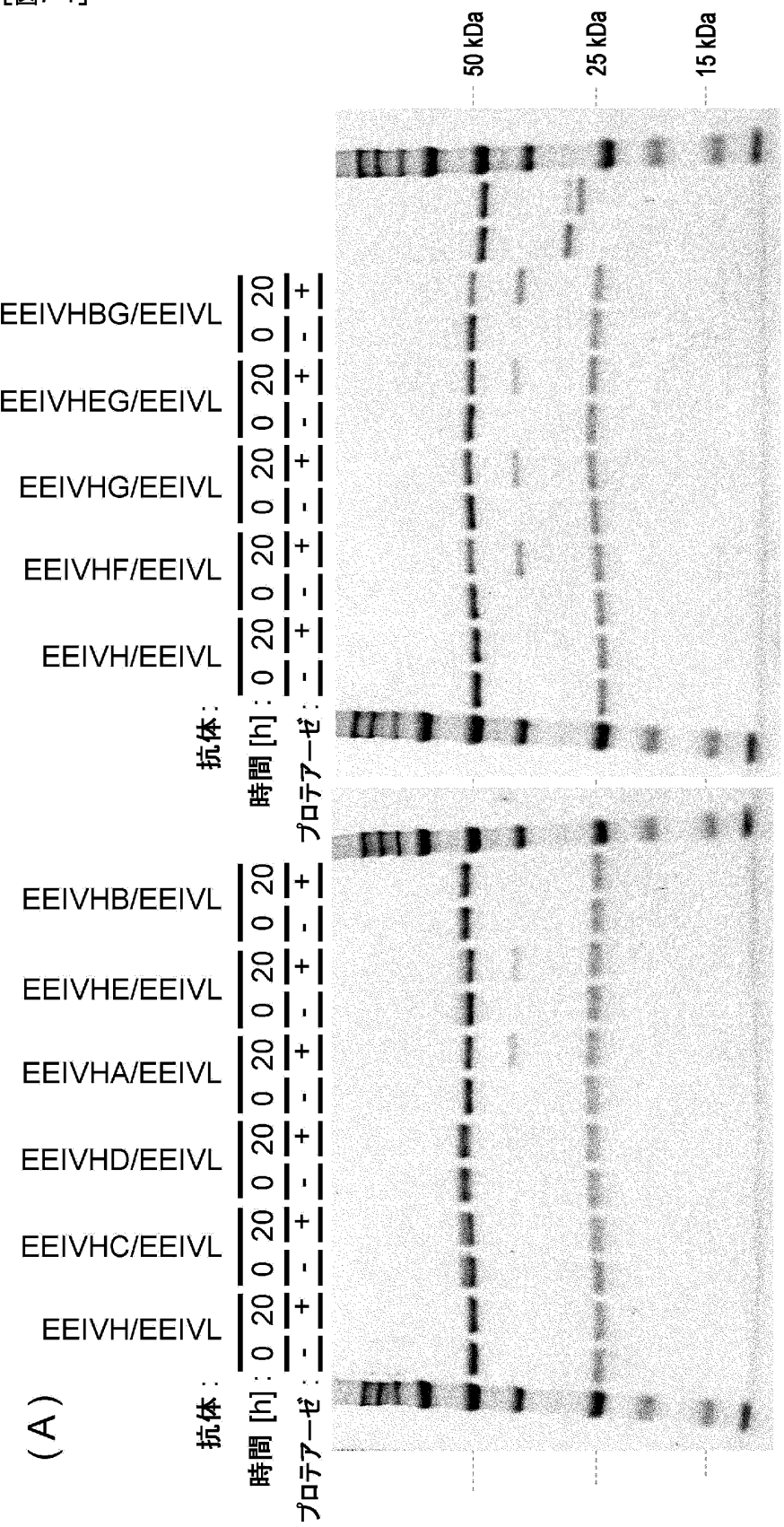
[図6A]



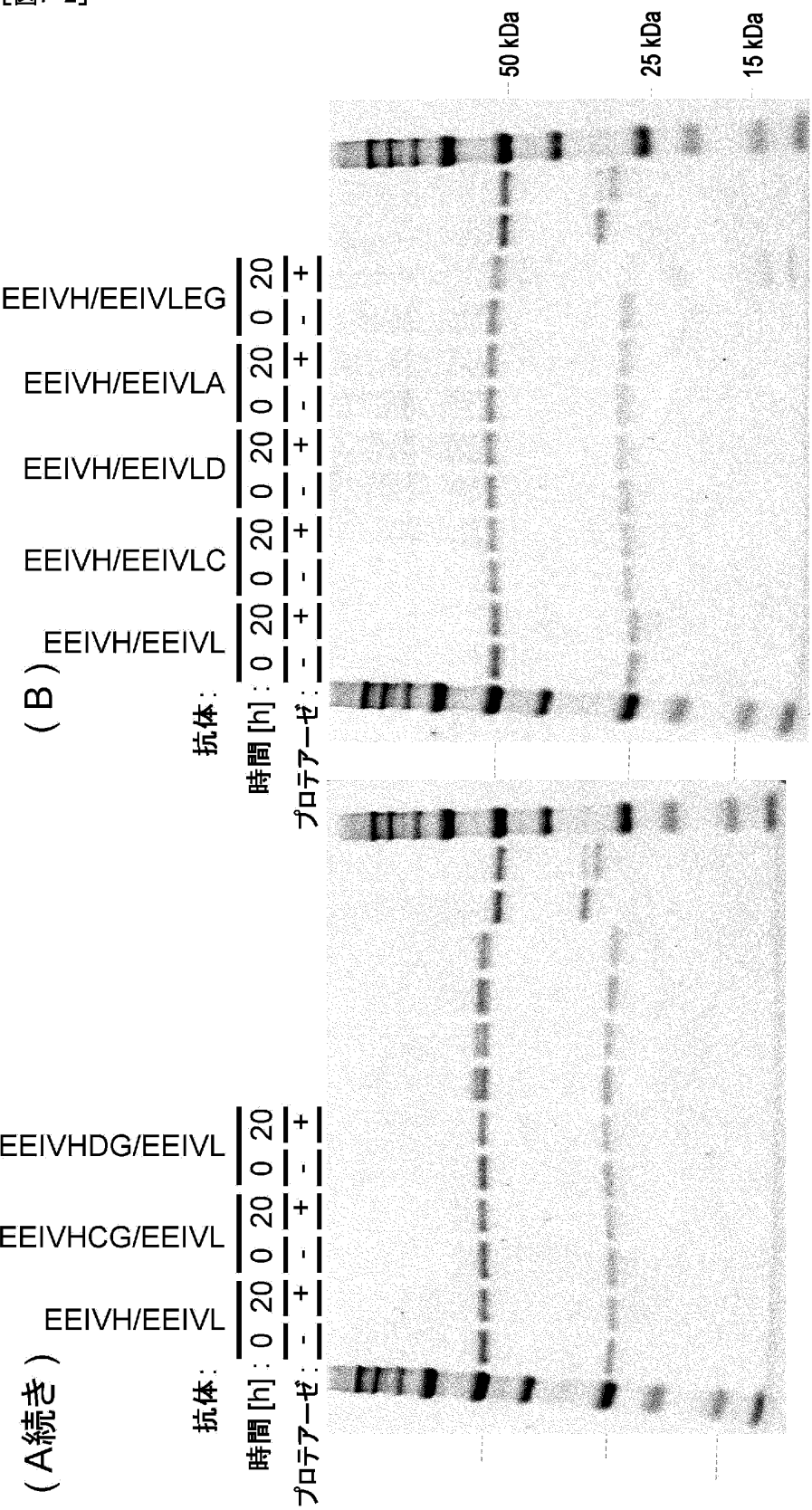
[図6B]



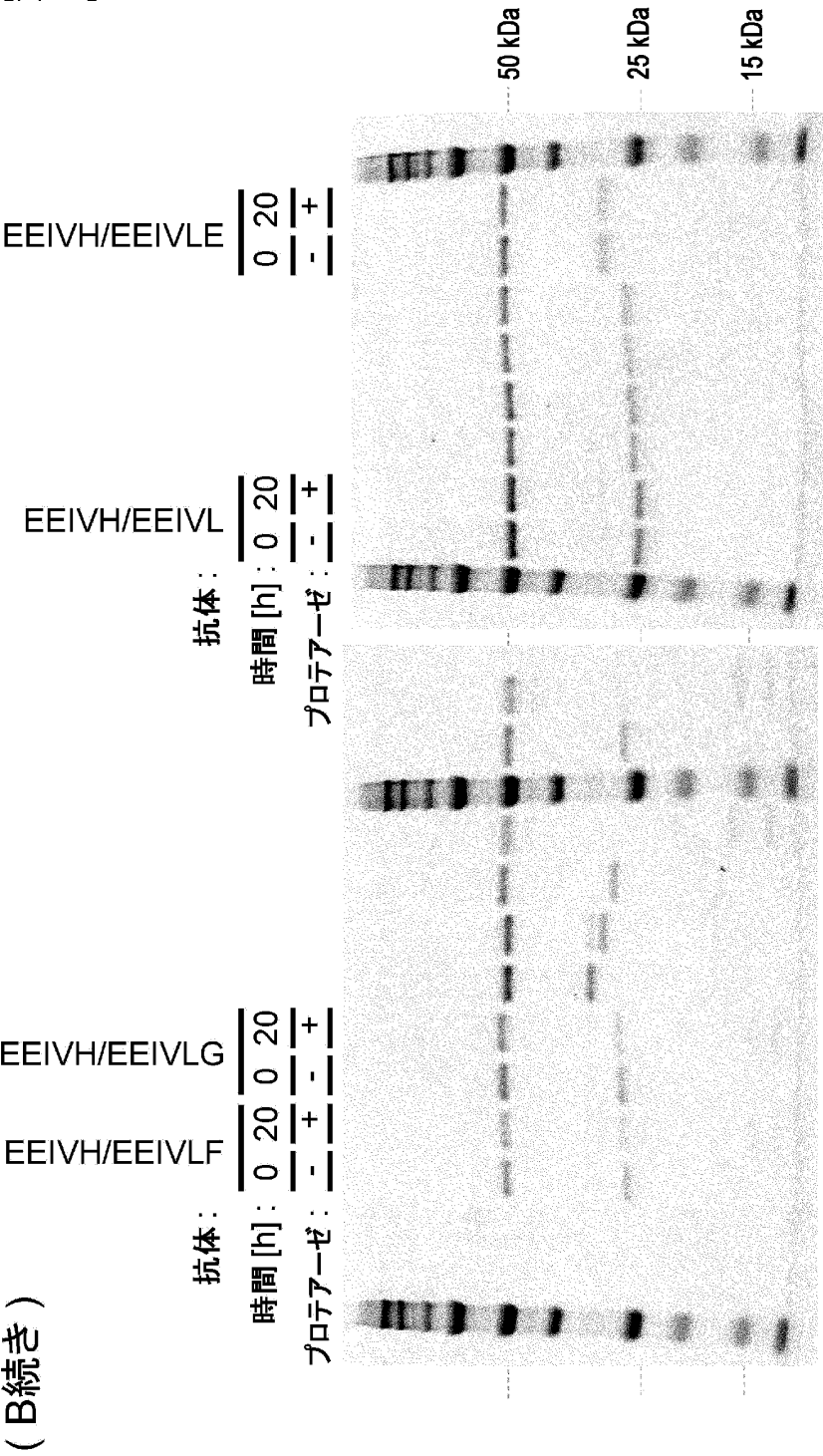
[図7-1]



[図7-2]



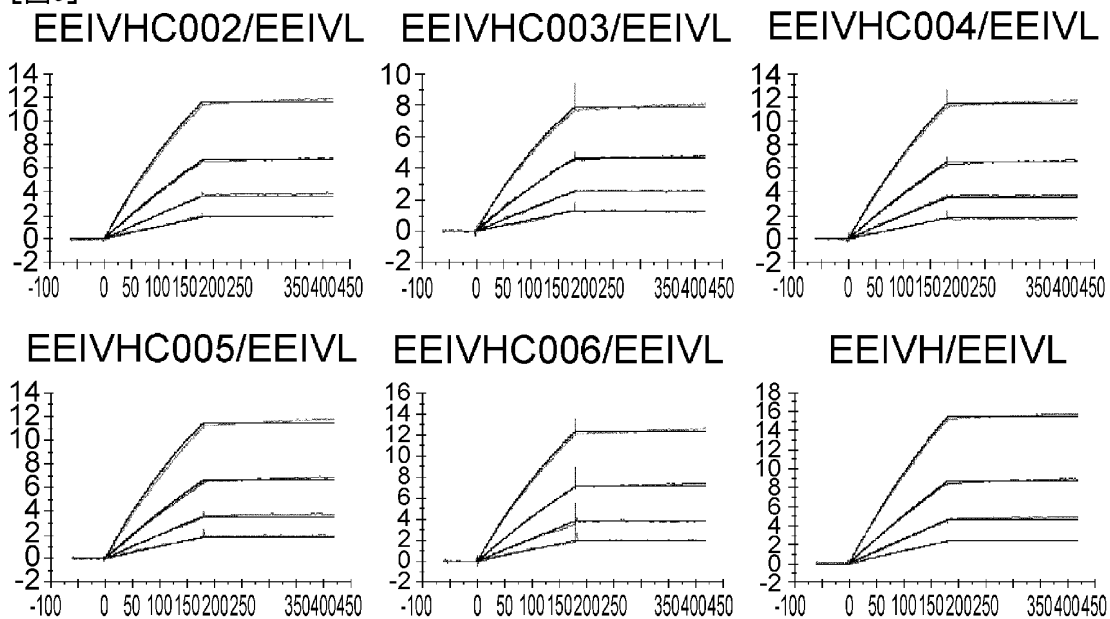
[図7-3]



[図8]

重鎖改変体名	プロテアーゼ切断配列挿入位置	挿入配列
EEIVHC	TV [insert] SSASTKGP	LSGRSDNH (配列番号 : 3)
EEIVHC002	TV [insert] SSASTKGP	GLSGRSDNHG (配列番号 : 28)
EEIVHC003	TV [insert] SSASTKGP	SGLSGRSDNHGS (配列番号 : 29)
EEIVHC004	TV [insert] SSASTKGP	GSGLSGRSDNHGSS (配列番号 : 30)
EEIVHC005	TV [insert] SSASTKGP	GGSGLSGRSDNHGSSG (配列番号 : 31)
EEIVHC006	TV [insert] SSASTKGP	SGSGLSGRSDNHGSSGT (配列番号 : 32)

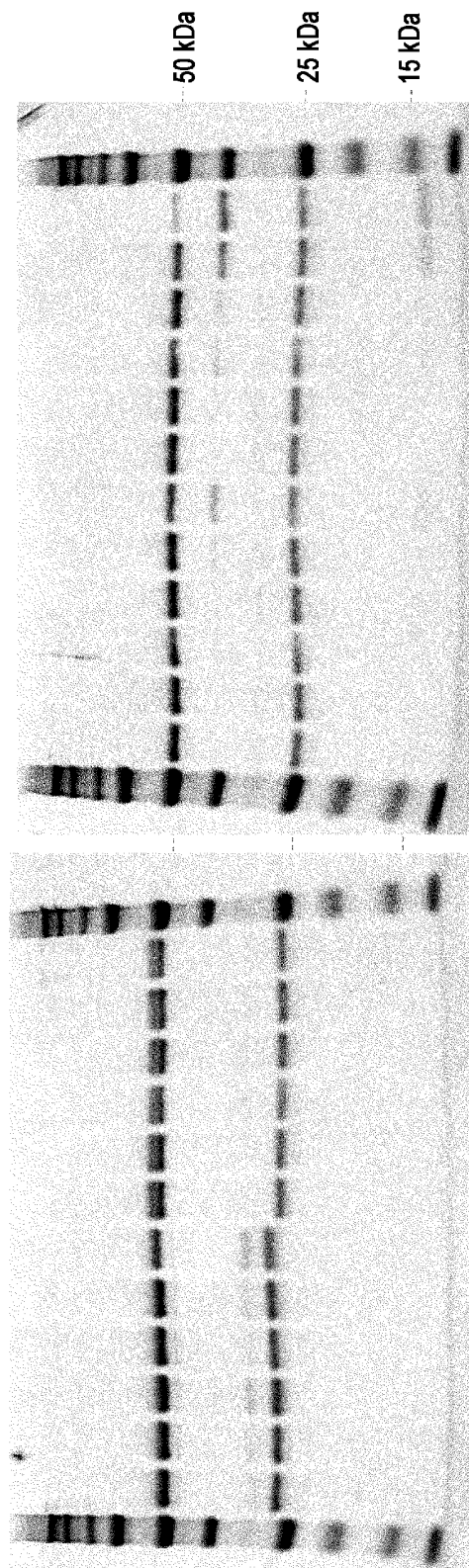
[図9]



[図10A]

(A)

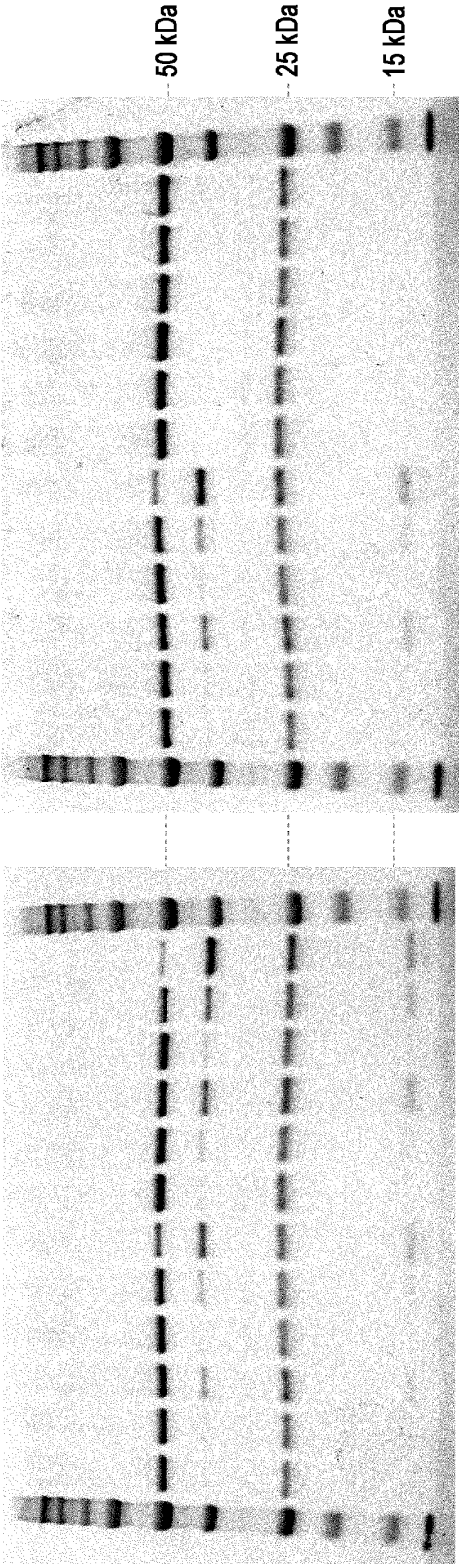
抗体：				抗体：				抗体：			
プロテアーゼ：				プロテアーゼ：				プロテアーゼ：			
時間 [h]：				時間 [h]：				時間 [h]：			
EEIVHC/EEIVL				EEIVHC002/EEIVL				EEIVHC003/EEIVL			
-	uPA	-	MT-SP1	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1
0	2	20	0	2	20	0	2	20	0	2	20



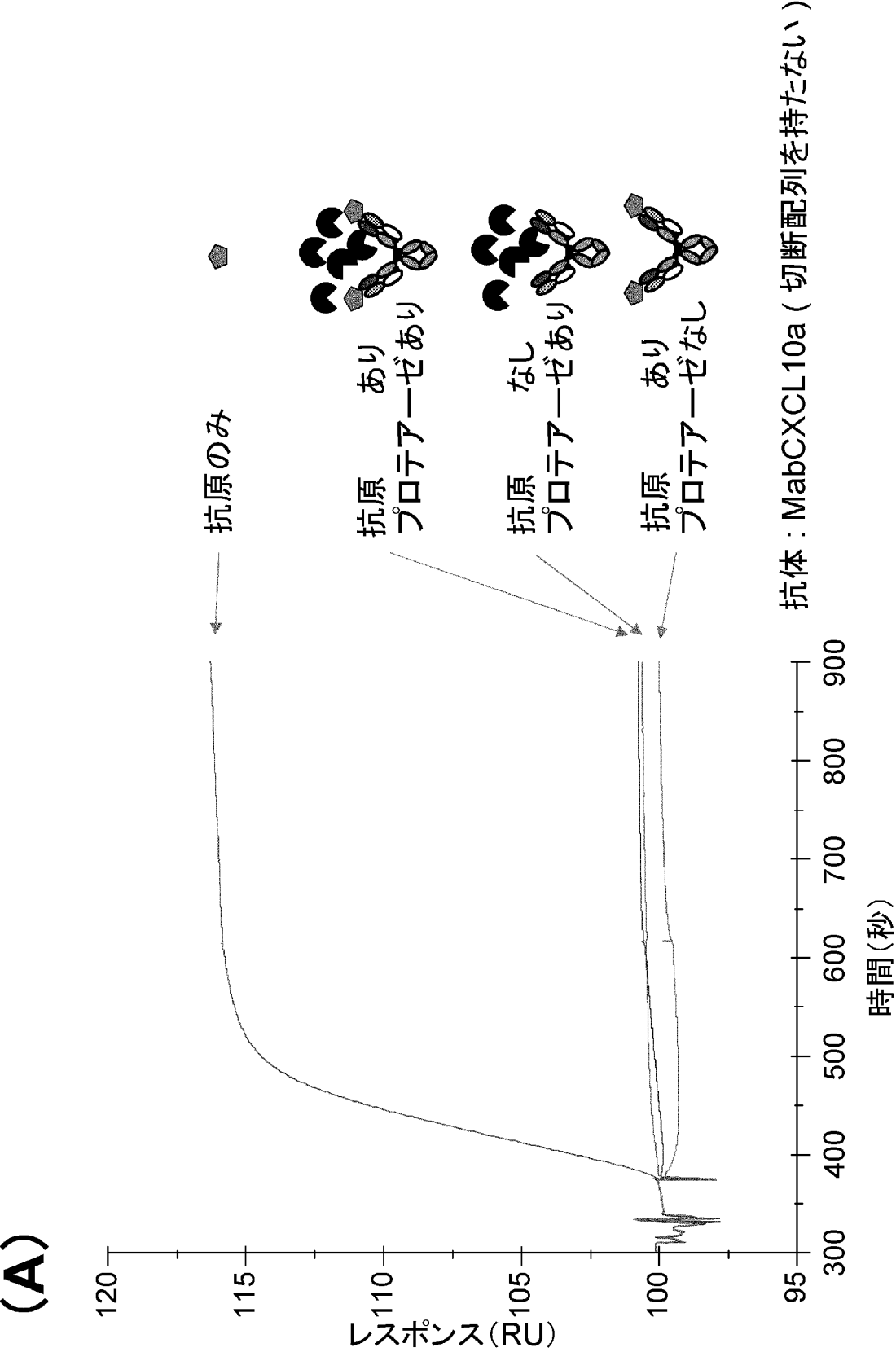
[図10B]

(B)

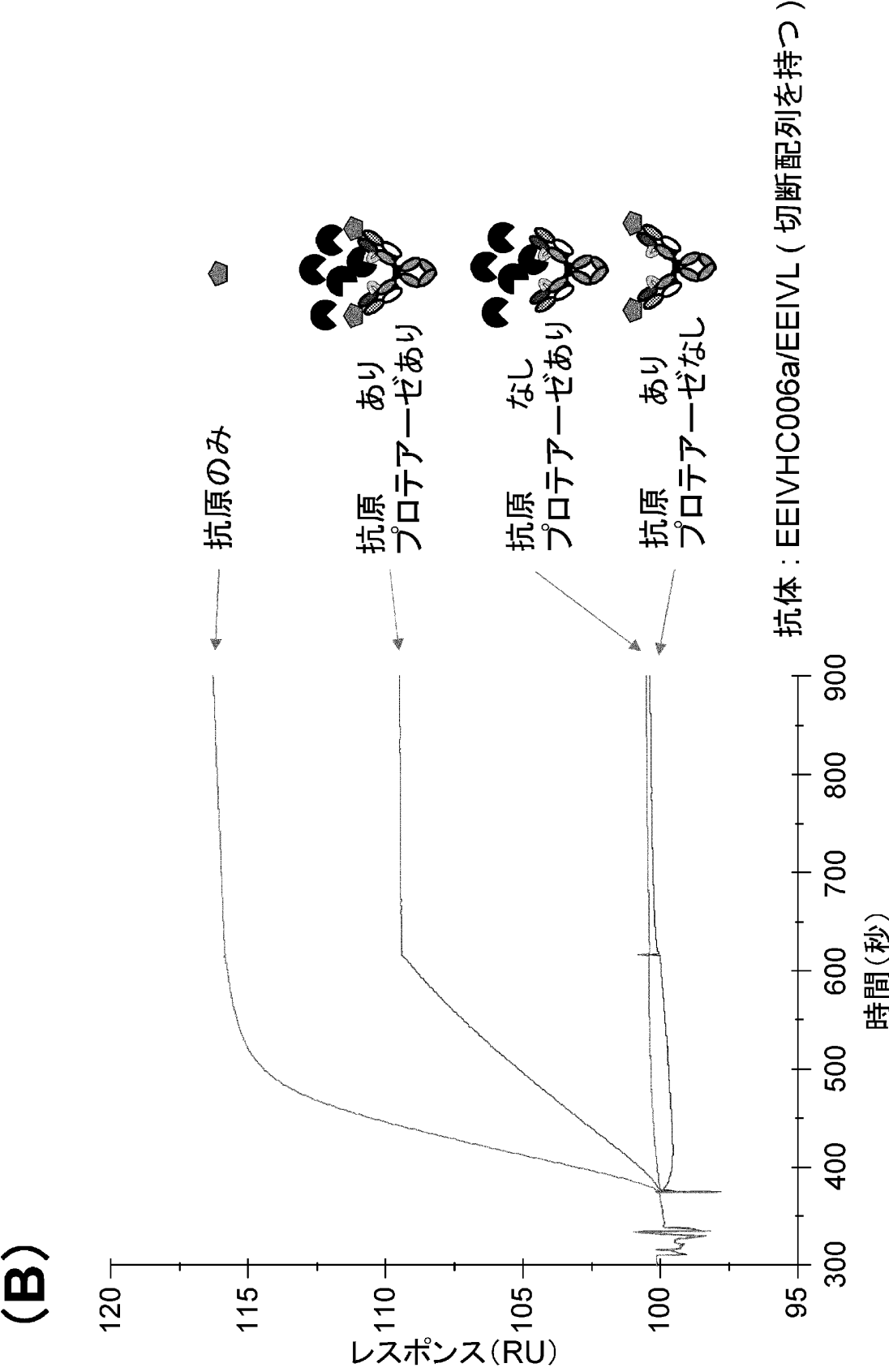
抗体： EEIVHC004/EEIVL				抗体： EEIVHC006/EEIVL				抗体： EEIVH/EEIVL			
プロテアーゼ： - uPA - MT-SP1				プロテアーゼ： - uPA - MT-SP1				プロテアーゼ： - uPA - MT-SP1			
時間 [h]： 0 2 20 0 2 20 0 2 20				時間 [h]： 0 2 20 0 2 20 0 2 20				時間 [h]： 0 2 20 0 2 20 0 2 20			



[図11A]



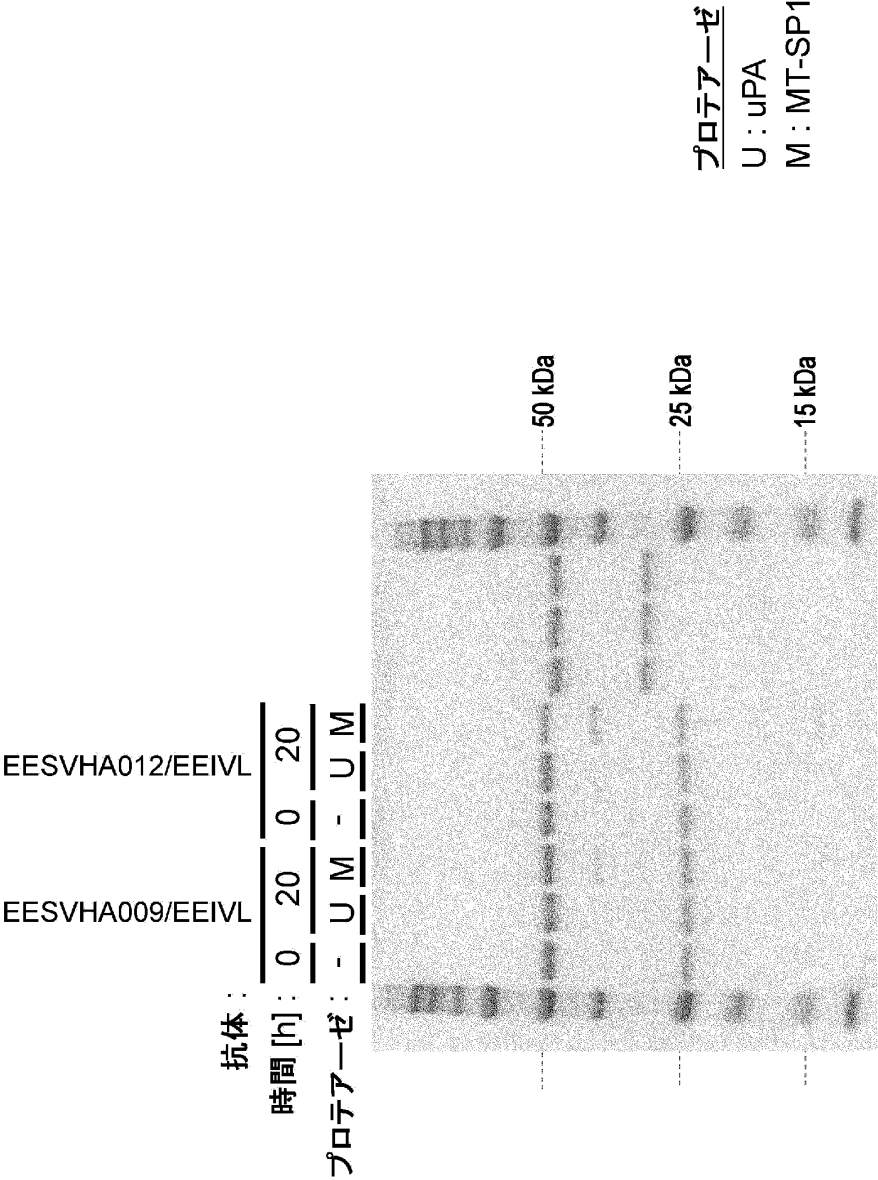
[図11B]



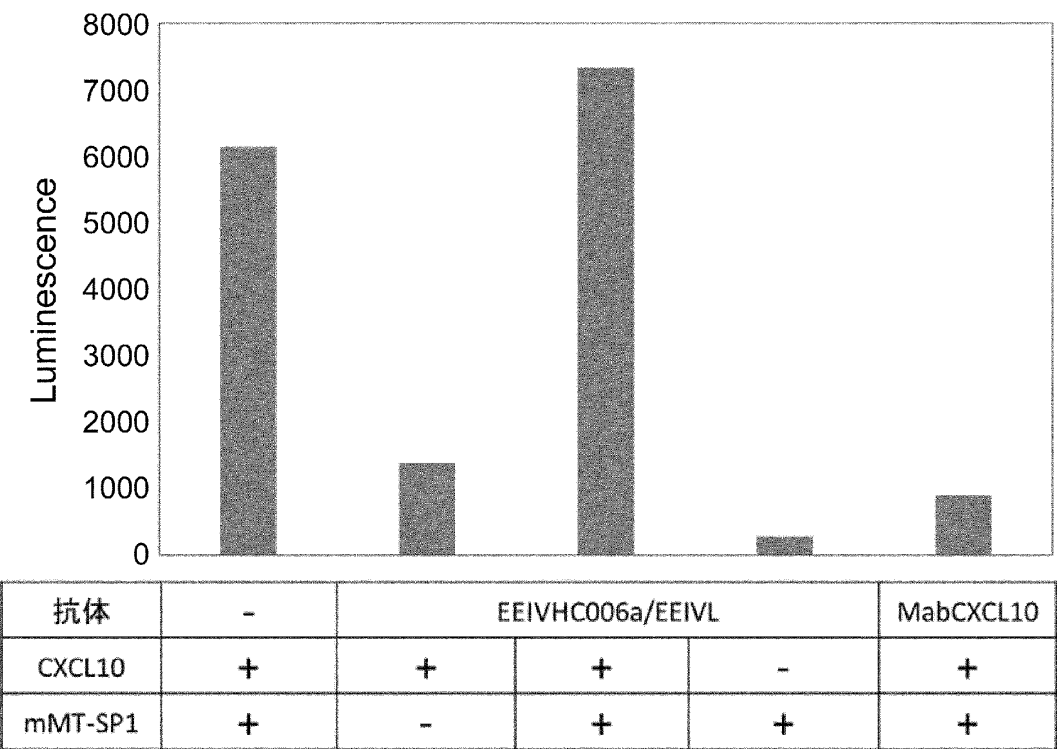
[図12]

重鎖改変体名	挿入位置及び改変位置	挿入配列	挿入及び改変後の配列
EESVHA009	TVSS [insert] A-STKGP	GLSGRSDNHGS (配列番号：61)	TVSSGLSGRSDNHGS STKGP (配列番号：62)
EESVHA012	TVSS [insert] A-STKGP	GGSLSGRSDNHGSSGT (配列番号：63)	TVSSGGSLSGRSDNHGSS GTSTKGP (配列番号：64)

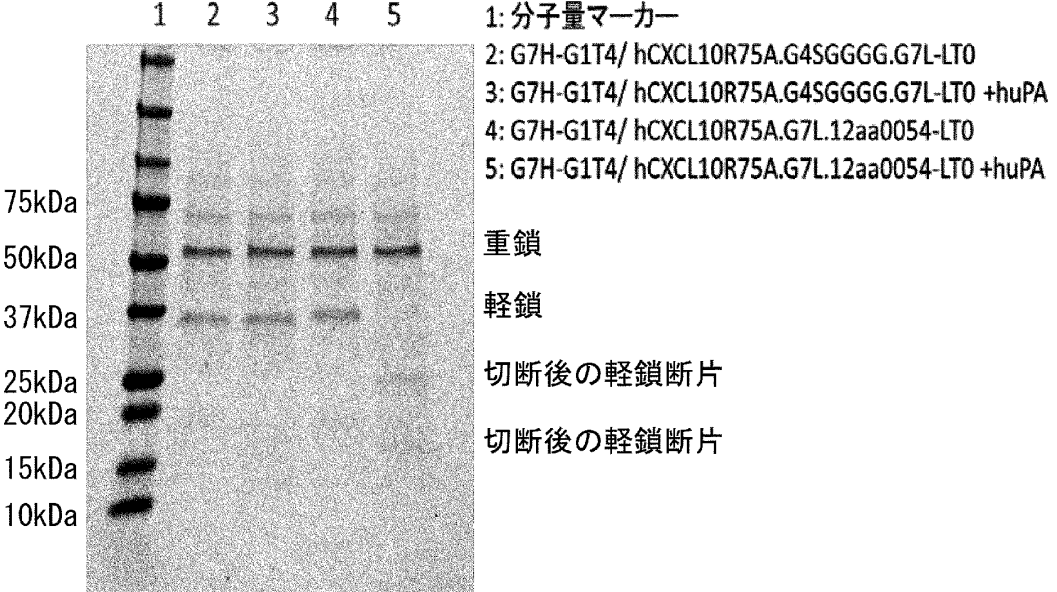
[図13]

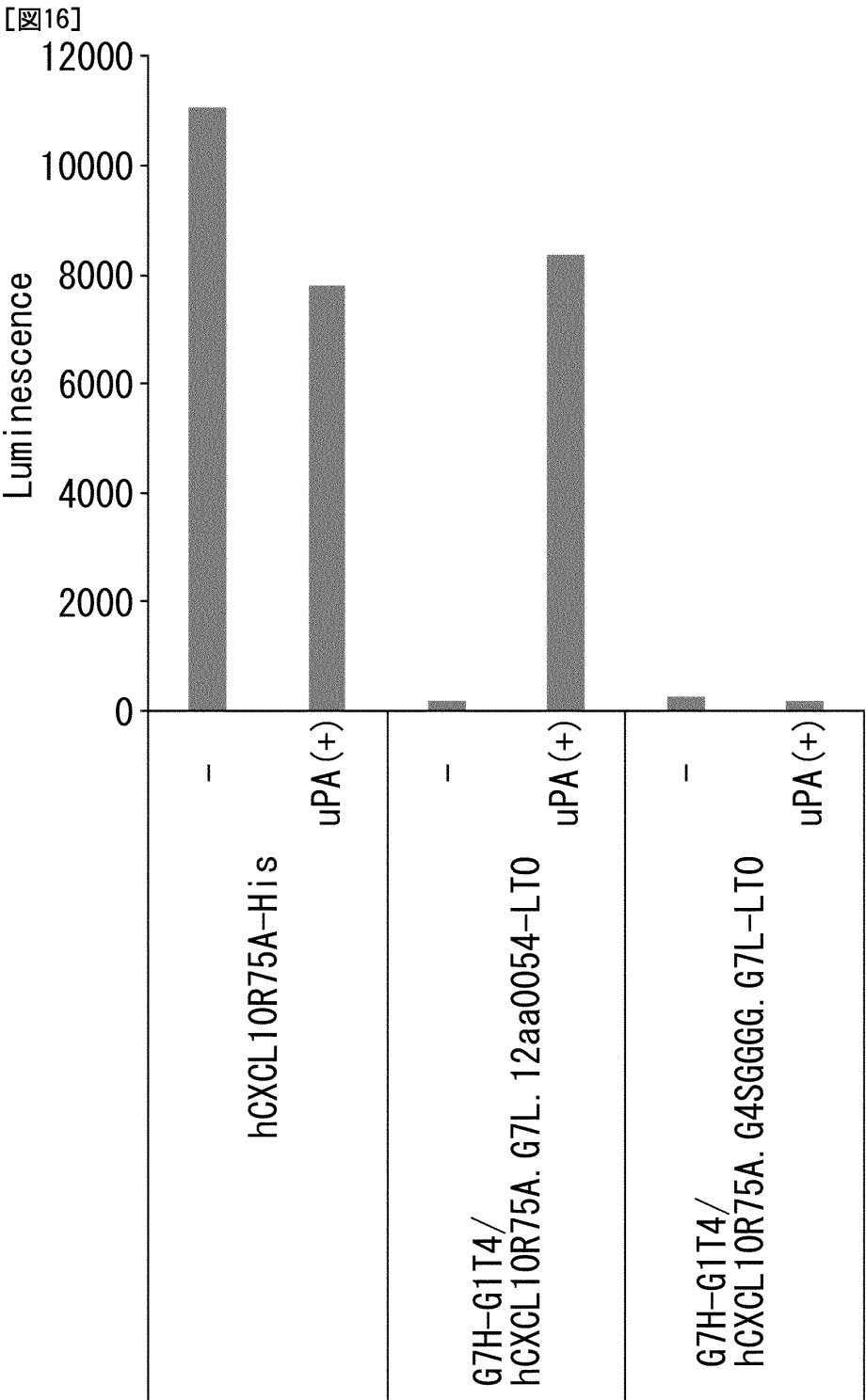


[図14]

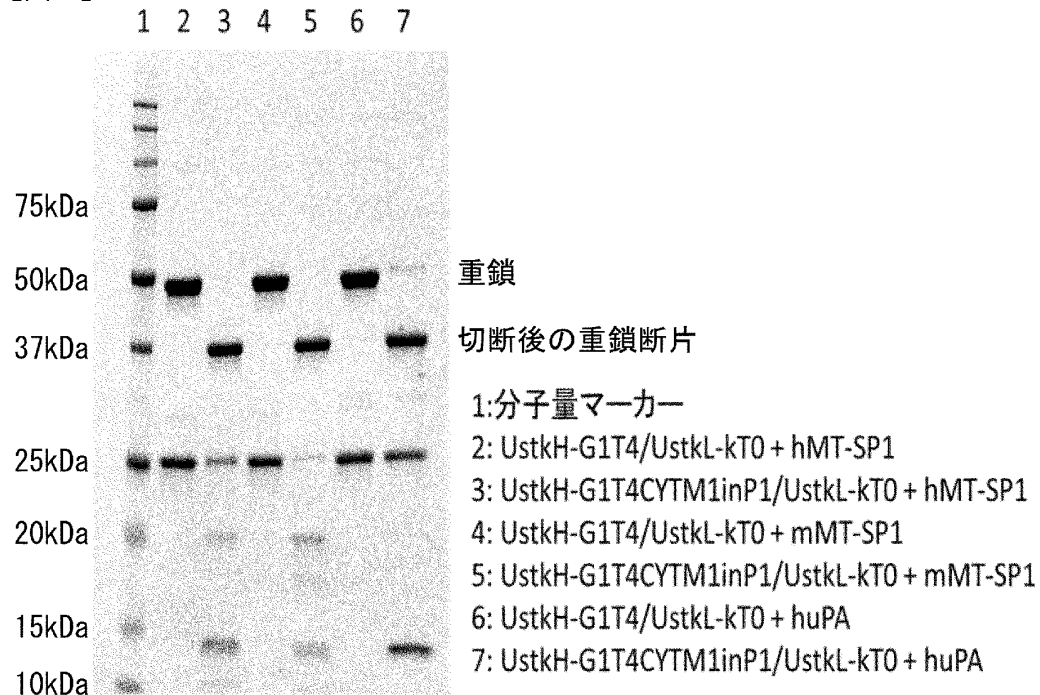


[図15]

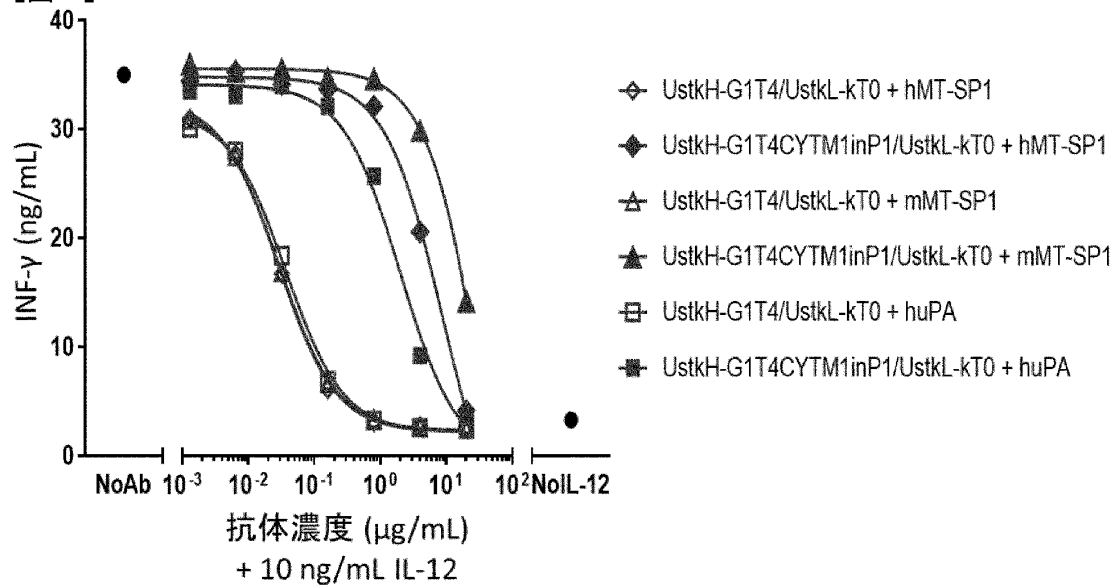




[図17]

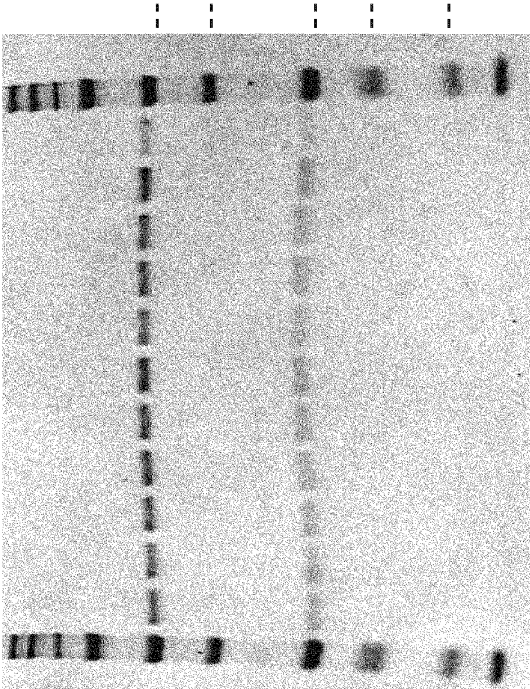


[図18]

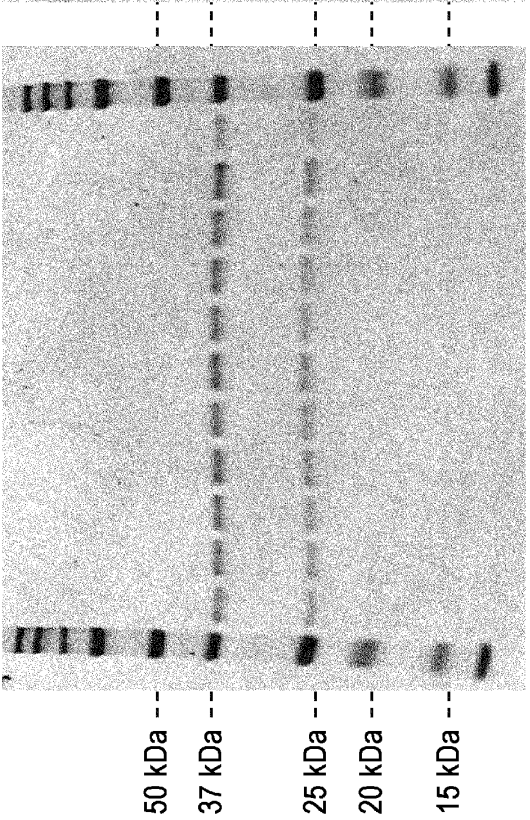


[図19A]

EldHA0003	MT-SP1	0 nM
EldHA12aa0126	MT-SP1	0 nM
EldHA12aa0103	MT-SP1	0 nM
EldHA12aa0095	MT-SP1	0 nM
EldHA12aa0089	MT-SP1	0 nM
EldHA12aa0081	MT-SP1	0 nM
EldHA12aa0063	MT-SP1	0 nM
EldHA12aa0054	MT-SP1	0 nM
EldHA12aa0016	MT-SP1	0 nM
EldHA12aa0010	MT-SP1	0 nM
EldHA12aa0004	MT-SP1	0 nM
抗体	プロテアーゼ	

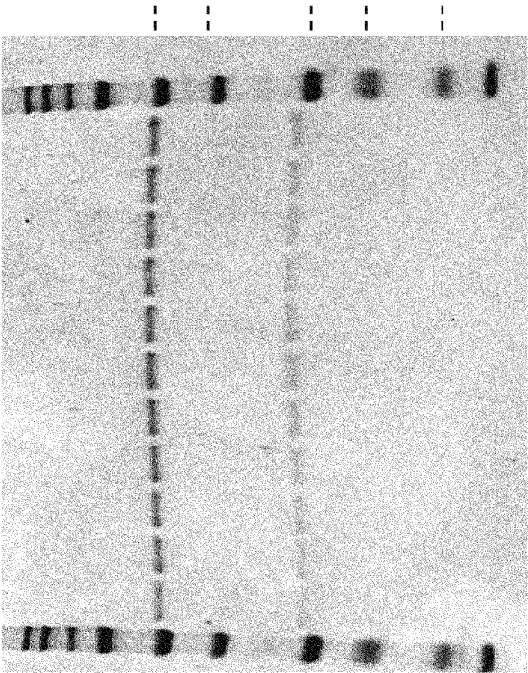


EldHA0003	MT-SP1	10 nM
EldHA12aa0126	MT-SP1	10 nM
EldHA12aa0103	MT-SP1	10 nM
EldHA12aa0095	MT-SP1	10 nM
EldHA12aa0089	MT-SP1	10 nM
EldHA12aa0081	MT-SP1	10 nM
EldHA12aa0063	MT-SP1	10 nM
EldHA12aa0054	MT-SP1	10 nM
EldHA12aa0016	MT-SP1	10 nM
EldHA12aa0010	MT-SP1	10 nM
EldHA12aa0004	MT-SP1	10 nM
抗体	プロテアーゼ	

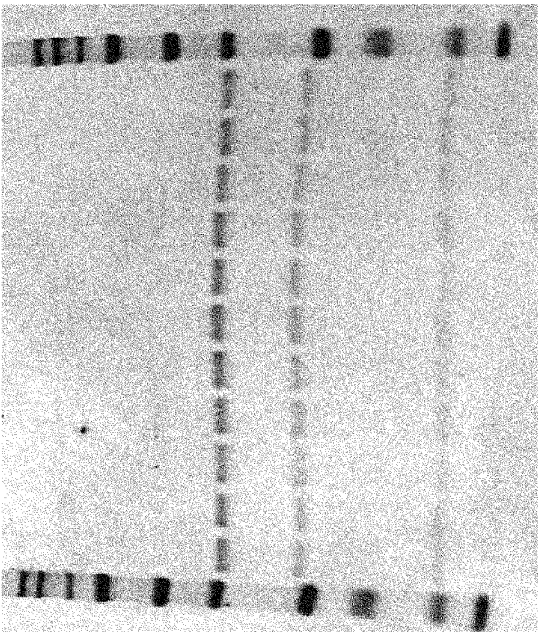


[図19B]

G7H.12aa	MT-SP1	0 nM
G7H.12aa0126	MT-SP1	0 nM
G7H.12aa0103	MT-SP1	0 nM
G7H.12aa0095	MT-SP1	0 nM
G7H.12aa0089	MT-SP1	0 nM
G7H.12aa0081	MT-SP1	0 nM
G7H.12aa0063	MT-SP1	0 nM
G7H.12aa0054	MT-SP1	0 nM
G7H.12aa0016	MT-SP1	0 nM
G7H.12aa0010	MT-SP1	0 nM
G7H.12aa0004	MT-SP1	0 nM
抗体	プロテアーゼ	

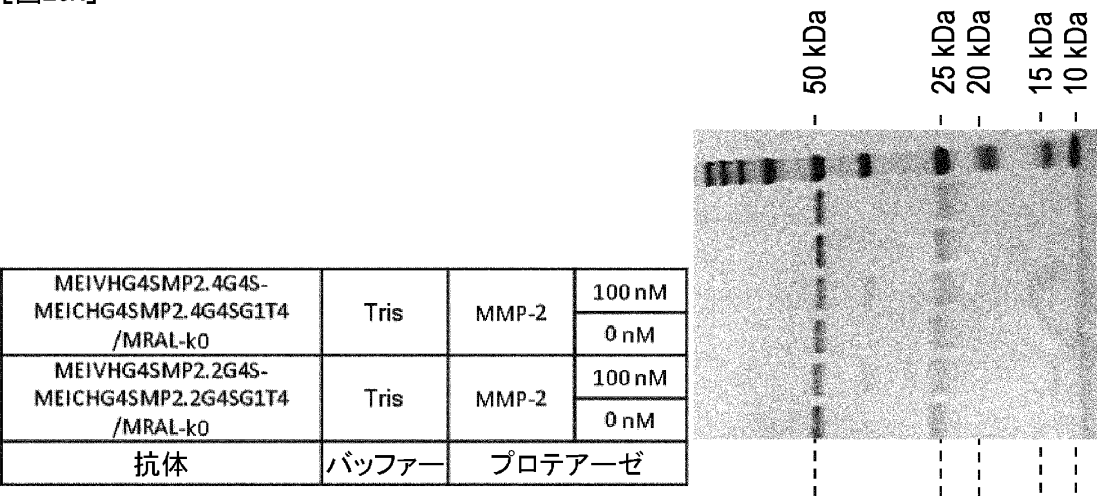


G7H.12aa	MT-SP1	10 nM
G7H.12aa0126	MT-SP1	10 nM
G7H.12aa0103	MT-SP1	10 nM
G7H.12aa0095	MT-SP1	10 nM
G7H.12aa0089	MT-SP1	10 nM
G7H.12aa0081	MT-SP1	10 nM
G7H.12aa0063	MT-SP1	10 nM
G7H.12aa0054	MT-SP1	10 nM
G7H.12aa0016	MT-SP1	10 nM
G7H.12aa0010	MT-SP1	10 nM
G7H.12aa0004	MT-SP1	10 nM
抗体	プロテアーゼ	

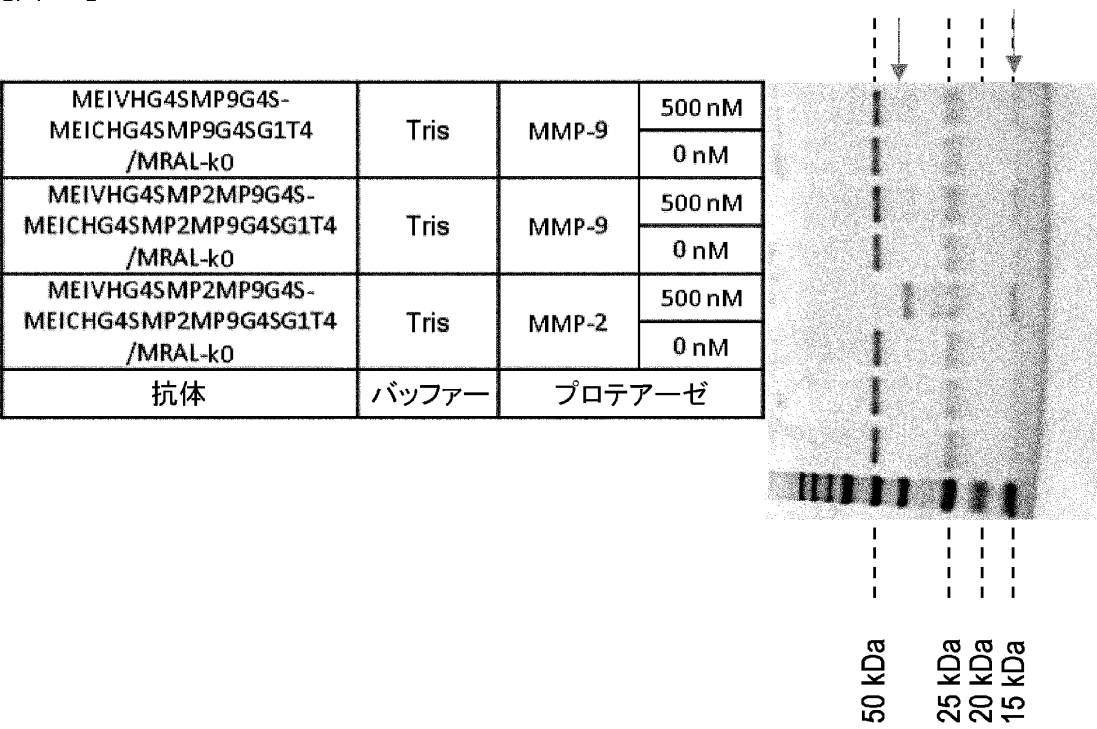


50 kDa
37 kDa
25 kDa
20 kDa
15 kDa

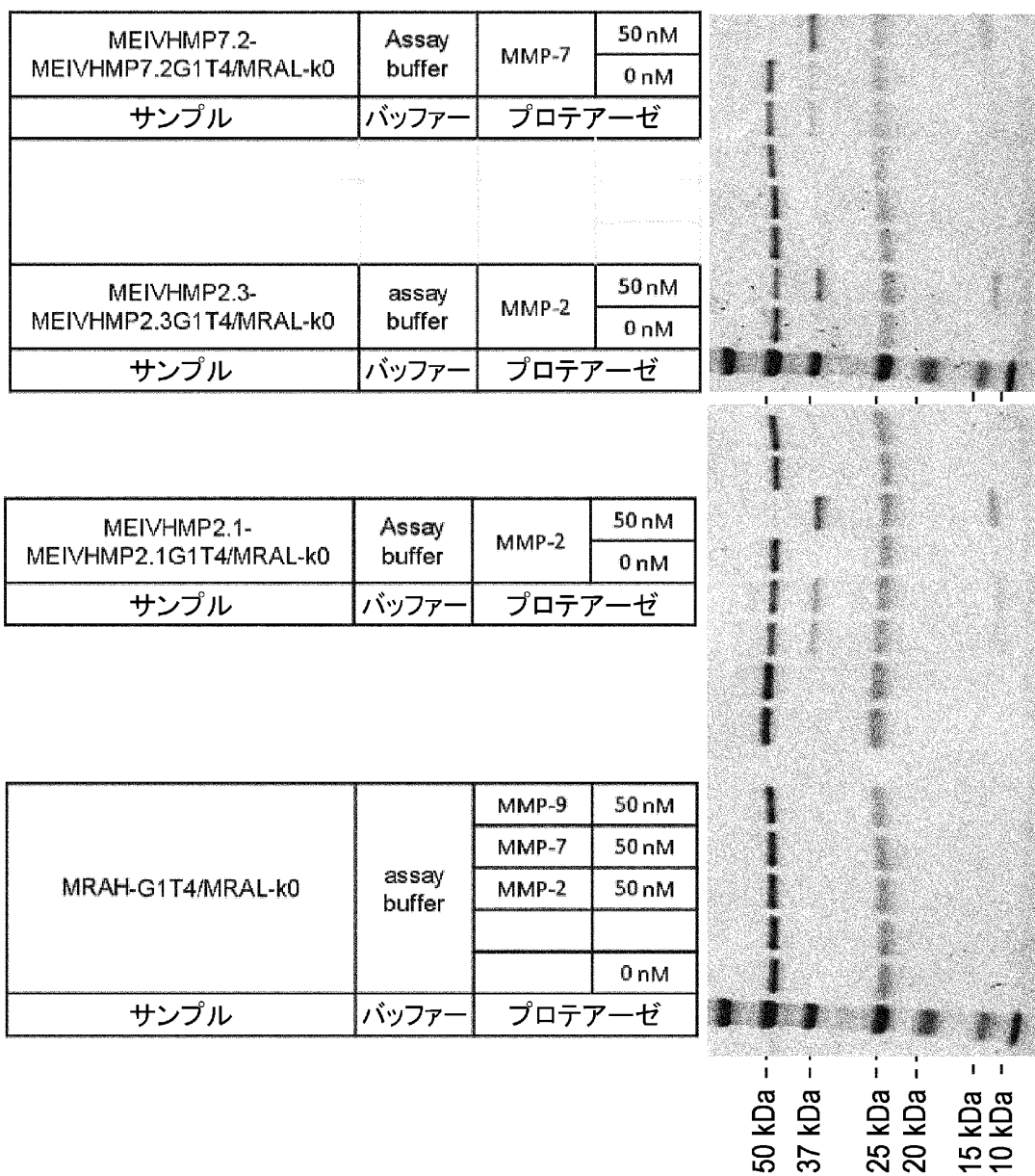
[図20A]



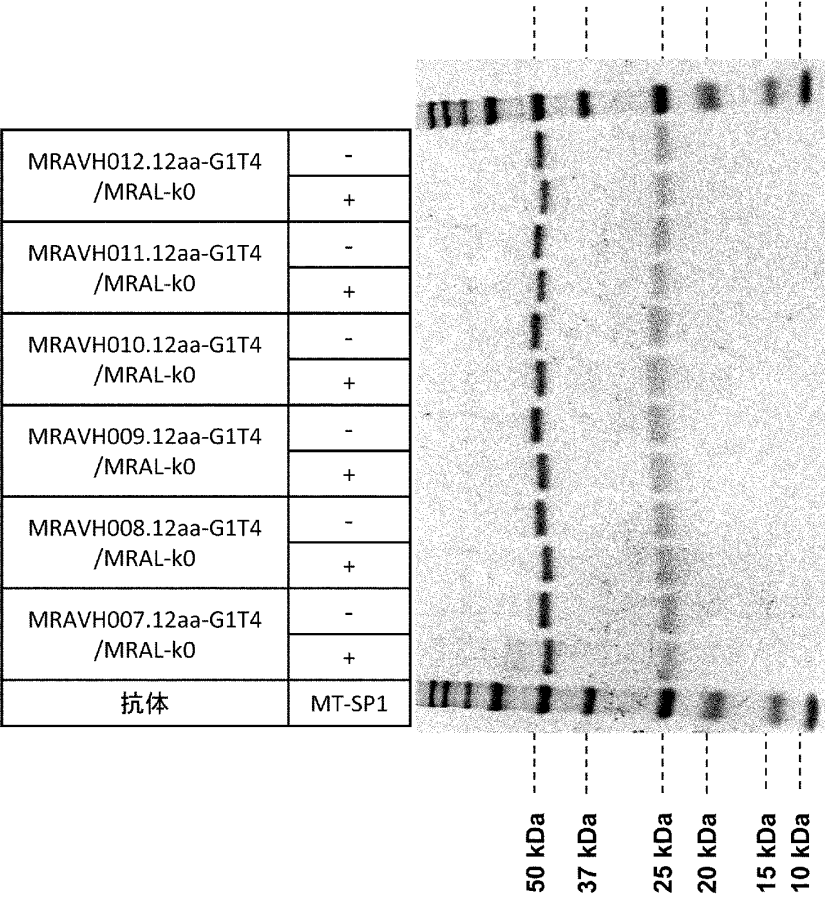
[図20B]



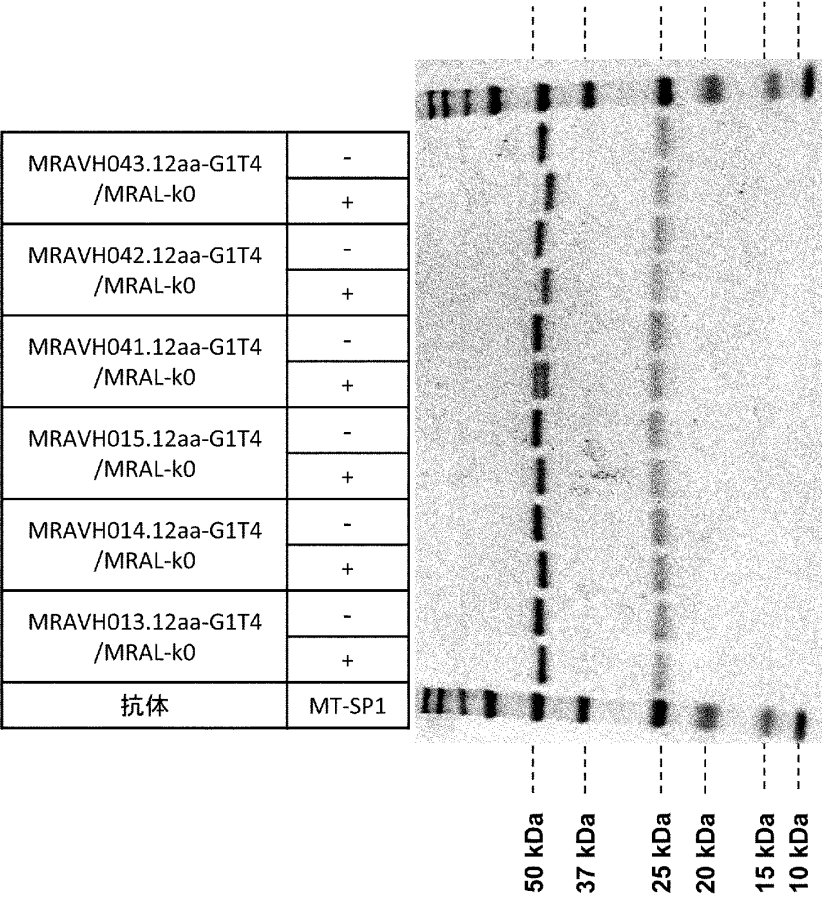
[図21]



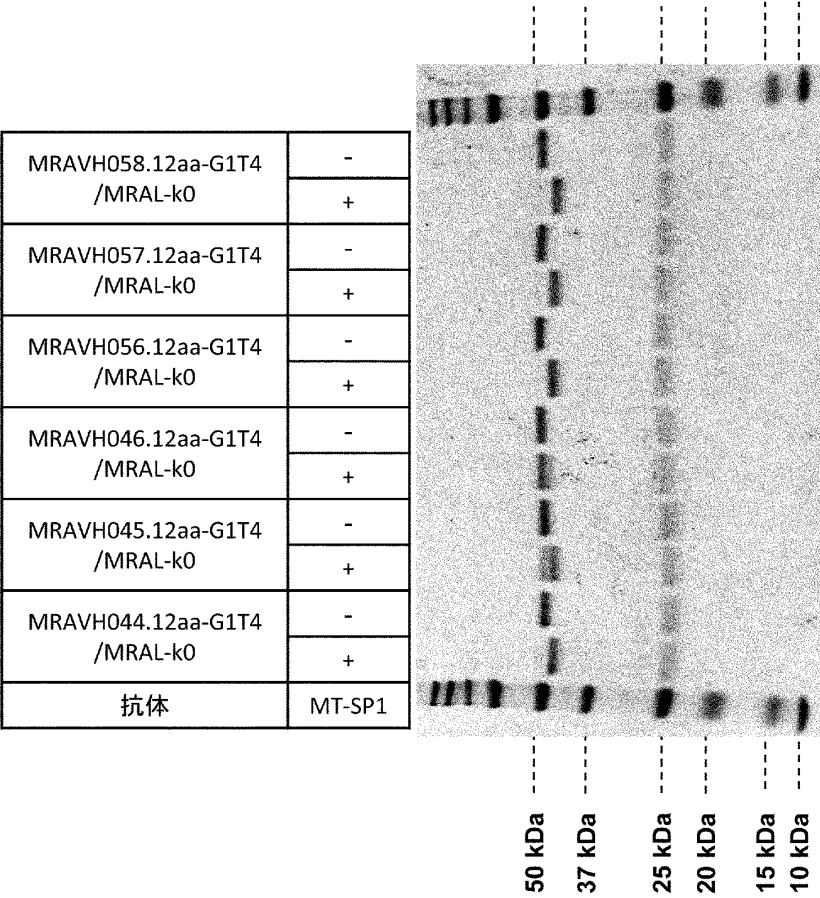
[図22A]



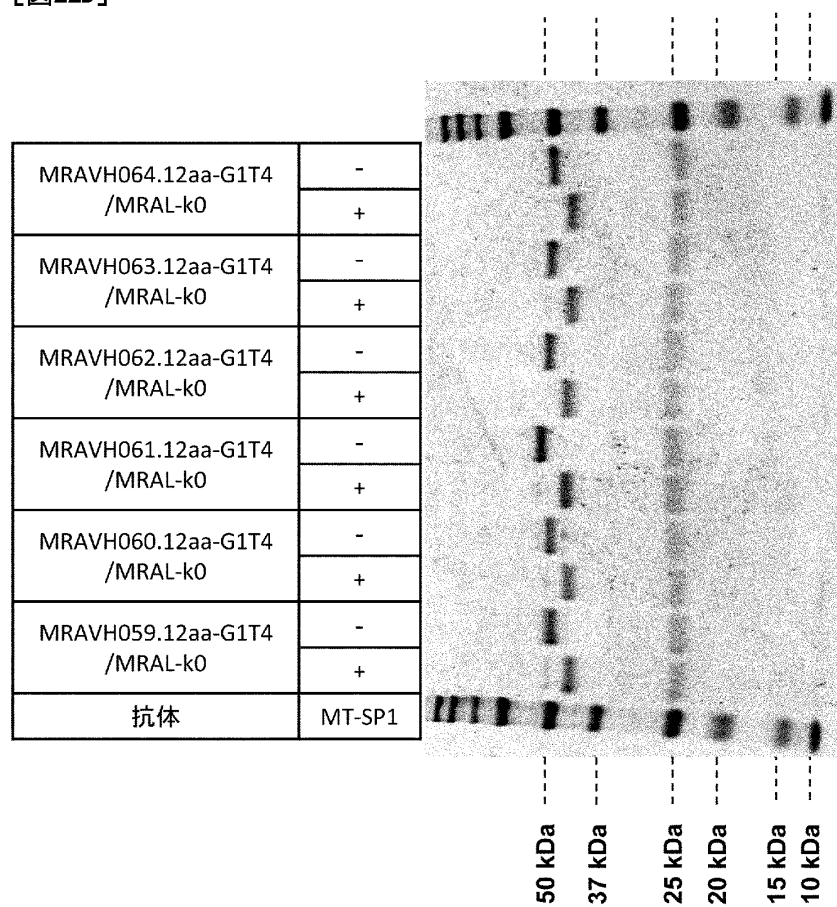
[図22B]



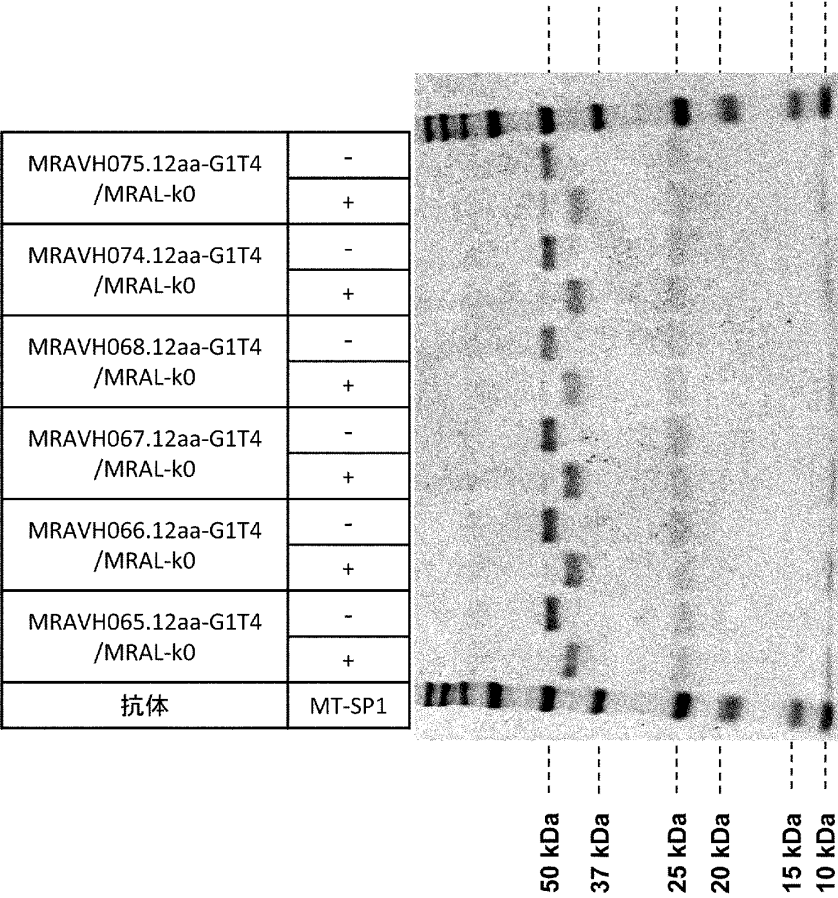
[図22C]



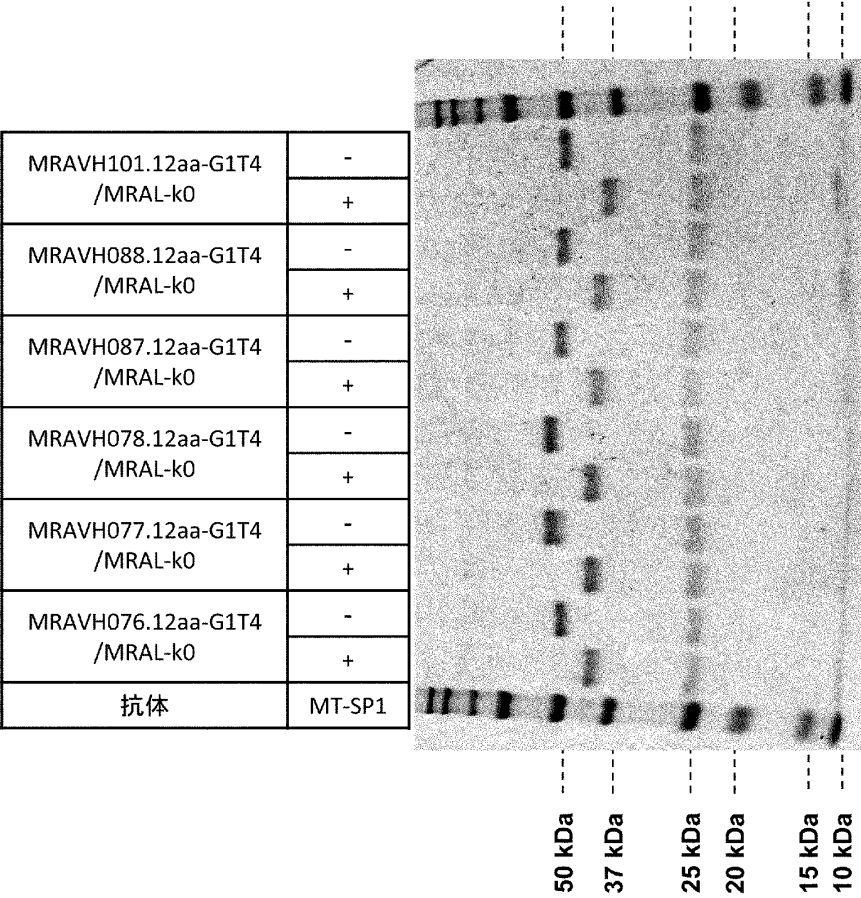
[図22D]



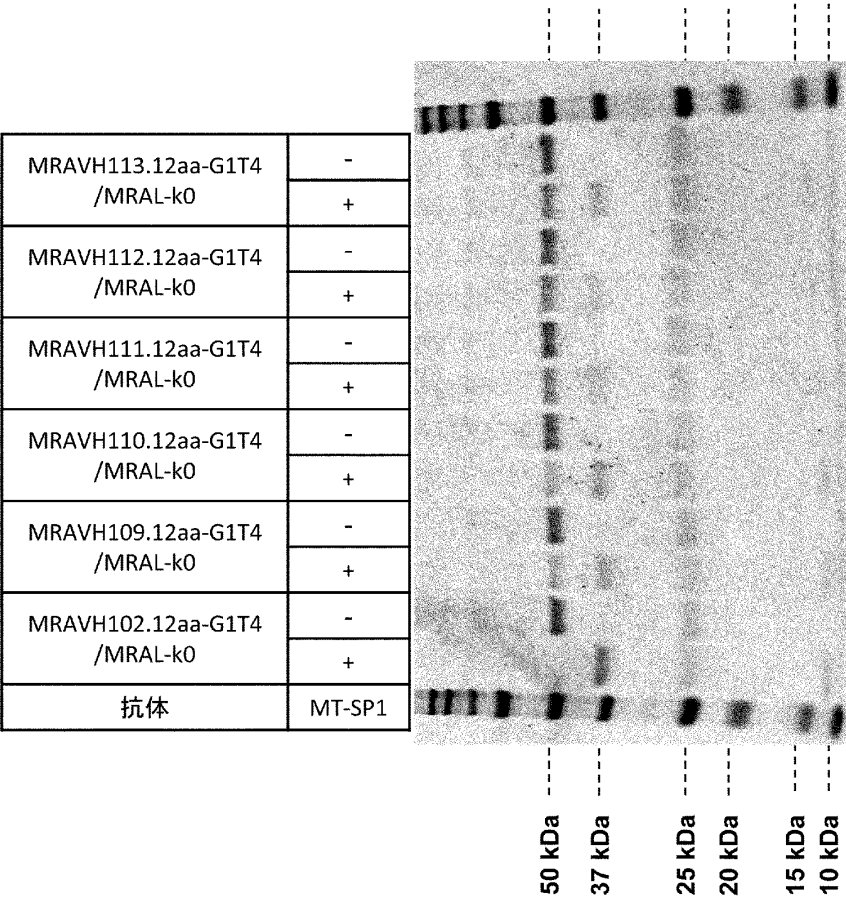
[図22E]



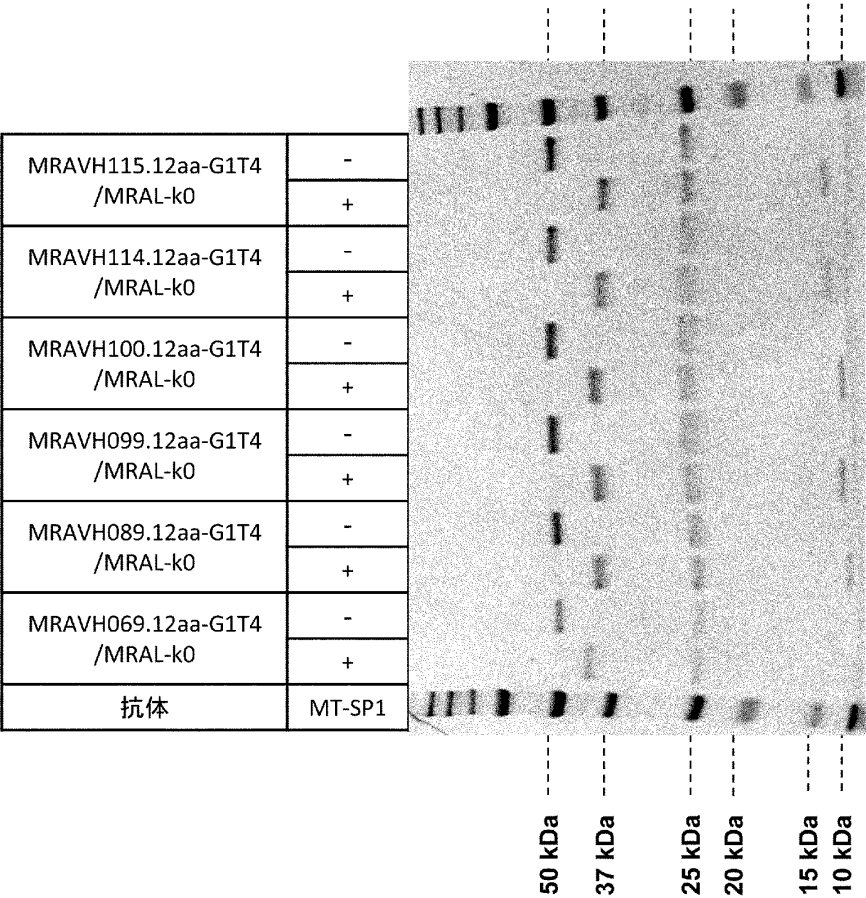
[図22F]



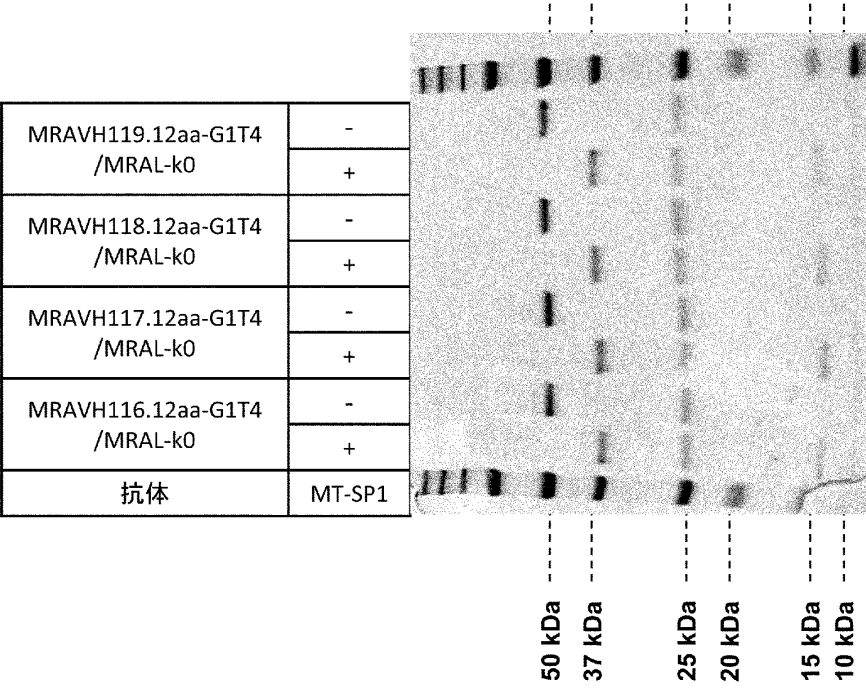
[図22G]



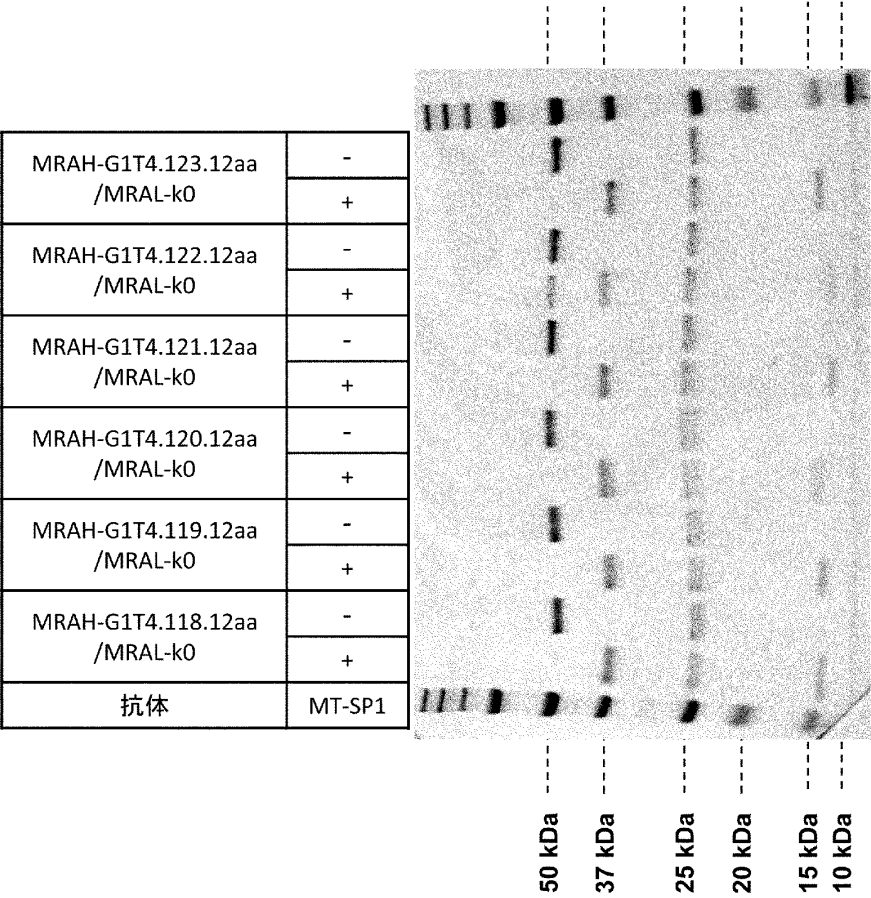
[図22H]



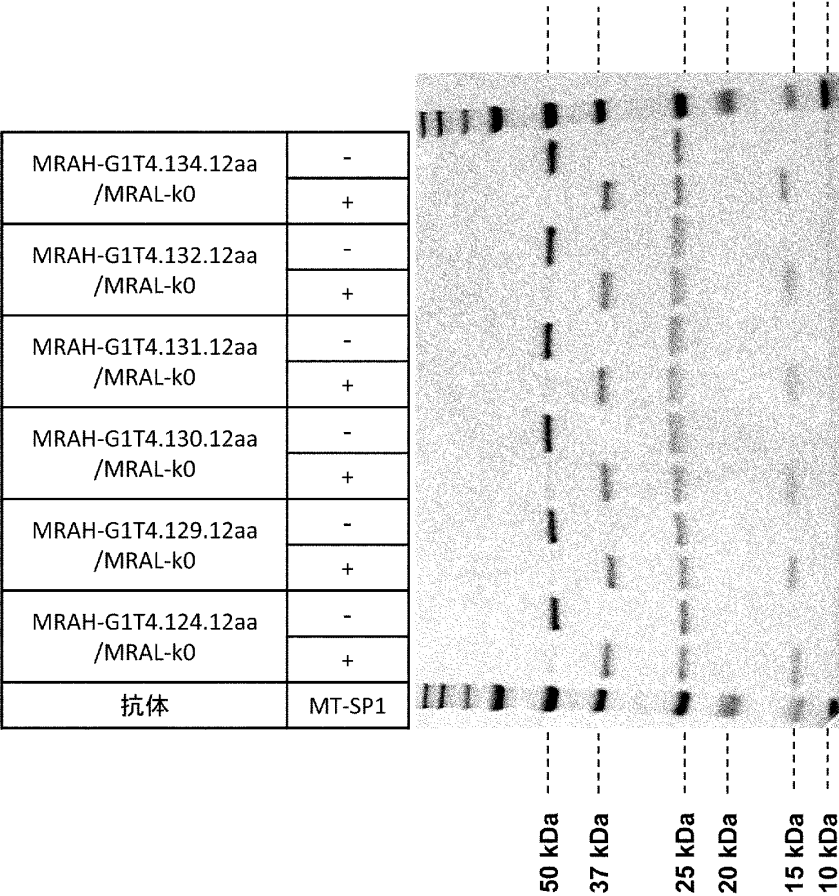
[図22I]



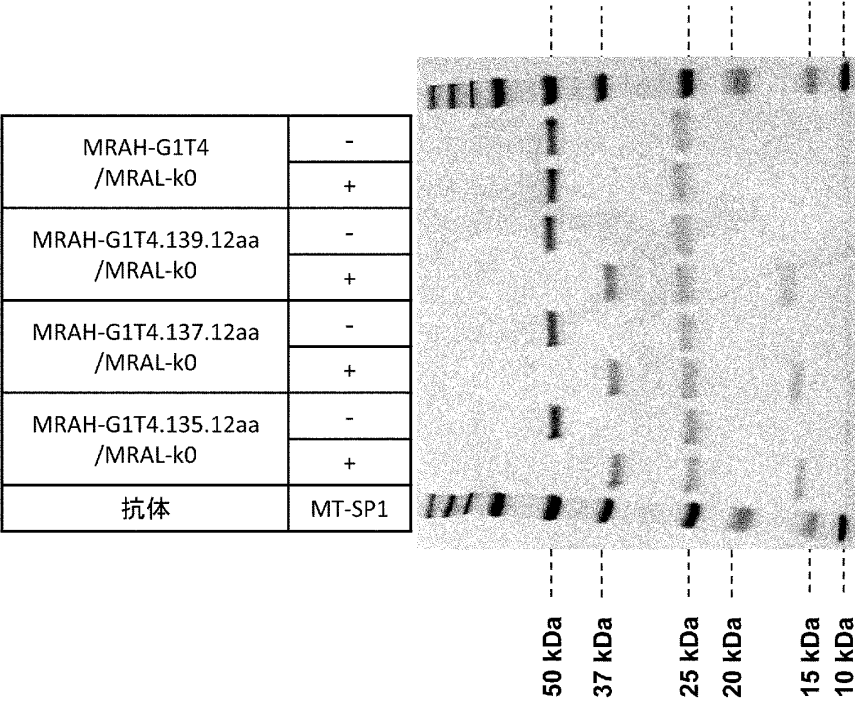
[図23A]



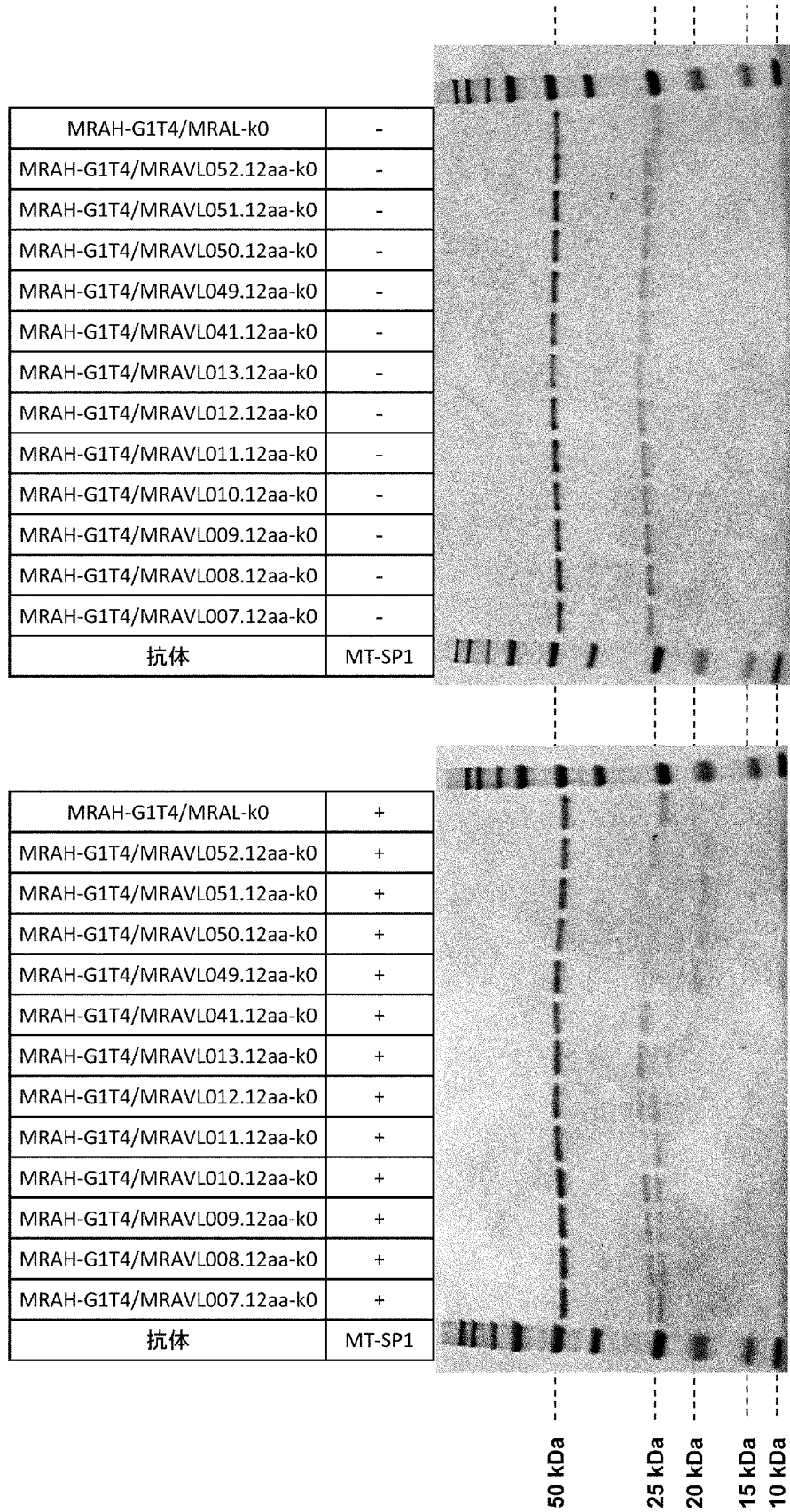
[図23B]



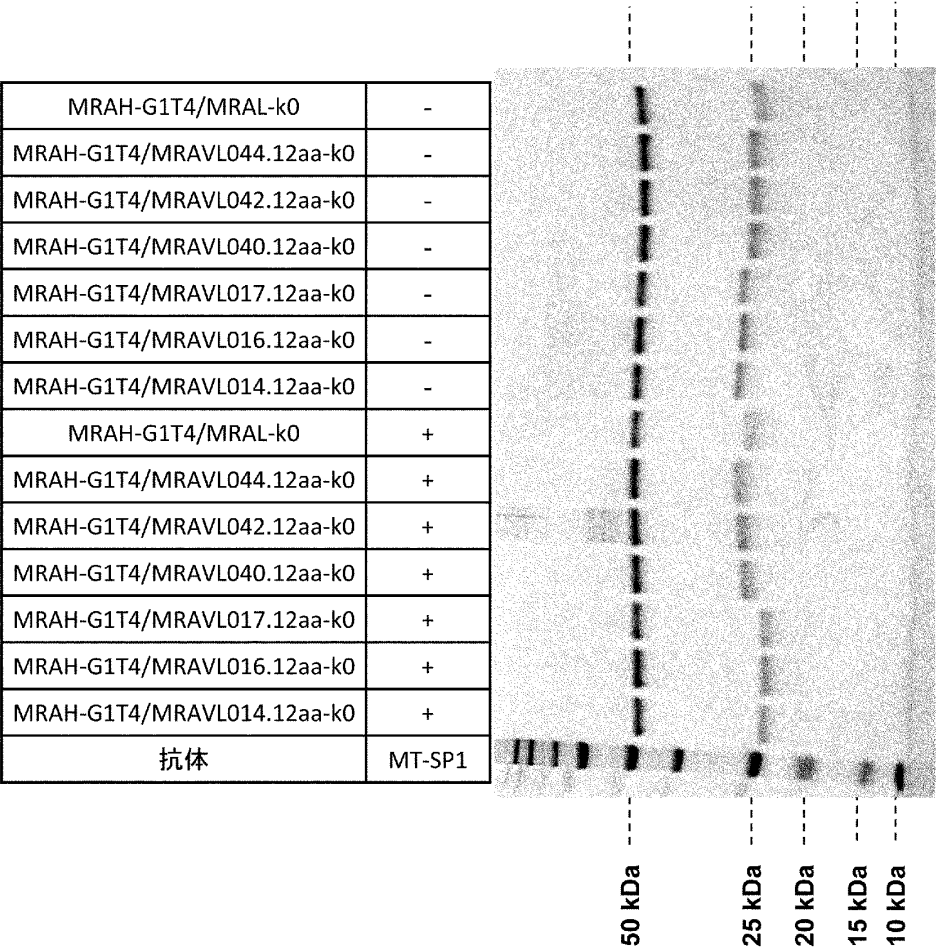
[図23C]



[図24A]

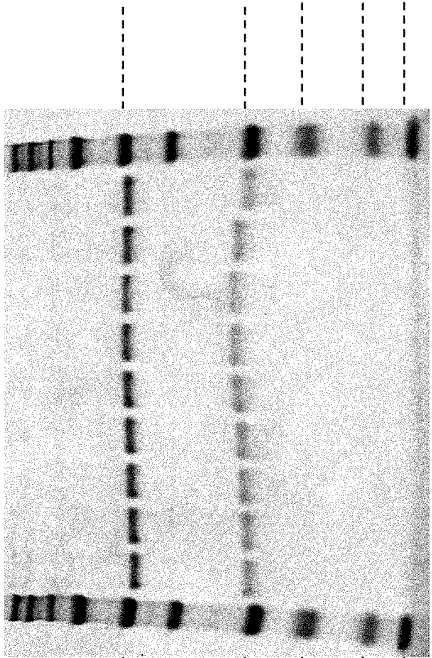


[図24B]

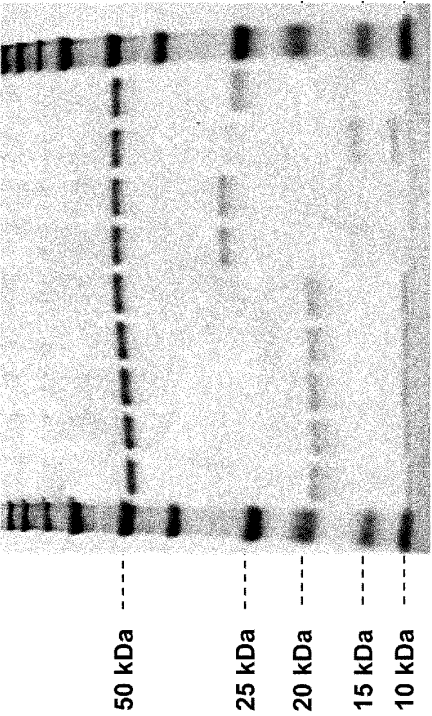


[図24C]

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	-
抗体	MT-SP1



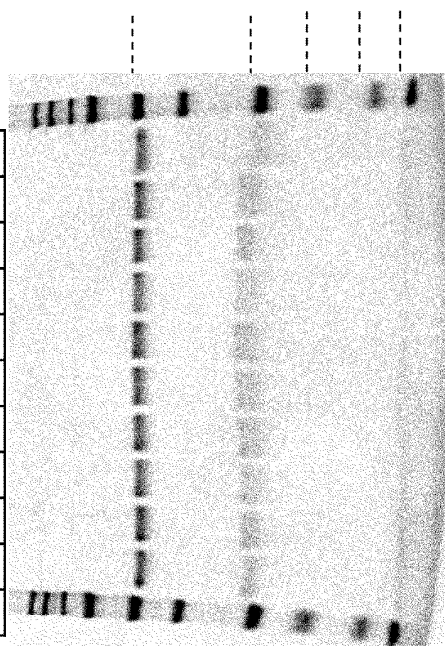
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL098.12aa-k0	+
MRAH-G1T4/MRAVL097.12aa-k0	+
MRAH-G1T4/MRAVL096.12aa-k0	+
MRAH-G1T4/MRAVL059.12aa-k0	+
MRAH-G1T4/MRAVL058.12aa-k0	+
MRAH-G1T4/MRAVL057.12aa-k0	+
MRAH-G1T4/MRAVL056.12aa-k0	+
MRAH-G1T4/MRAVL054.12aa-k0	+
抗体	MT-SP1



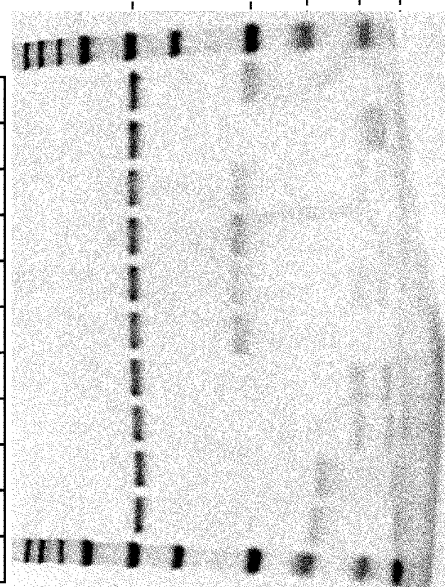
50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

[図24D]

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	-
抗体	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	+
抗体	MT-SP1



50 kDa

25 kDa

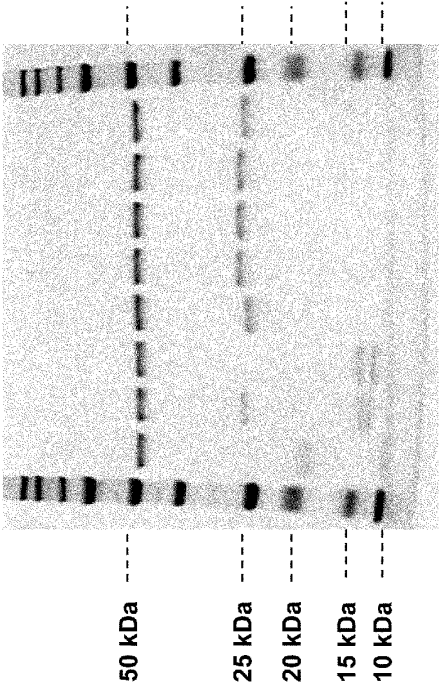
20 kDa

15 kDa

10 kDa

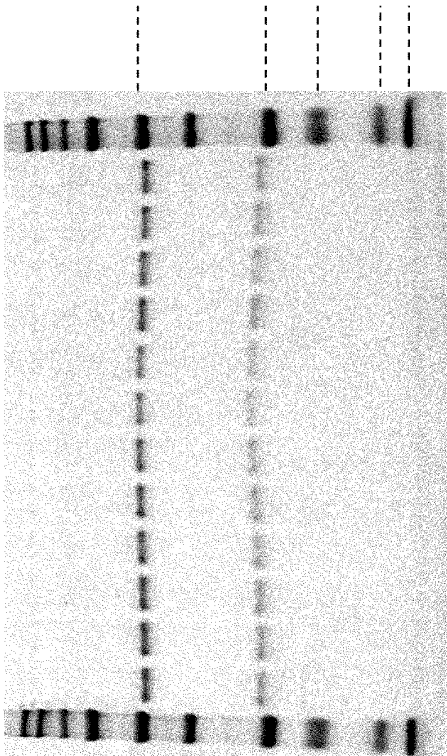
[図24E]

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	+
抗体	MT-SP1

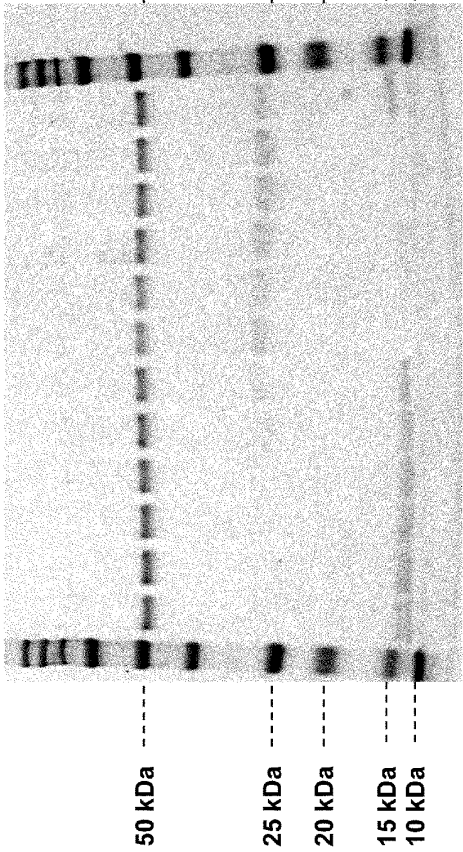


[図25A]

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	-
抗体	MT-SP1

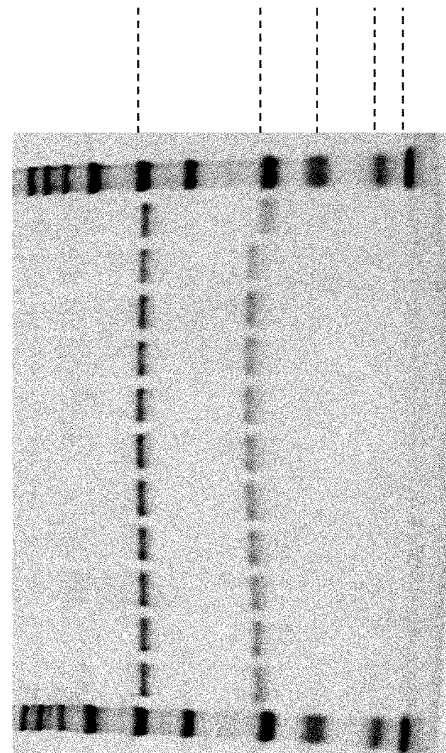


MRAH-G1T4/MRAL-k0.120.12aa	+
MRAH-G1T4/MRAL-k0.119.12aa	+
MRAH-G1T4/MRAL-k0.118.12aa	+
MRAH-G1T4/MRAL-k0.117.12aa	+
MRAH-G1T4/MRAL-k0.116.12aa	+
MRAH-G1T4/MRAL-k0.115.12aa	+
MRAH-G1T4/MRAL-k0.113.12aa	+
MRAH-G1T4/MRAL-k0.112.12aa	+
MRAH-G1T4/MRAL-k0.111.12aa	+
MRAH-G1T4/MRAL-k0.110.12aa	+
MRAH-G1T4/MRAL-k0.109.12aa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

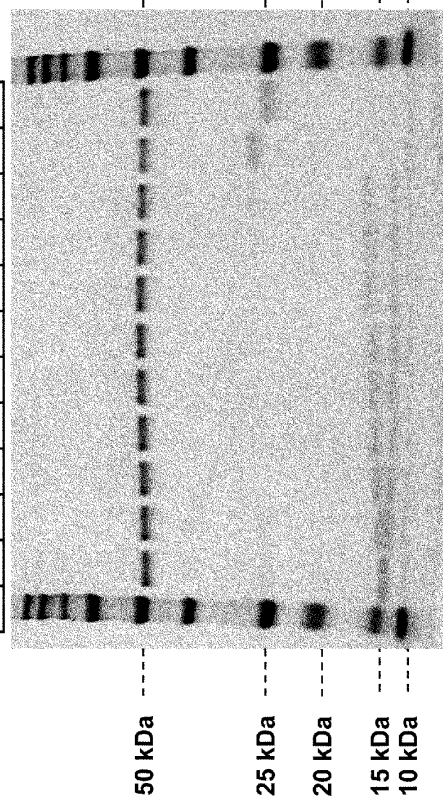


[図25B]

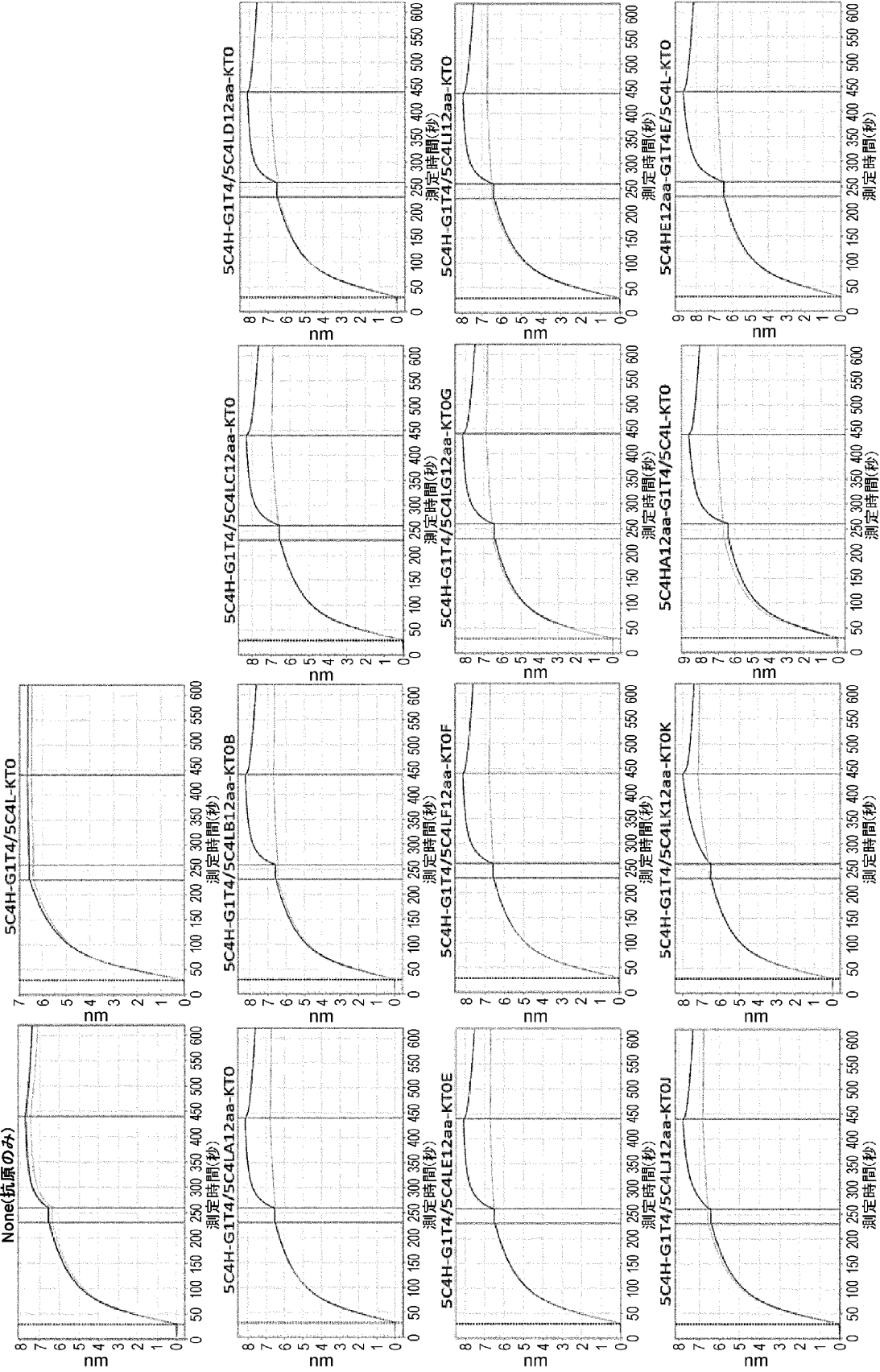
MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAL-k0.130.12aa	-
MRAH-G1T4/MRAL-k0.129.12aa	-
MRAH-G1T4/MRAL-k0.128.12aa	-
MRAH-G1T4/MRAL-k0.127.12aa	-
MRAH-G1T4/MRAL-k0.126.12aa	-
MRAH-G1T4/MRAL-k0.125.12aa	-
MRAH-G1T4/MRAL-k0.124.12aa	-
MRAH-G1T4/MRAL-k0.123.12aa	-
MRAH-G1T4/MRAL-k0.122.12aa	-
MRAH-G1T4/MRAL-k0.121.12aa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



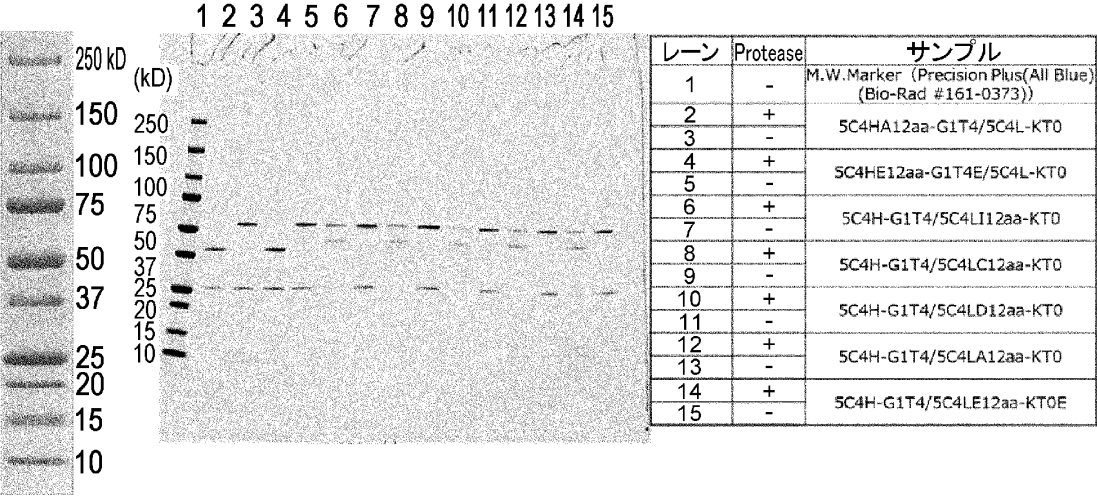
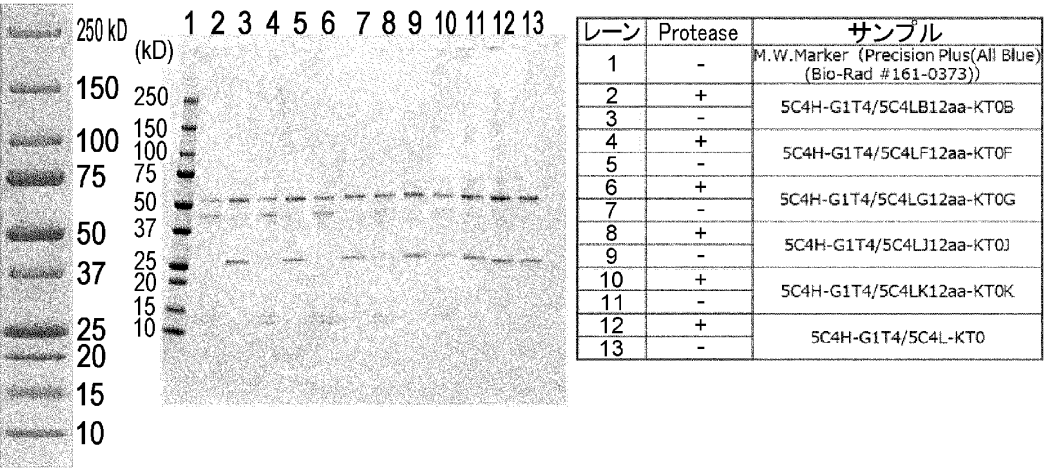
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAL-k0.130.12aa	+
MRAH-G1T4/MRAL-k0.129.12aa	+
MRAH-G1T4/MRAL-k0.128.12aa	+
MRAH-G1T4/MRAL-k0.127.12aa	+
MRAH-G1T4/MRAL-k0.126.12aa	+
MRAH-G1T4/MRAL-k0.125.12aa	+
MRAH-G1T4/MRAL-k0.124.12aa	+
MRAH-G1T4/MRAL-k0.123.12aa	+
MRAH-G1T4/MRAL-k0.122.12aa	+
MRAH-G1T4/MRAL-k0.121.12aa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



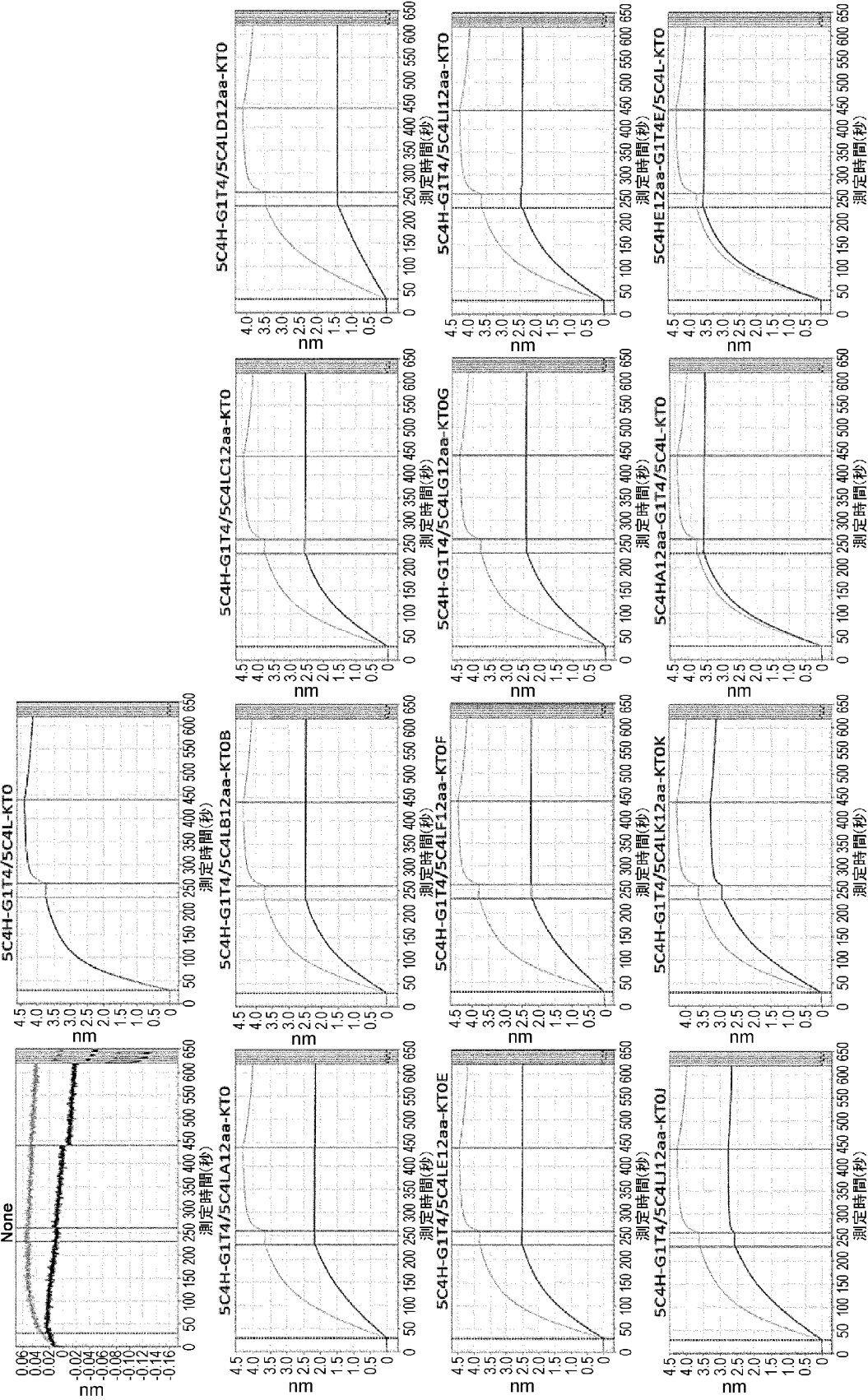
[図26]



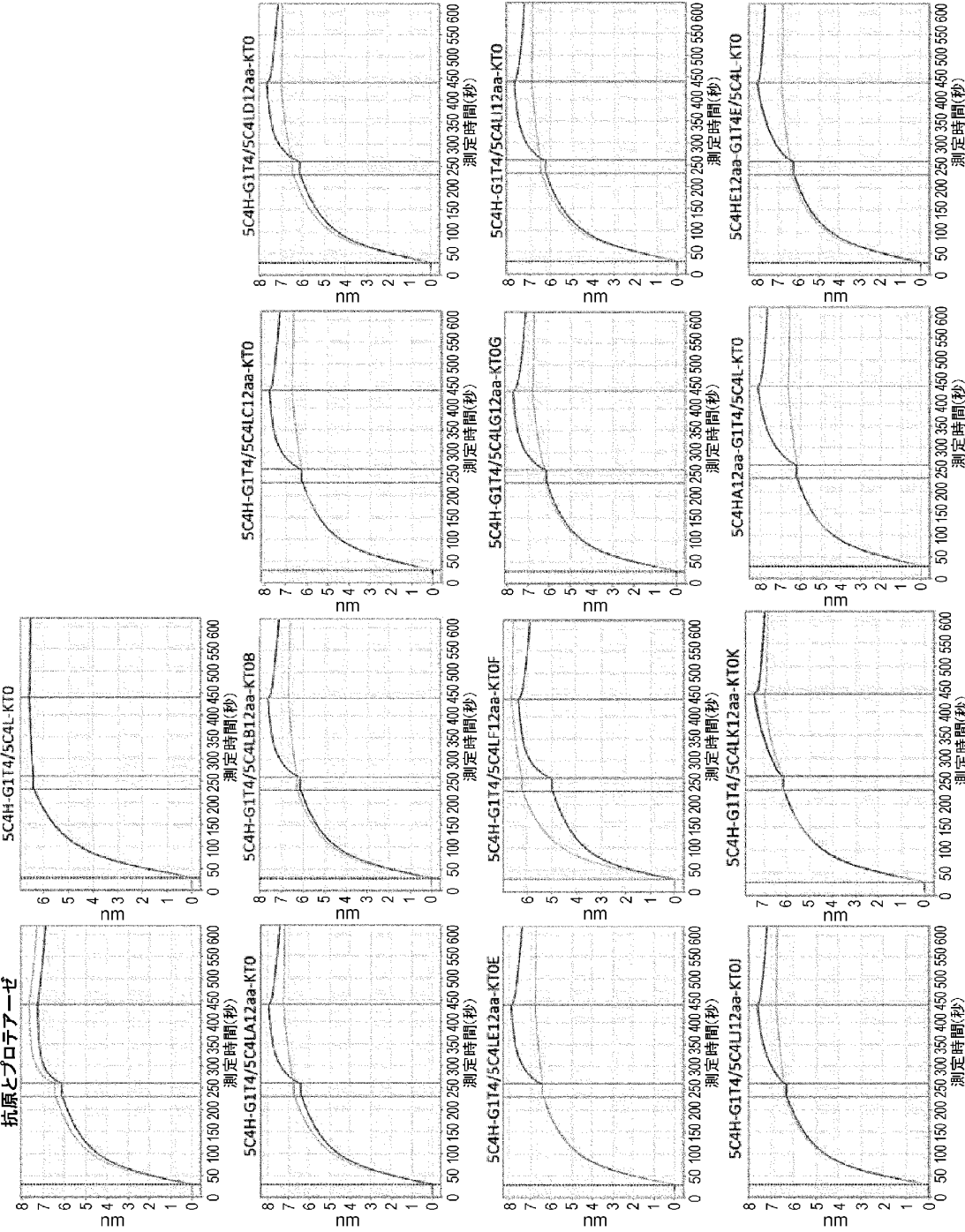
[図27]



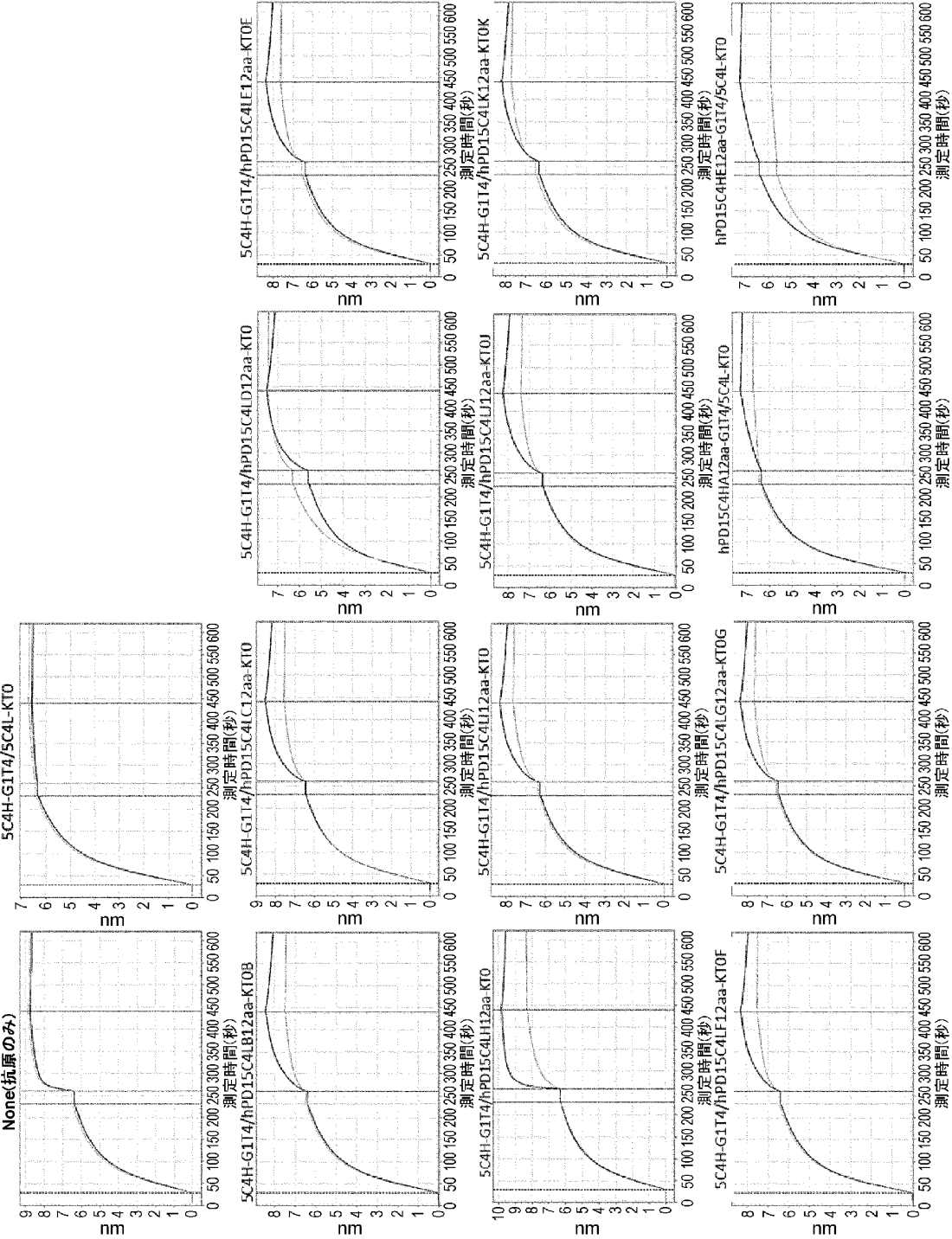
[図28]



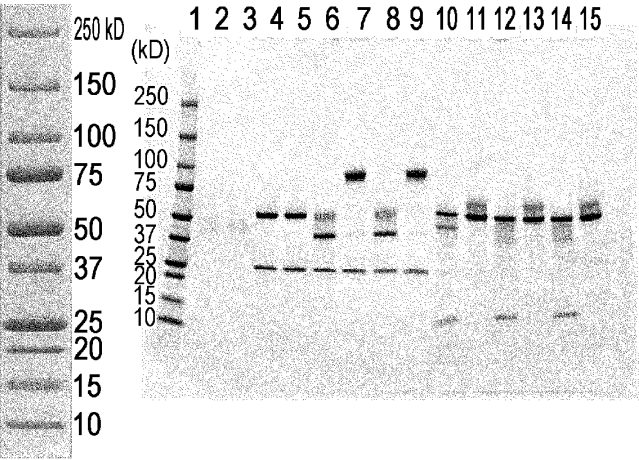
[図29]



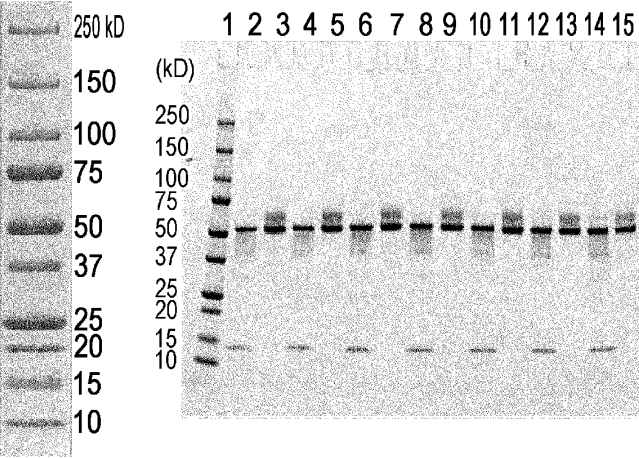
[図30]



[図31]



レーン	Protease	サンプル
1	-	M.W.Marker(Precision Plus(All Blue) (Bio-Rad #161-0373))
2	+	PD1
3	-	
4	+	5C4H-G1T4/5C4L-KT0
5	-	
6	+	hPD15C4HA12aa-G1T4/5C4L-KT0
7	-	
8	+	hPD15C4HE12aa-G1T4E/5C4L-KT0
9	-	
10	+	5C4H-G1T4/hPD15C4LH12aa-KT0
11	-	
12	+	5C4H-G1T4/hPD15C4LI12aa-KT0
13	-	
14	+	5C4H-G1T4/hPD15C4LC12aa-KT0
15	-	



レーン	Protease	サンプル
1	-	M.W.Marker (Precision Plus(All Blue) (Bio-Rad #161-0373))
2	+	5C4H-G1T4/hPD15C4LD12aa-KT0
3	-	
4	+	5C4H-G1T4/hPD15C4LE12aa-KT0E
5	-	
6	+	5C4H-G1T4/hPD15C4LB12aa-KT0B
7	-	
8	+	5C4H-G1T4/hPD15C4LF12aa-KT0F
9	-	
10	+	5C4H-G1T4/hPD15C4LG12aa-KT0G
11	-	
12	+	5C4H-G1T4/hPD15C4LJ12aa-KT0J
13	-	
14	+	5C4H-G1T4/hPD15C4LK12aa-KT0K
15	-	

[図32]

