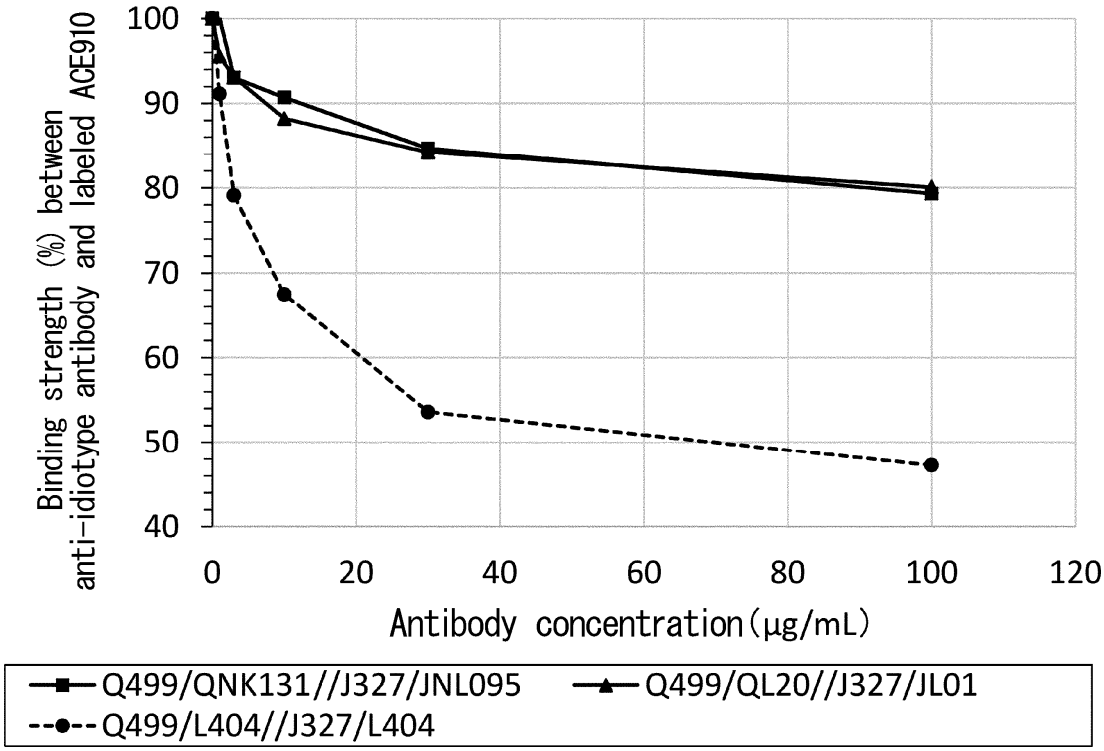

Binding strength (%) between anti-idiotype antibody and labeled ACE910
100
90
80
70
60
50
40
0
20
40
60
80
100
120
Antibody concentration (μg/mL)
Q499/QNK131//J327/JNL095
Q499/QL20//J327/JL01
Q499/L404//J327/L404

FIG. 1

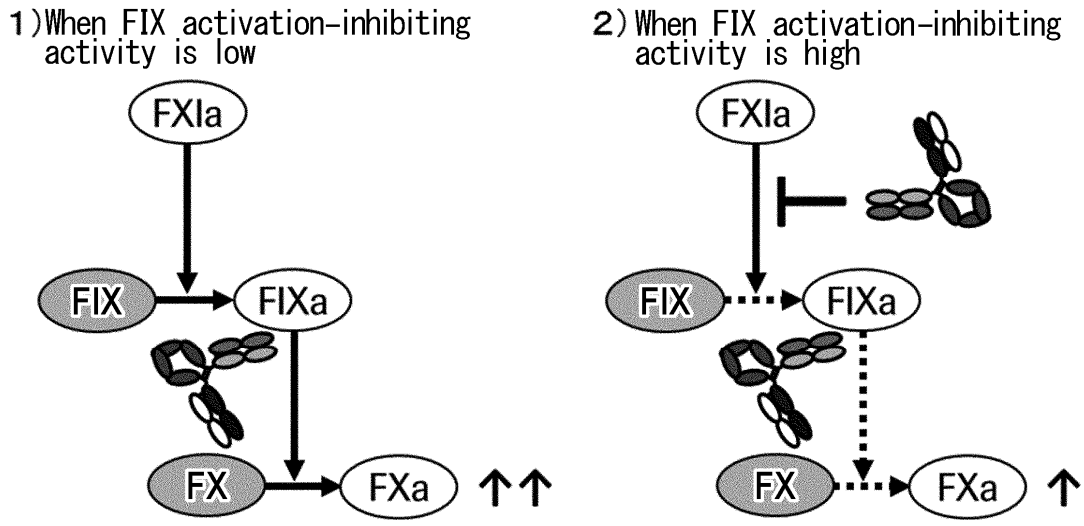
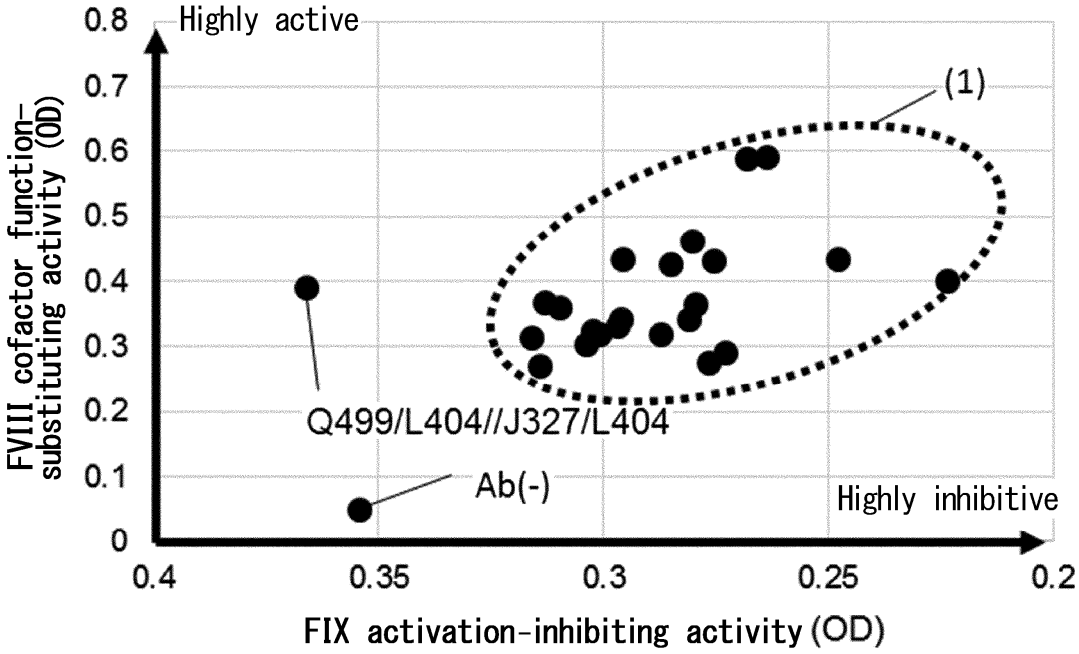

1)When FIX activation-inhibiting activity is low
FXIa
FIX
FIXa
FX
FXa
↑↑
2)When FIX activation-inhibiting activity is high
FXIa
FIX
FIXa
FX
FXa
↑

FIG. 2

(1)


Highly active
(1)
FVIII cofactor function-substituting activity (OD)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Q499/L404//J327/L404
Ab(-)
Highly inhibitive
0.4
0.35
0.3
0.25
0.2
FIX activation-inhibiting activity (OD)

(2)

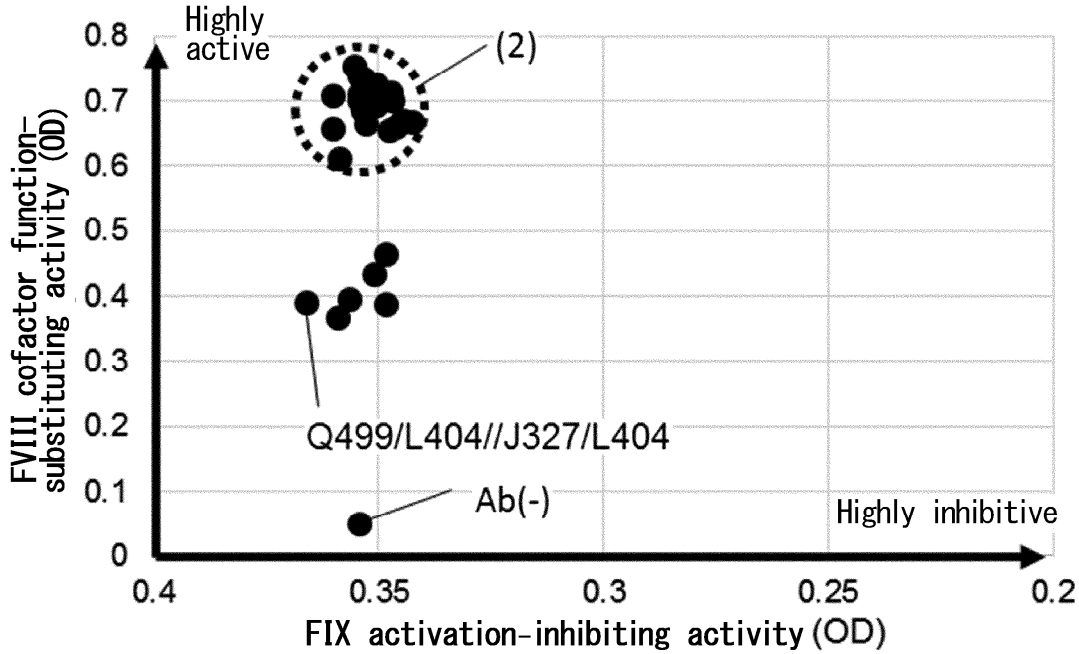

Highly active
(2)
FVIII cofactor function-substituting activity (OD)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Q499/L404//J327/L404
Ab(-)
Highly inhibitive
0.4
0.35
0.3
0.25
0.2
FIX activation-inhibiting activity (OD)

FIG. 3

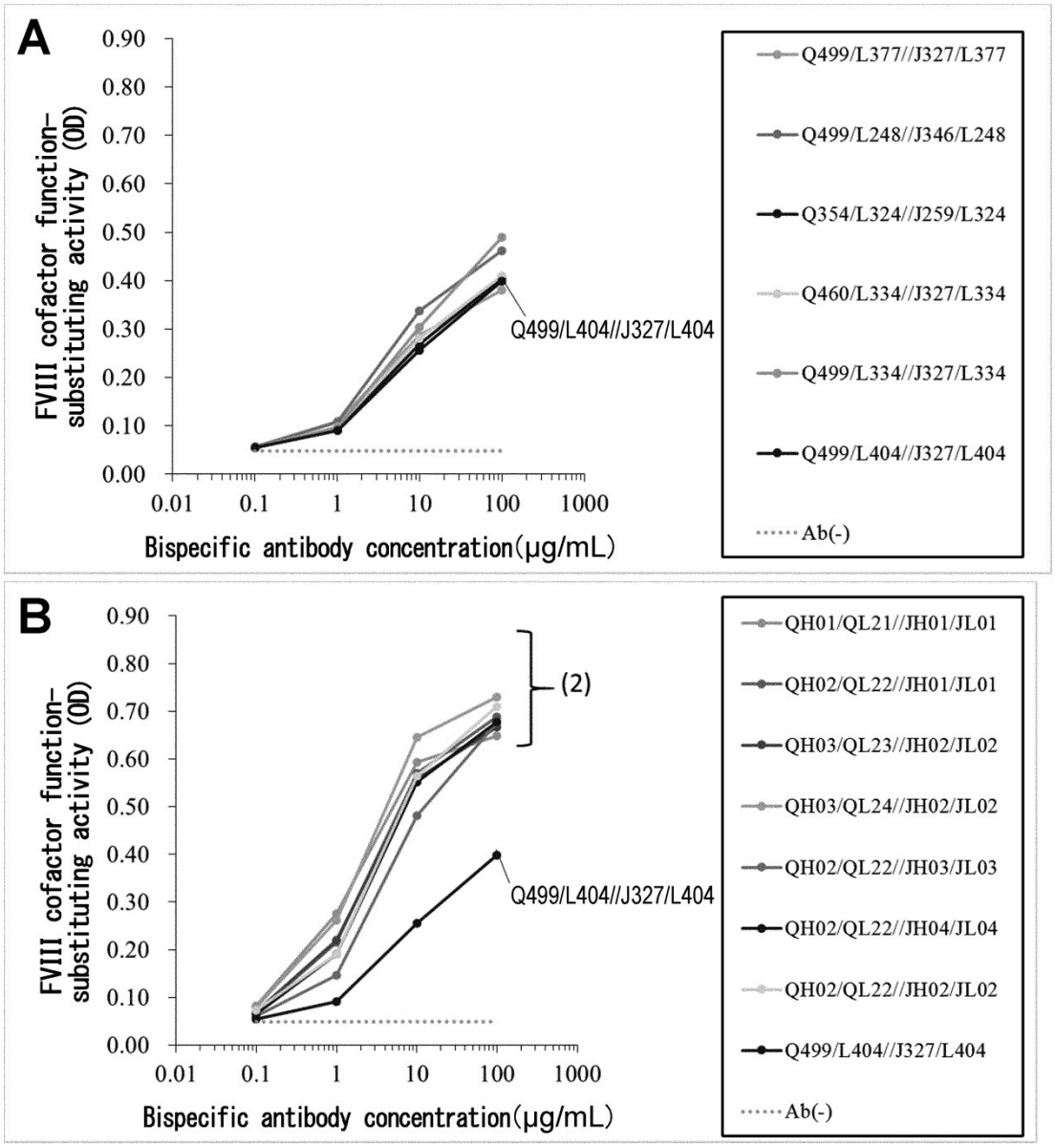

A
FVIII cofactor function-substituting activity (OD)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.01
0.1
1
10
100
1000
Bispecific antibody concentration(μg/mL)
Q499/L404//J327/L404
Q499/L377//J327/L377
Q499/L248//J346/L248
Q354/L324//J259/L324
Q460/L334//J327/L334
Q499/L334//J327/L334
Q499/L404//J327/L404
Ab(-)
B
FVIII cofactor function-substituting activity (OD)
(2)
Q499/L404//J327/L404
QH01/QL21//JH01/JL01
QH02/QL22//JH01/JL01
QH03/QL23//JH02/JL02
QH03/QL24//JH02/JL02
QH02/QL22//JH03/JL03
QH02/QL22//JH04/JL04
QH02/QL22//JH02/JL02
Q499/L404//J327/L404
Ab(-)

FIG. 4-1

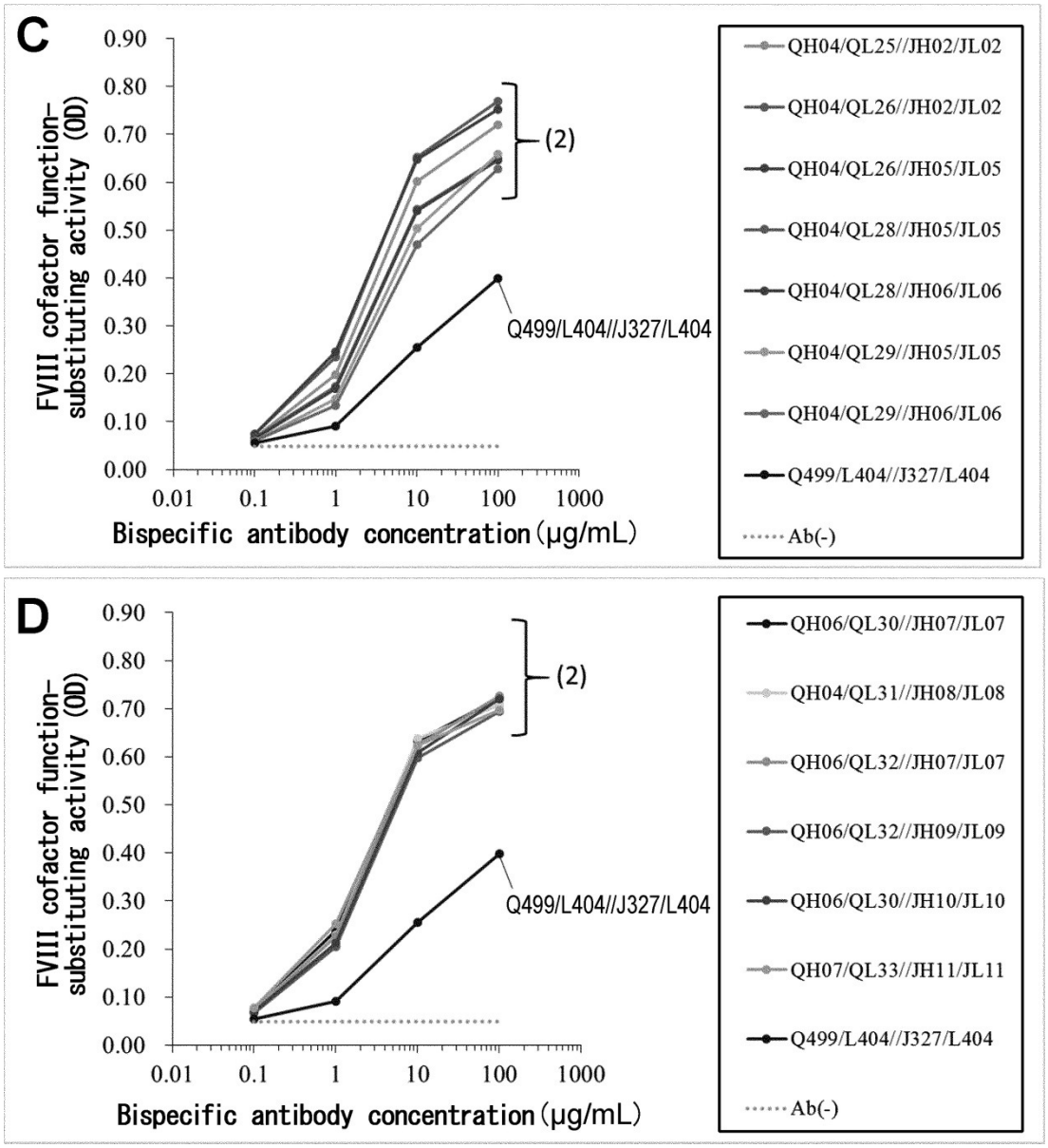

C
FVIII cofactor function-substituting activity (OD)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.01
0.1
1
10
100
1000
Bispecific antibody concentration (μg/mL)
(2)
Q499/L404//J327/L404
QH04/QL25//JH02/JL02
QH04/QL26//JH02/JL02
QH04/QL26//JH05/JL05
QH04/QL28//JH05/JL05
QH04/QL28//JH06/JL06
QH04/QL29//JH05/JL05
QH04/QL29//JH06/JL06
Q499/L404//J327/L404
Ab(-)
D
FVIII cofactor function-substituting activity (OD)
Bispecific antibody concentration (μg/mL)
(2)
Q499/L404//J327/L404
QH06/QL30//JH07/JL07
QH04/QL31//JH08/JL08
QH06/QL32//JH07/JL07
QH06/QL32//JH09/JL09
QH06/QL30//JH10/JL10
QH07/QL33//JH11/JL11
Q499/L404//J327/L404
Ab(-)

FIG. 4-2

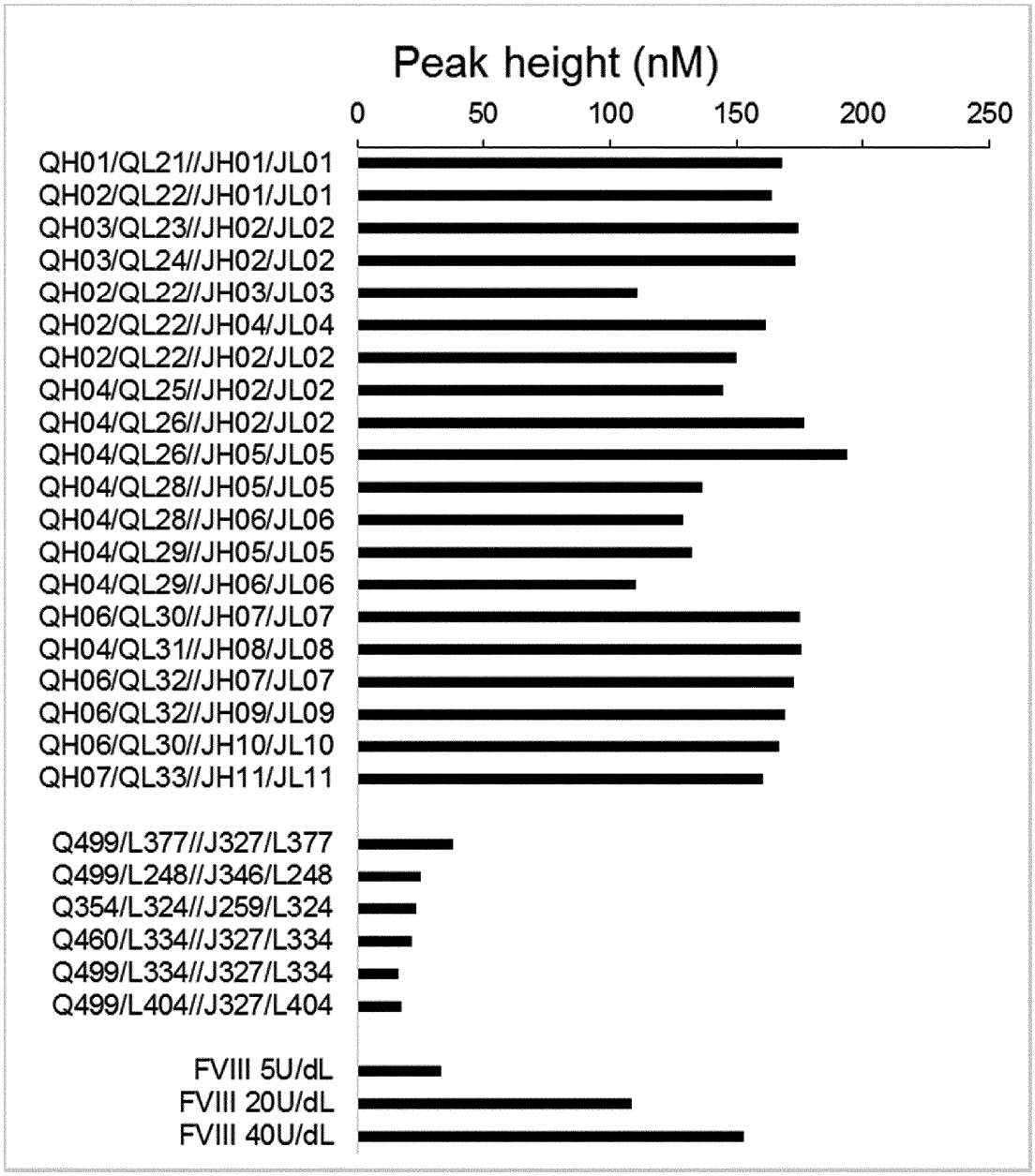

Peak height (nM)
0
50
100
150
200
250
QH01/QL21//JH01/JL01
QH02/QL22//JH01/JL01
QH03/QL23//JH02/JL02
QH03/QL24//JH02/JL02
QH02/QL22//JH03/JL03
QH02/QL22//JH04/JL04
QH02/QL22//JH02/JL02
QH04/QL25//JH02/JL02
QH04/QL26//JH02/JL02
QH04/QL26//JH05/JL05
QH04/QL28//JH05/JL05
QH04/QL28//JH06/JL06
QH04/QL29//JH05/JL05
QH04/QL29//JH06/JL06
QH06/QL30//JH07/JL07
QH04/QL31//JH08/JL08
QH06/QL32//JH07/JL07
QH06/QL32//JH09/JL09
QH06/QL30//JH10/JL10
QH07/QL33//JH11/JL11
Q499/L377//J327/L377
Q499/L248//J346/L248
Q354/L324//J259/L324
Q460/L334//J327/L334
Q499/L334//J327/L334
Q499/L404//J327/L404
FVIII 5U/dL
FVIII 20U/dL
FVIII 40U/dL

FIG. 5

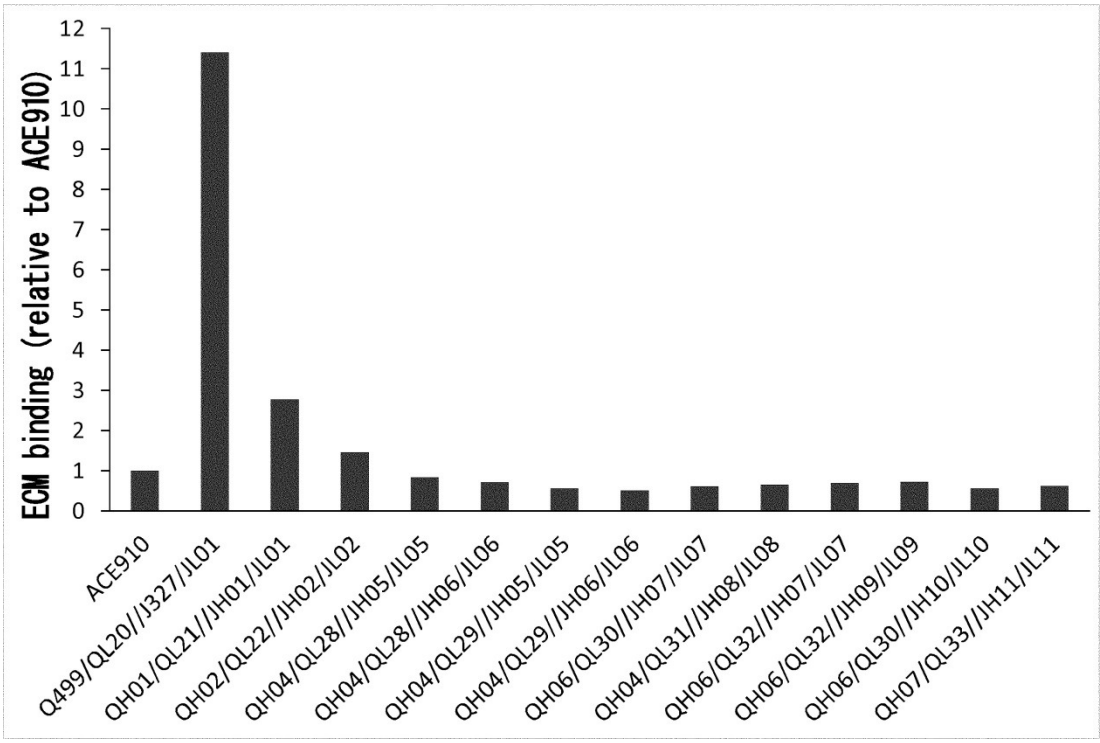

ECM binding (relative to ACE910)
12
11
10
9
8
7
6
5
4
3
2
1
0
ACE910
Q499/QL20//J327/JL01
QH01/QL21//JH01/JL01
QH02/QL22//JH02/JL02
QH04/QL28//JH05/JL05
QH04/QL28//JH06/JL06
QH04/QL29//JH05/JL05
QH04/QL29//JH06/JL06
QH06/QL30//JH07/JL07
QH04/QL31//JH08/JL08
QH06/QL32//JH07/JL07
QH06/QL32//JH09/JL09
QH06/QL30//JH10/JL10
QH07/QL33//JH11/JL11

FIG. 6

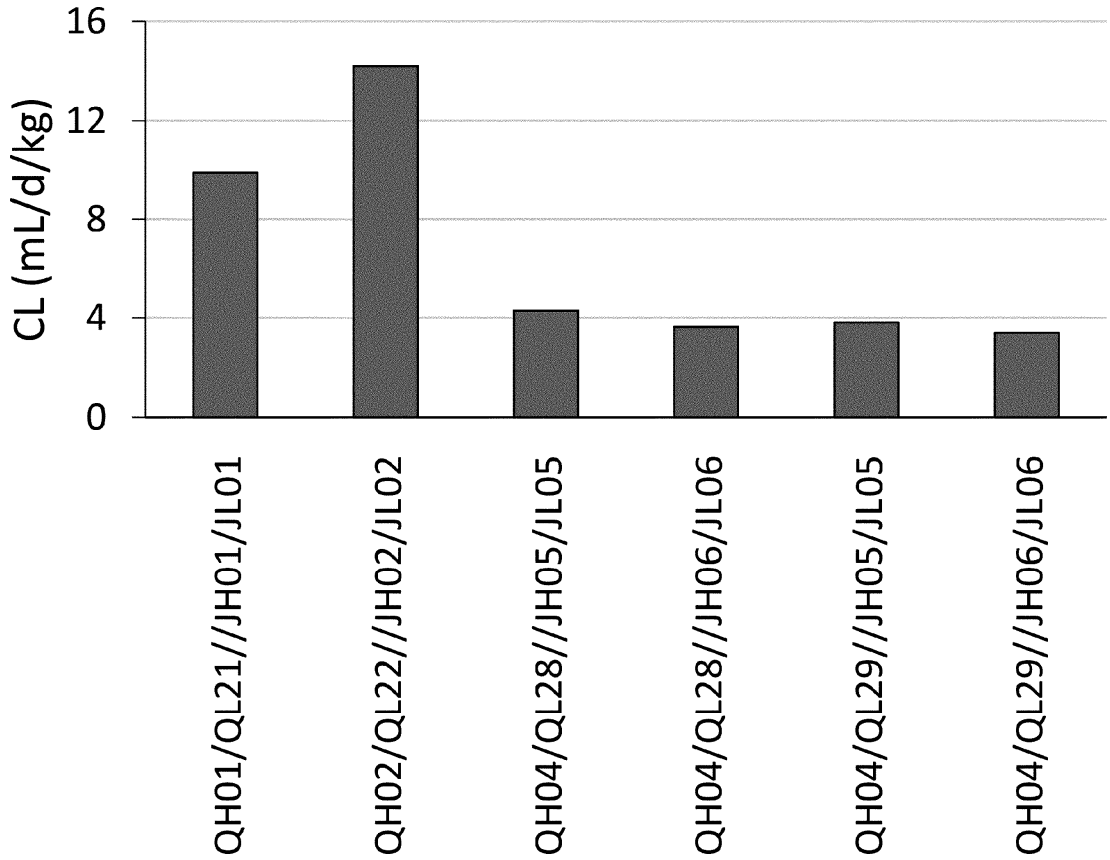

16
12
8
4
0
CL (mL/d/kg)
QH01/QL21//JH01/JL01
QH02/QL22//JH02/JL02
QH04/QL28//JH05/JL05
QH04/QL28//JH06/JL06
QH04/QL29//JH05/JL05
QH04/QL29//JH06/JL06

FIG. 7

Mispairs that may be expressed in theory

No.	Name	No.	Name
①	**HA/LA//HB/LB**	⑥	**HA/LB//HA/LA**
②	**HB/LB//HB/LB**	⑦	**HA/LB//HA/LB**
③	**HB/LA//HB/LB**	⑧	**HA/LB//HB/LB**
④	**HB/LA//HB/LA**	⑨	**HA/LB//HB/LA**
⑤	**HA/LA//HA/LA**	⑩	**HA/LA//HB/LA**

Mispairs after KiH introduction

No.	Name	No.	Name
①	**Q/κ//J/λ**	⑥	Q/λ//Q/κ
②	J/λ//J/λ	⑦	Q/λ//Q/λ
③	J/κ//J/λ	⑧	**Q/λ//J/λ**
④	J/κ//J/κ	⑨	**Q/λ//J/κ**
⑤	Q/κ//Q/κ	⑩	**Q/κ//J/κ**

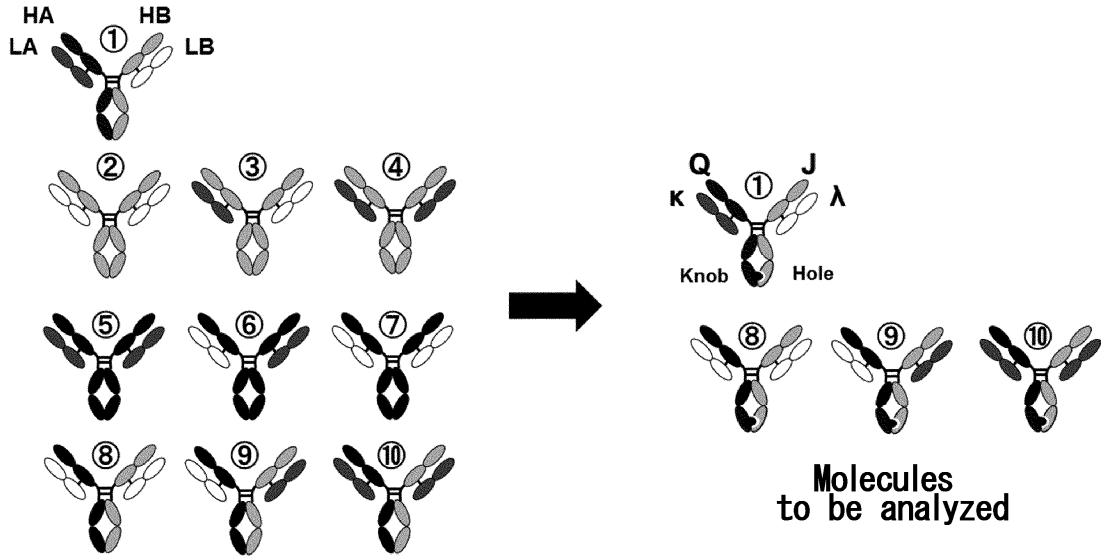


FIG. 8

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)				L chain (λ)					Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
1	K	-	K	-	E	-	E	E	E	E	E	E	E	K	K	K	K	K	KiH	100.00	99.63	99.84

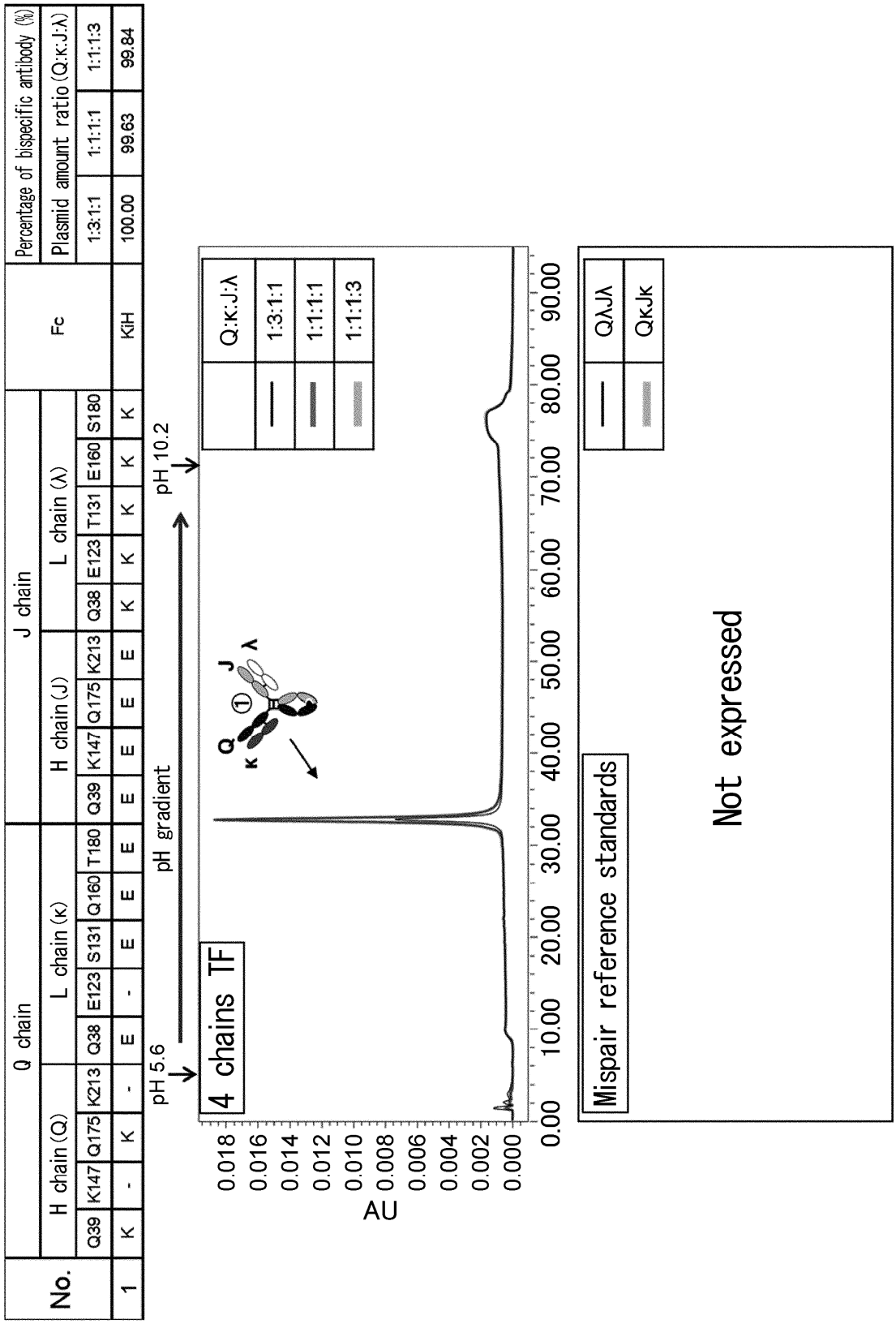


FIG. 9-1

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)				L chain (λ)					Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
2	-	-	K	-	-	-	E	E	E	-	E	E	E	-	K	K	K	K	KiH	99.89	99.80	99.58

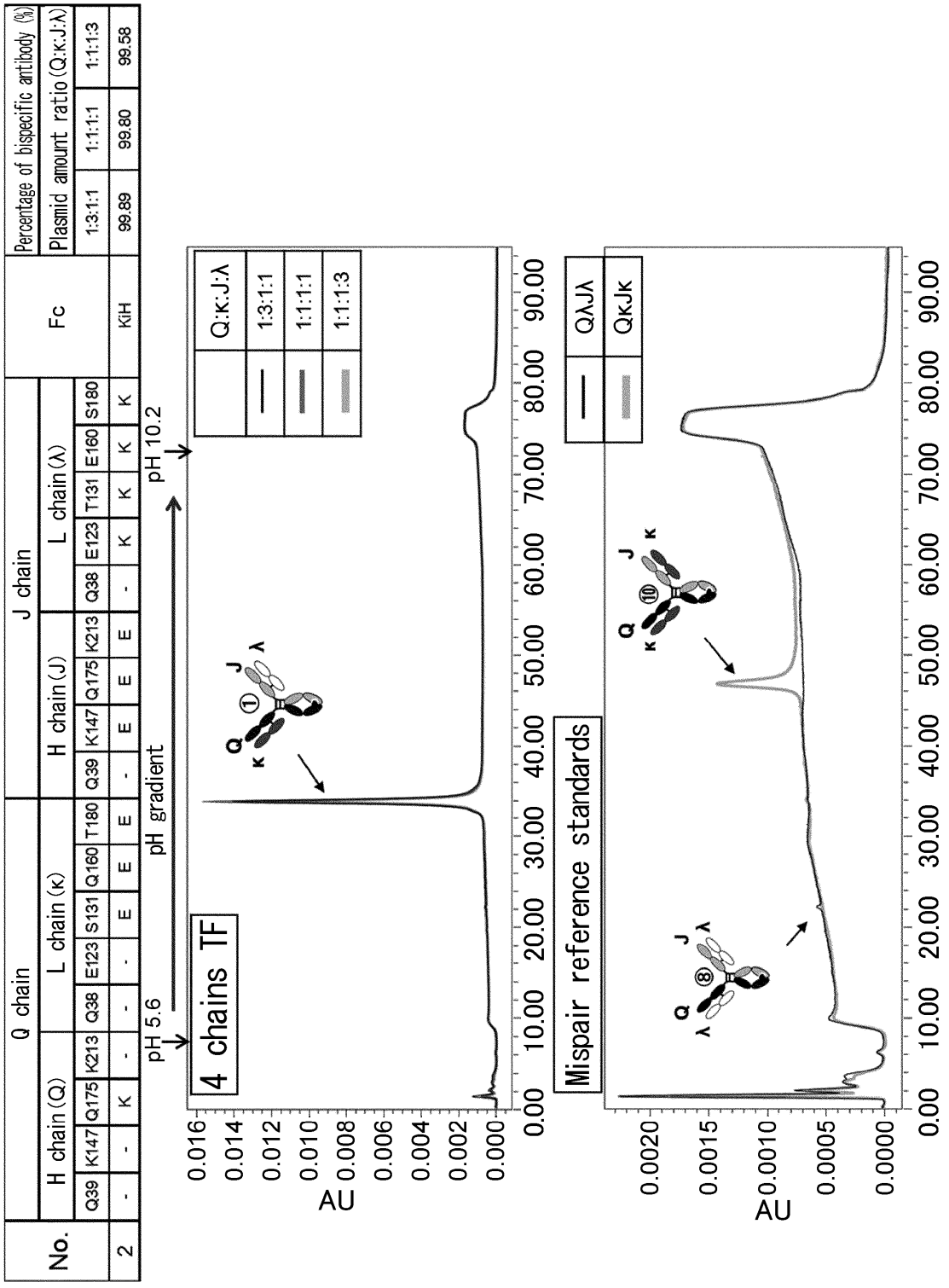


FIG. 9-2

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)	
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)	
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
3	-	-	-	-	-	-	E	-	E	-	E	-	-	-	-	K	-	K	KiH	94.57	95.90	94.63

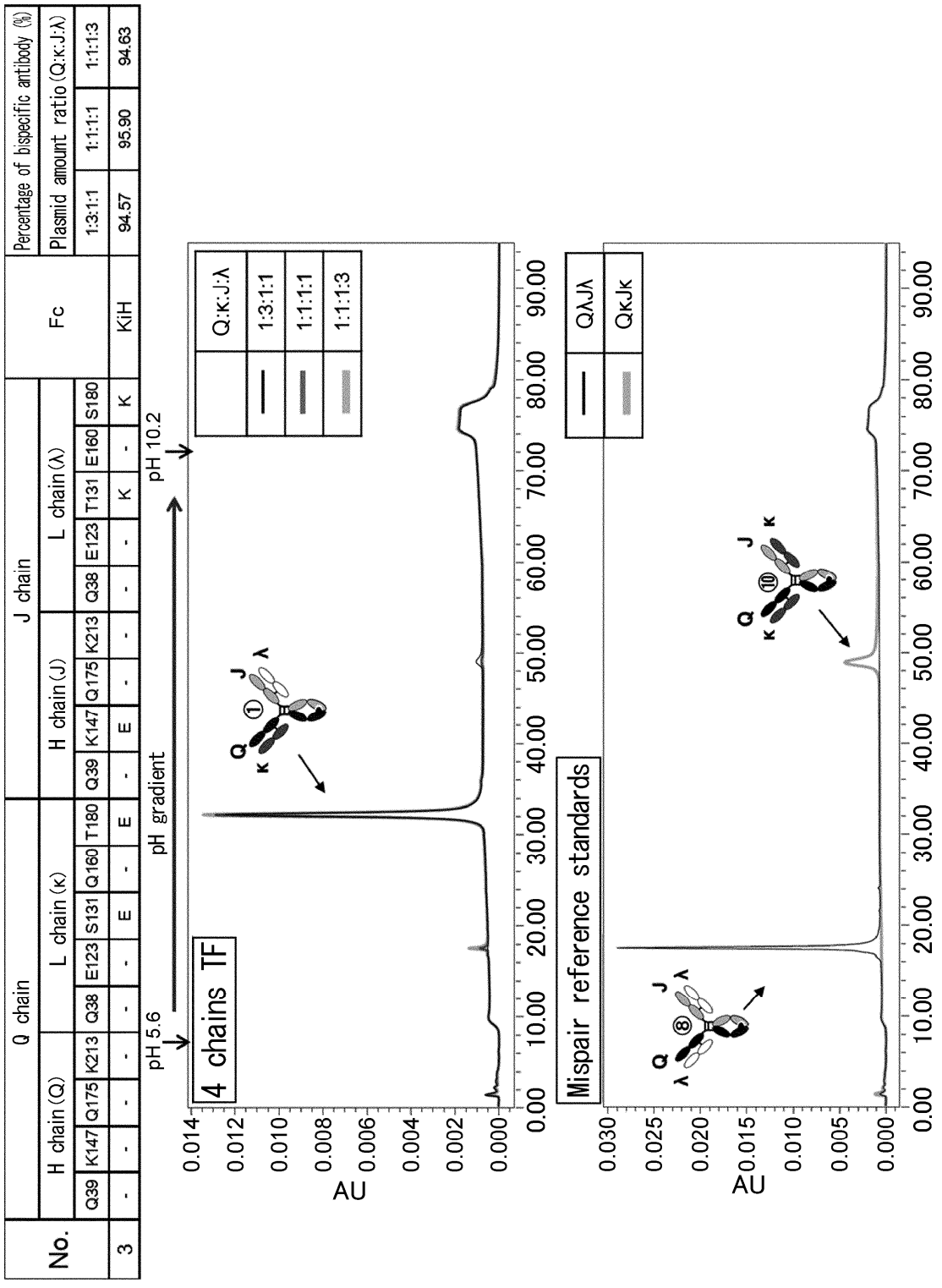


FIG. 9-3

No.	Q chain									J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)						H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180	1:3:1:1	1:1:1:1	1:1:1:3
4	-	-	K	-	-	-	-	E	E	-	E	E	-	-	-	-	K	K	KiH	96.95	98.67	49.99

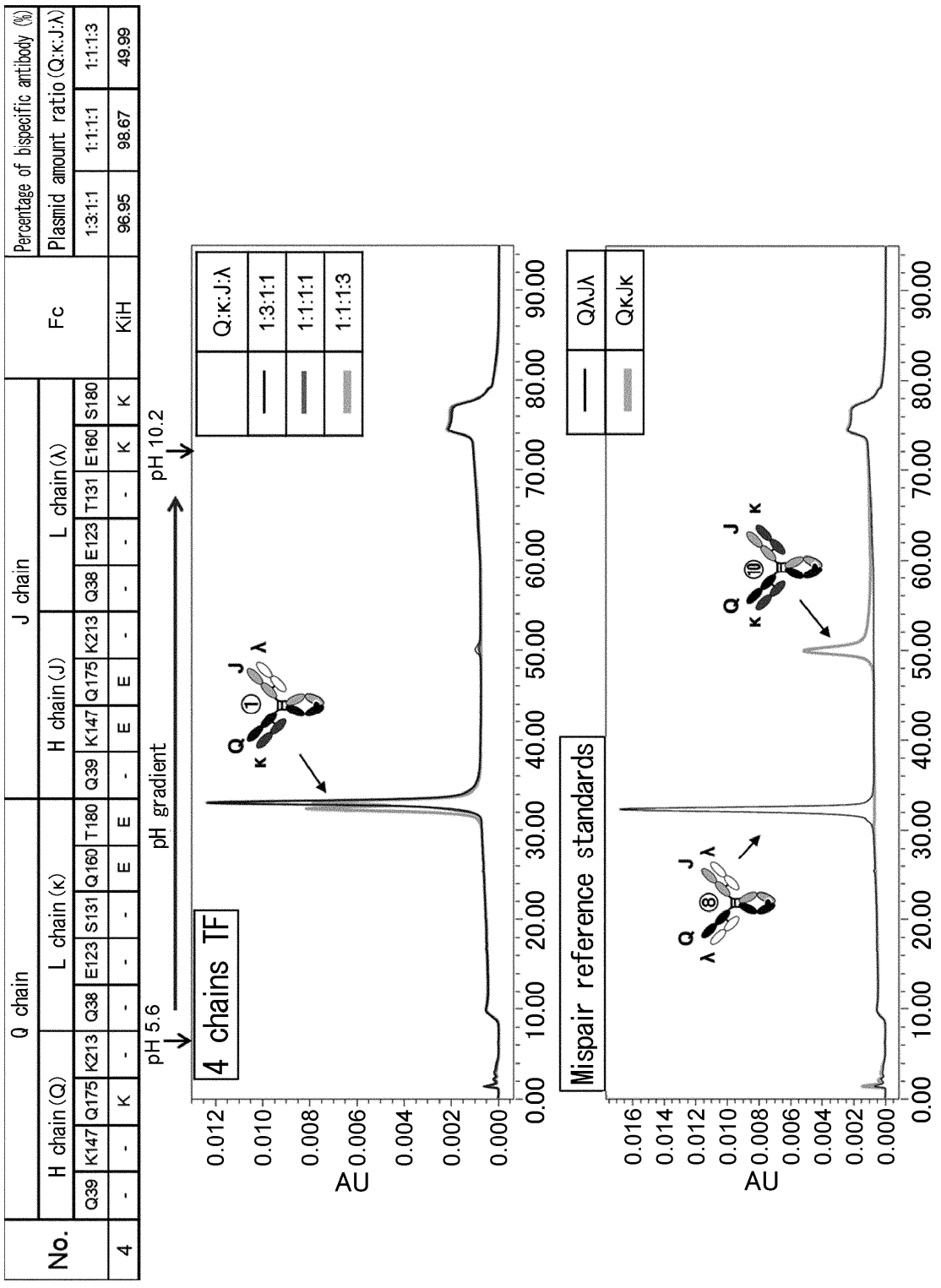


FIG. 9-4

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
5	-	-	-	-	-	-	-	-	E	-	E	-	E	-	K	-	-	K	KiH	1:3:1:1	1:1:1:1	1:1:1:3
																				89.47	82.13	42.25

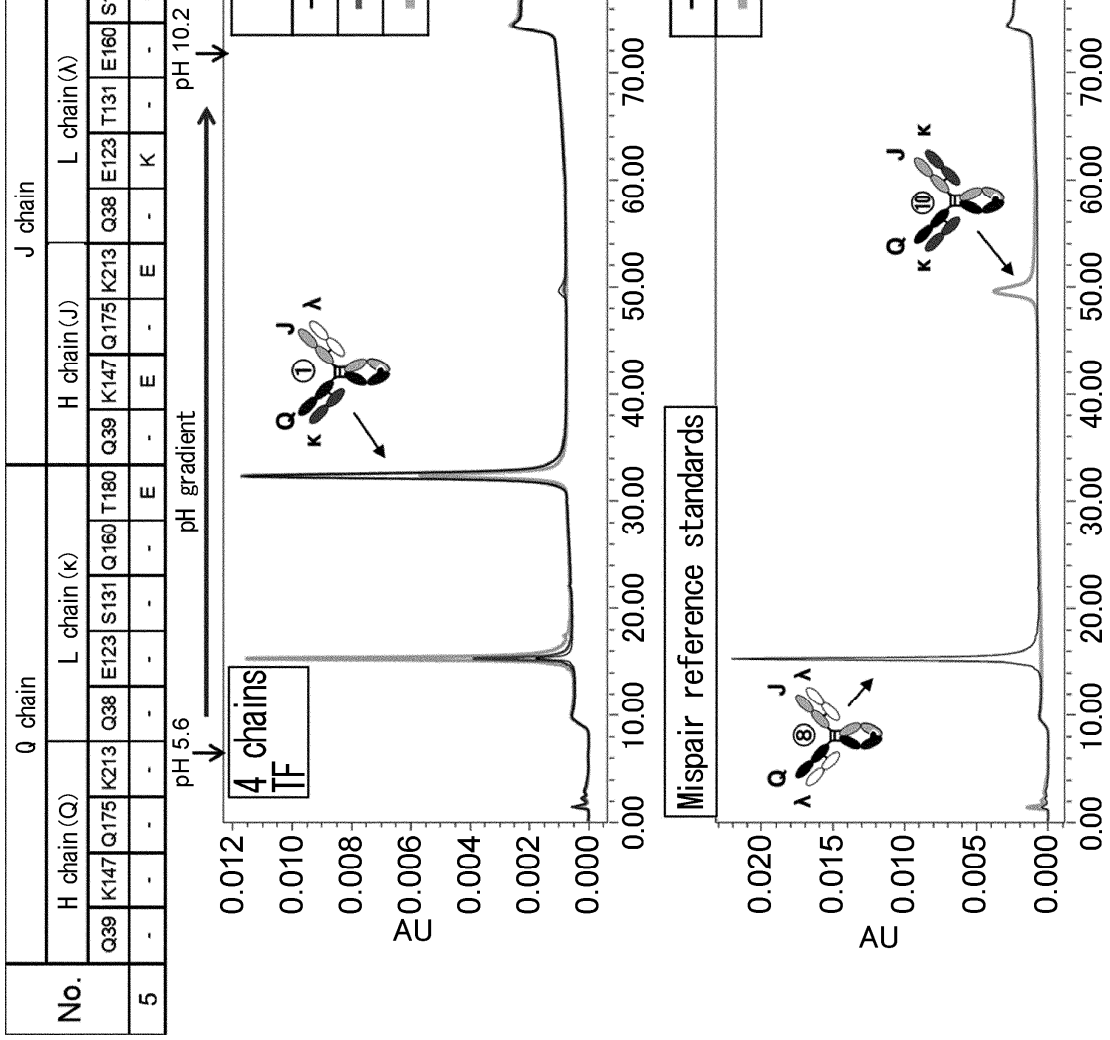


FIG. 9-5

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180	1:3:1:1	1:1:1:1	1:1:1:3
6	-	-	K	-	-	-	E	E	-	-	E	E	-	-	-	K	K	-	KiH	99.15	98.98	95.23

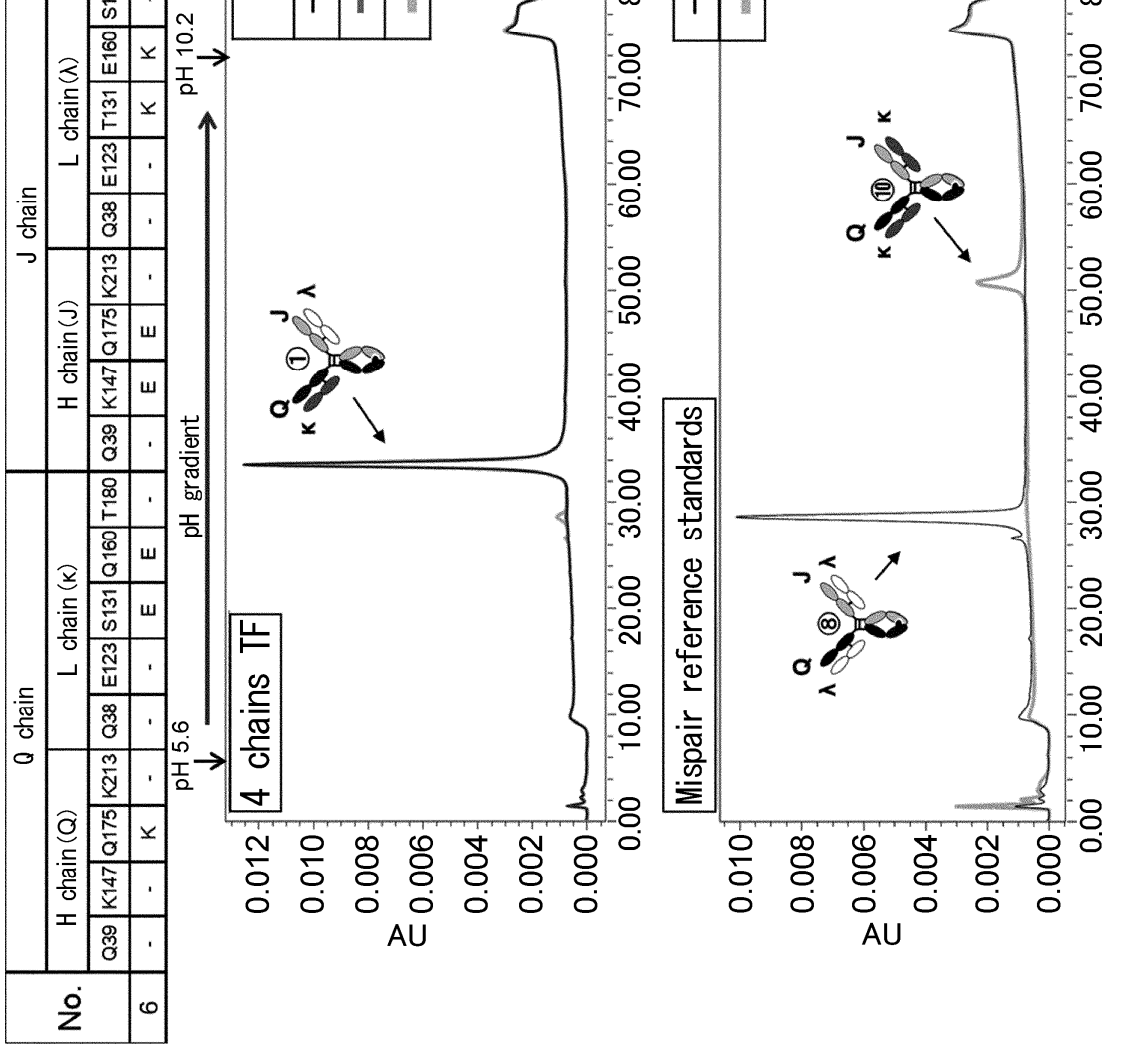


FIG. 9-6

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
7	-	-	-	-	-	-	E	-	-	-	E	-	E	-	K	K	-	-	1:3:1:1	1:1:1:1	1:1:1:3
																		KiH	98.19	93.81	81.57

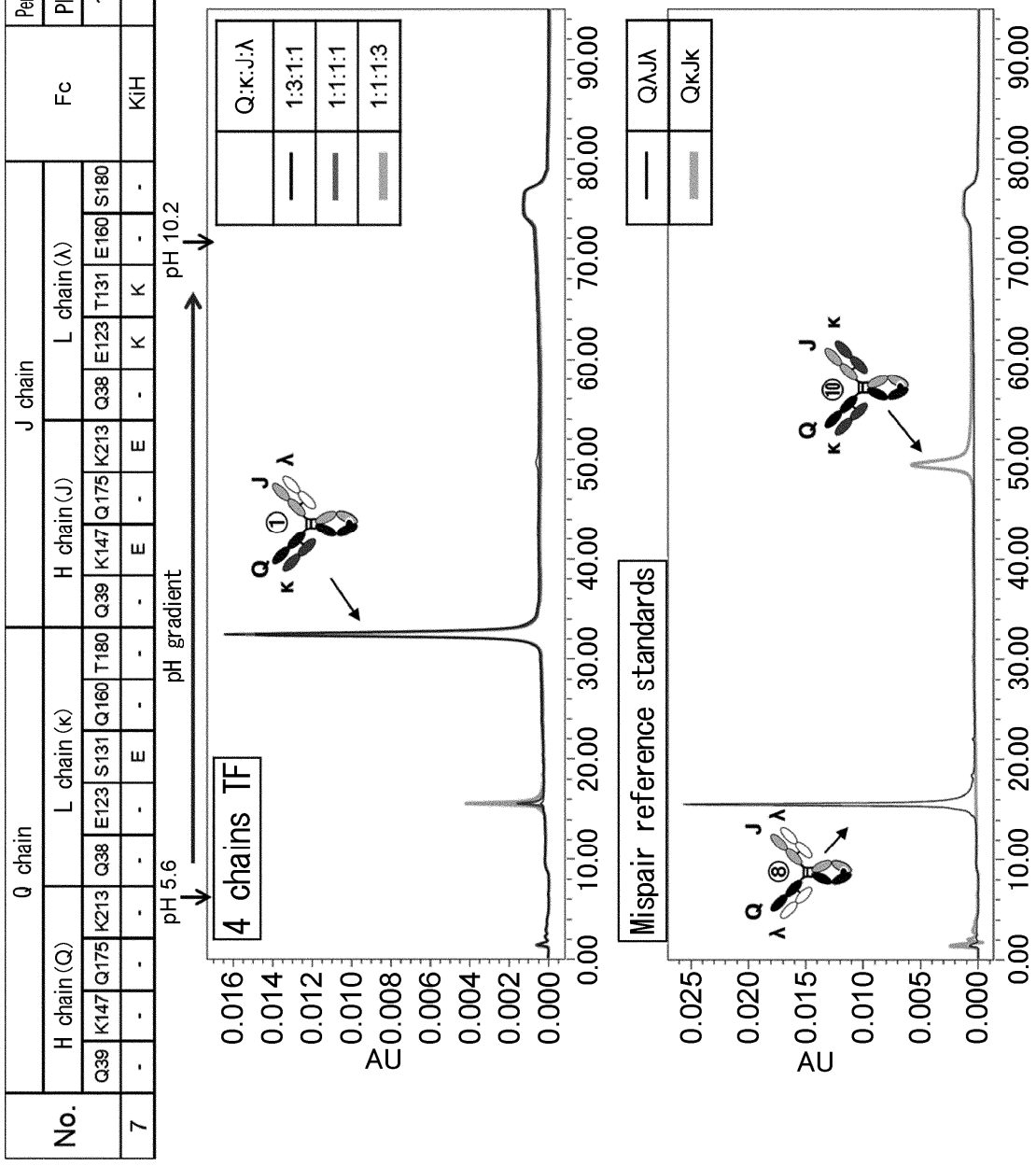


FIG. 9-7

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)				L chain (λ)					Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
8	-	-	K	-	-	-	-	E	-	-	-	E	E	-	K	-	K	-	KiH	83.85	79.87	23.41

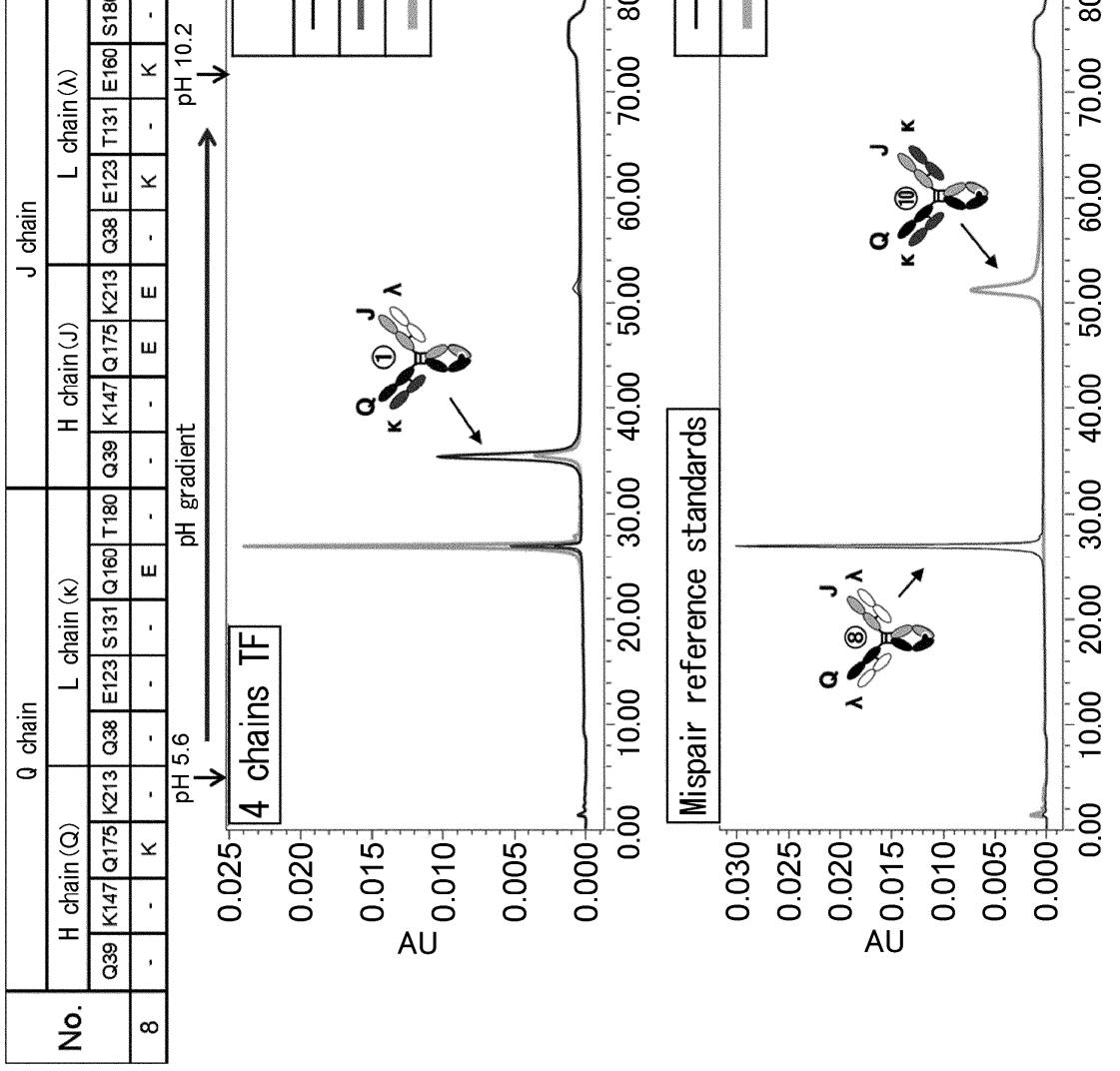


FIG. 9-8

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
9	-	-	K	-	-	-	E	-	E	-	E	E	-	-	-	K	-	K	1:3:1:1	1:1:1:1	1:1:1:3
																		KiH	97.53	97.75	96.62

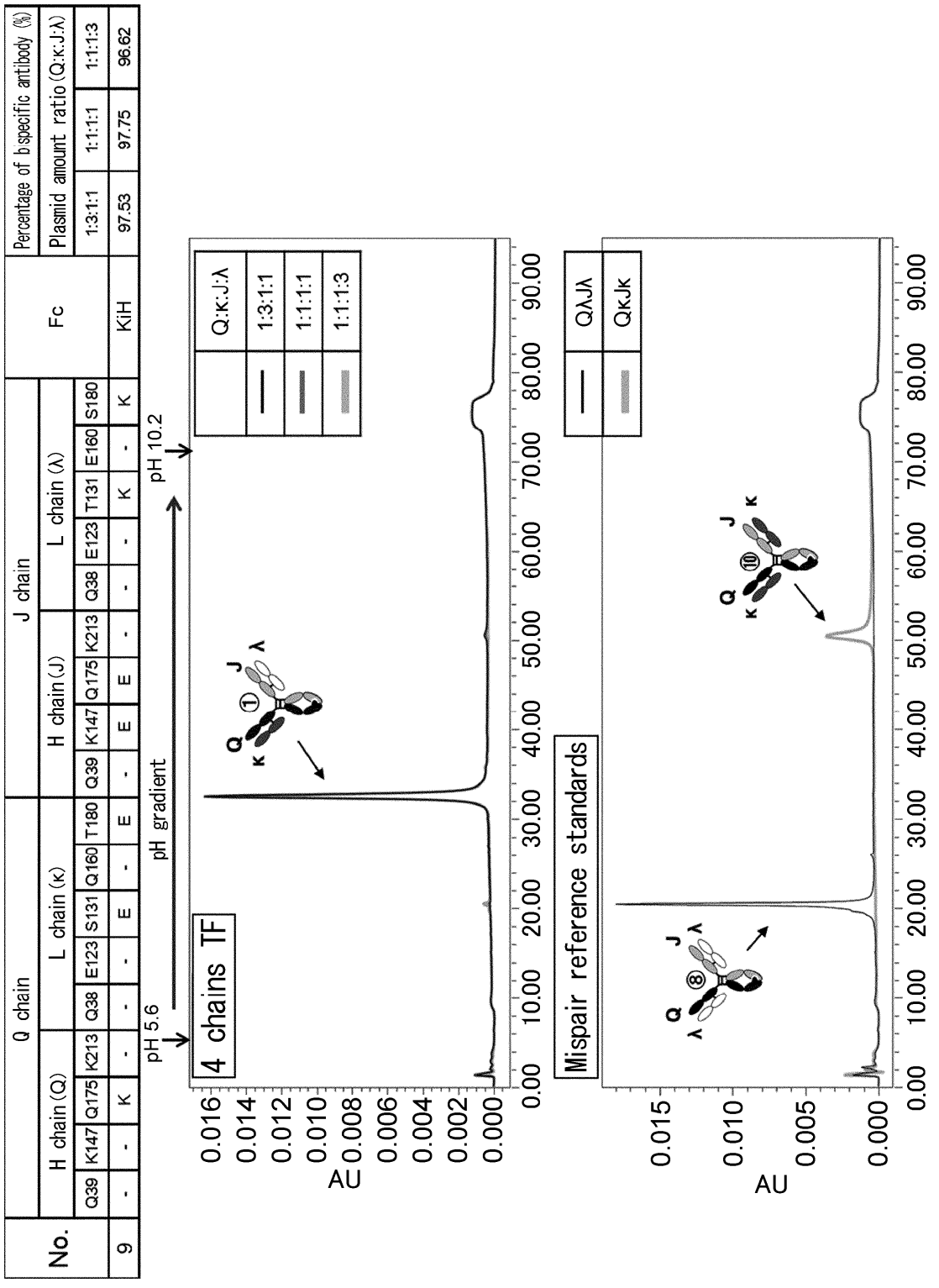


FIG. 9-9

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
10	-	-	-	-	-	-	-	-	E	-	E	-	-	-	-	-	-	K	1:3:1:1	1:1:1:1	1:1:1:3
																		KiH	83.72	78.41	43.93

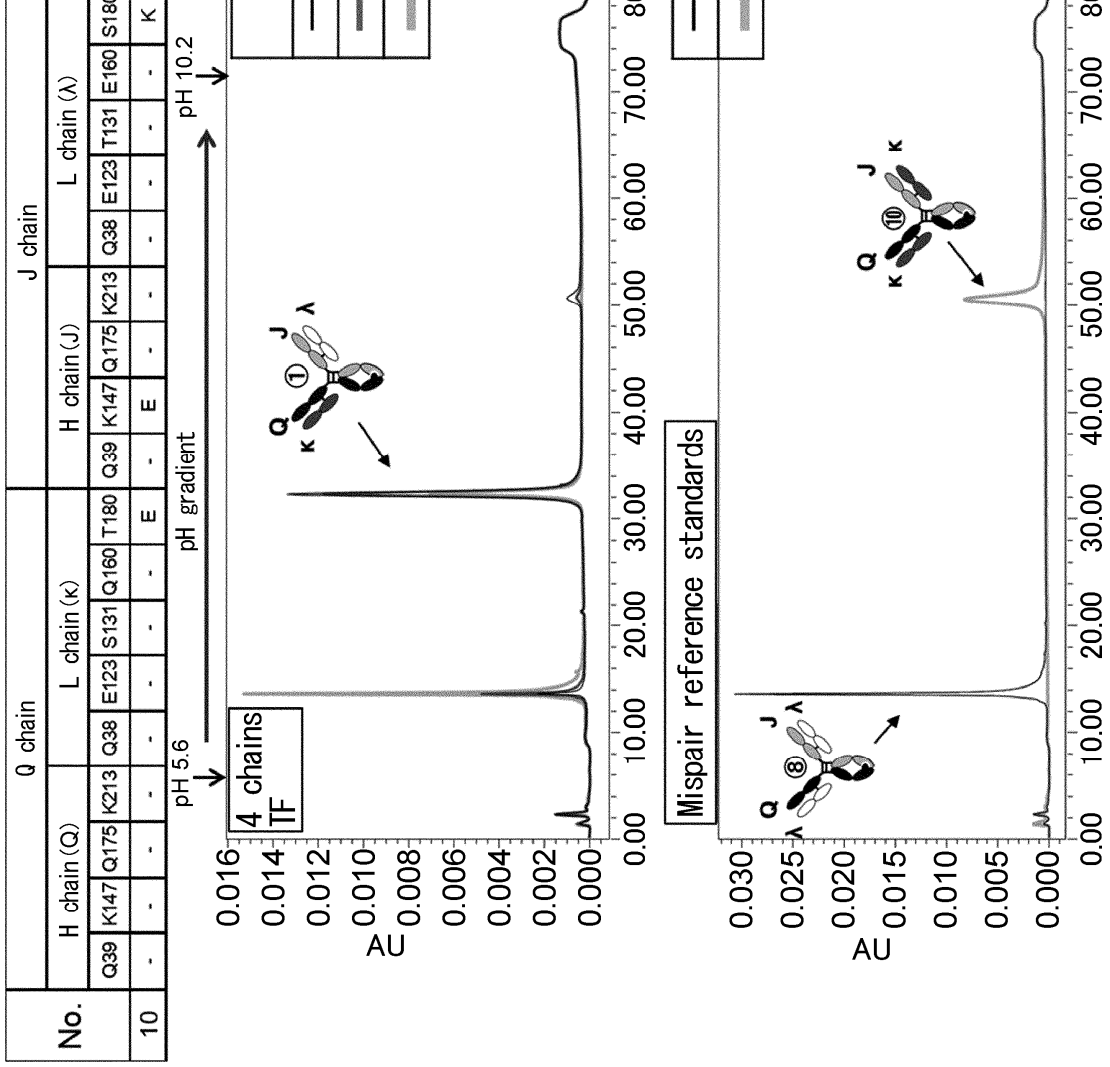


FIG. 9-10

No.	Q chain									J chain									Fc	Percentage of bispecific antibody (%)	
	H chain (Q)			L chain (κ)						H chain (J)			L chain (λ)							Plasmid amount ratio (Q:κ:J:λ)	
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	91.72
11	-	-	-	-	-	-	E	-	-	-	E	-	-	-	-	K	-	-	KiH	1:1:1:1	86.80
																				1:1:1:3	36.37

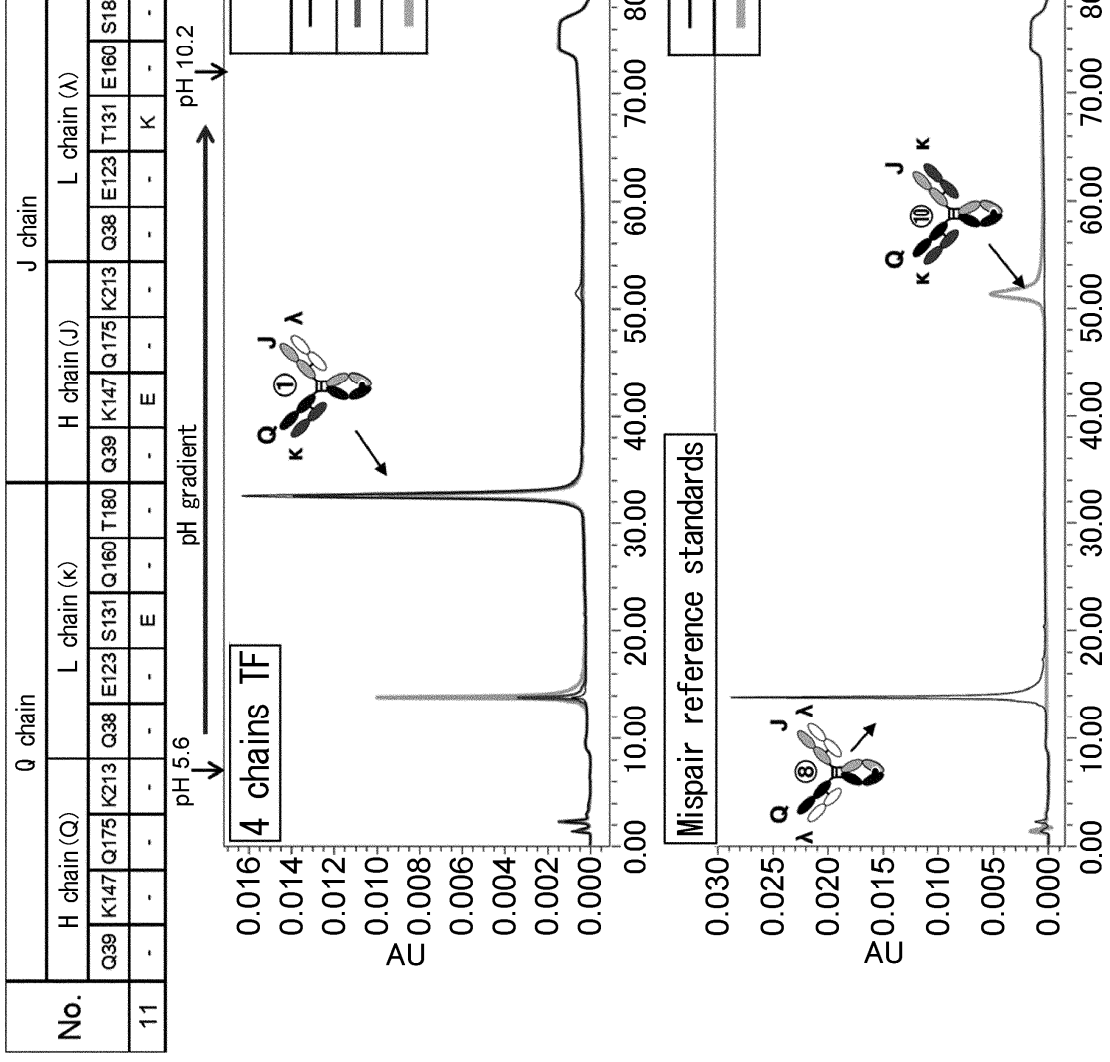


FIG. 9-11

No.	Q chain									J chain									Fc	Percentage of bispecific antibody (%)		
	H chain (Q)				L chain (κ)					H chain (J)				L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
12	-	-	K	-	-	-	-	E	-	-	-	E	-	-	-	-	K	-	KiH	82.07	78.11	23.14

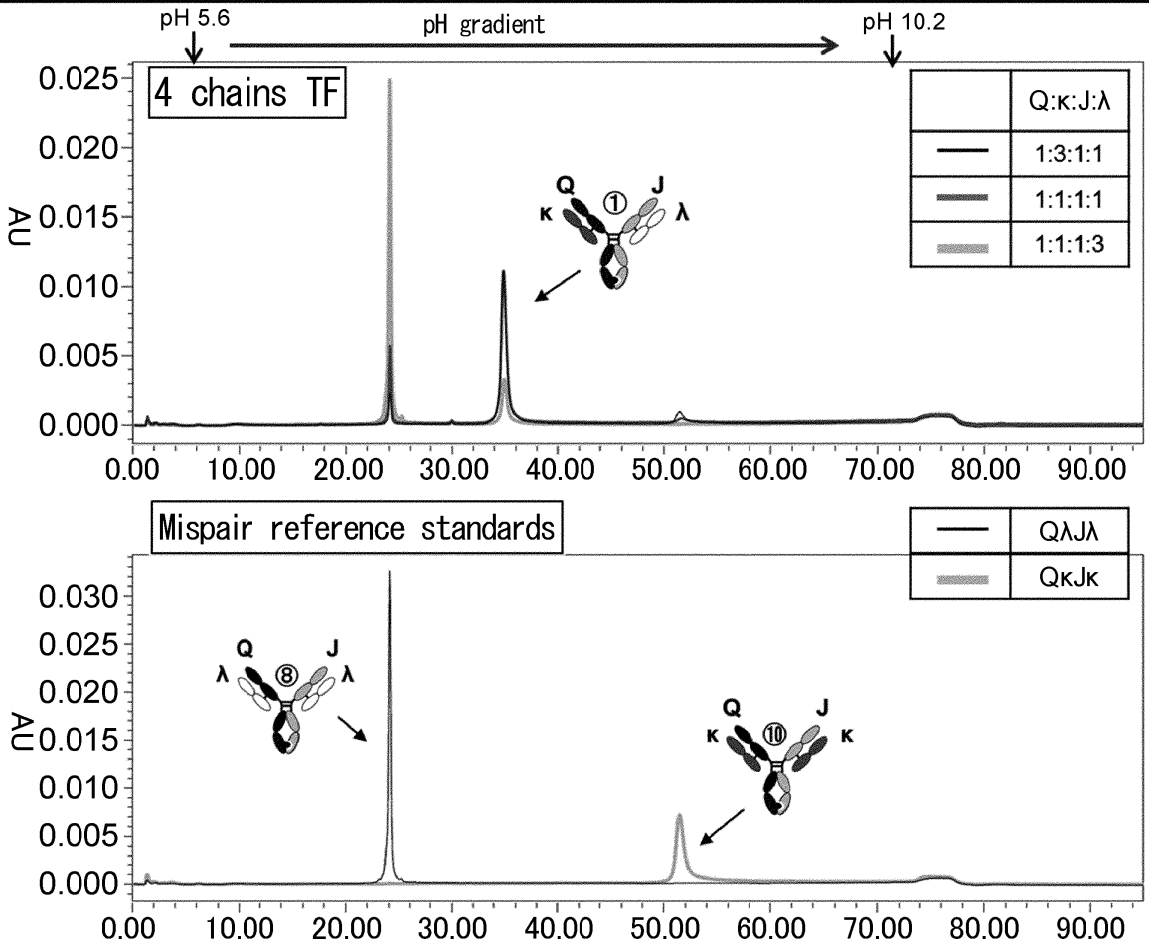


FIG. 9-12

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
13	-	-	-	-	-	-	-	-	-	-	-	-	E	-	K	-	-	-		1:3:1:1	1:1:1:1	1:1:1:3
																			KiH	73.93	66.69	24.94

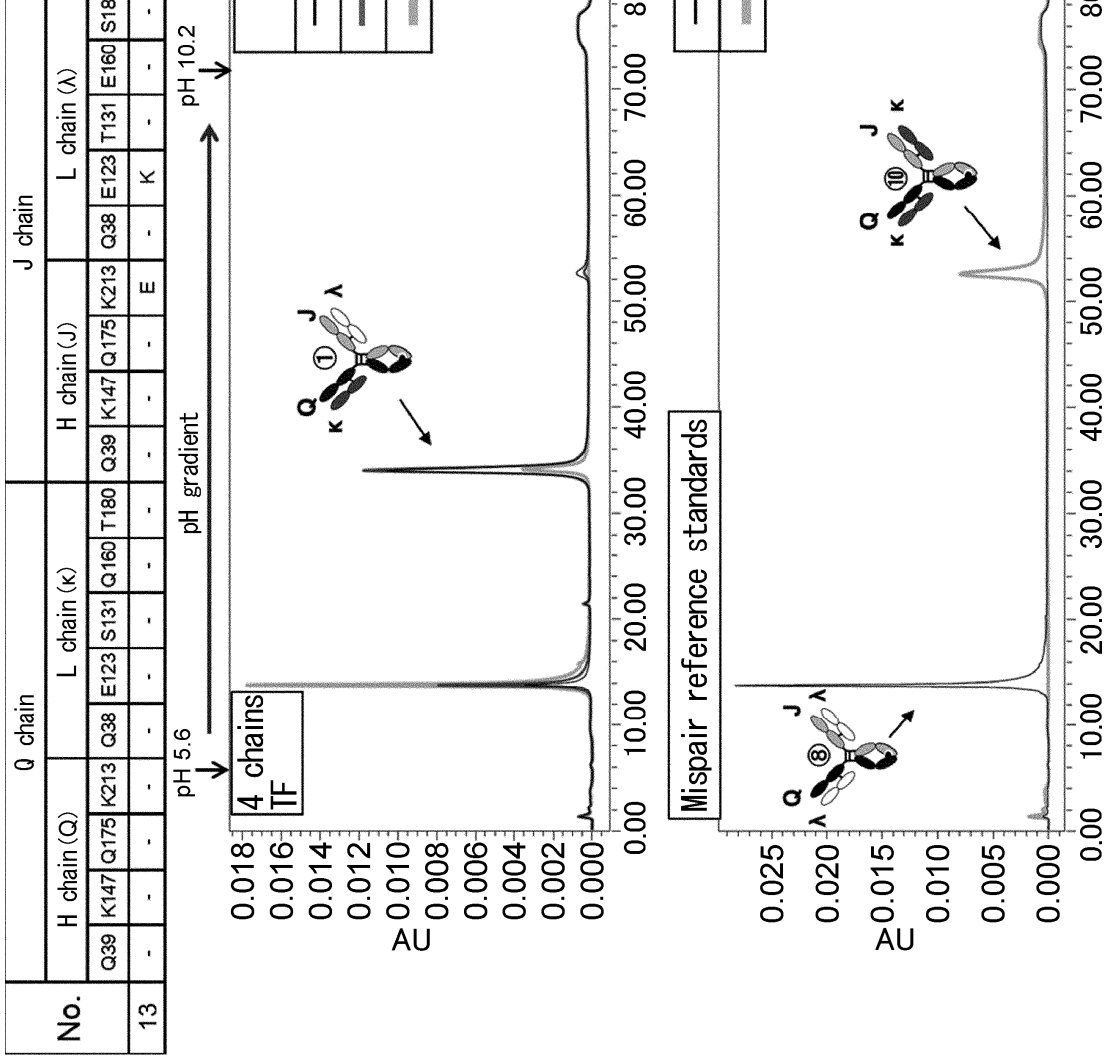


FIG. 9-13

No.	Q chain									J chain									Fc	Percentage of bispecific antibody (%)		
	H chain (Q)				L chain (κ)					H chain (J)				L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
14	-	-	K	-	-	-	-	-	E	-	-	E	-	-	-	-	-	K	KiH	80.27	76.61	46.85

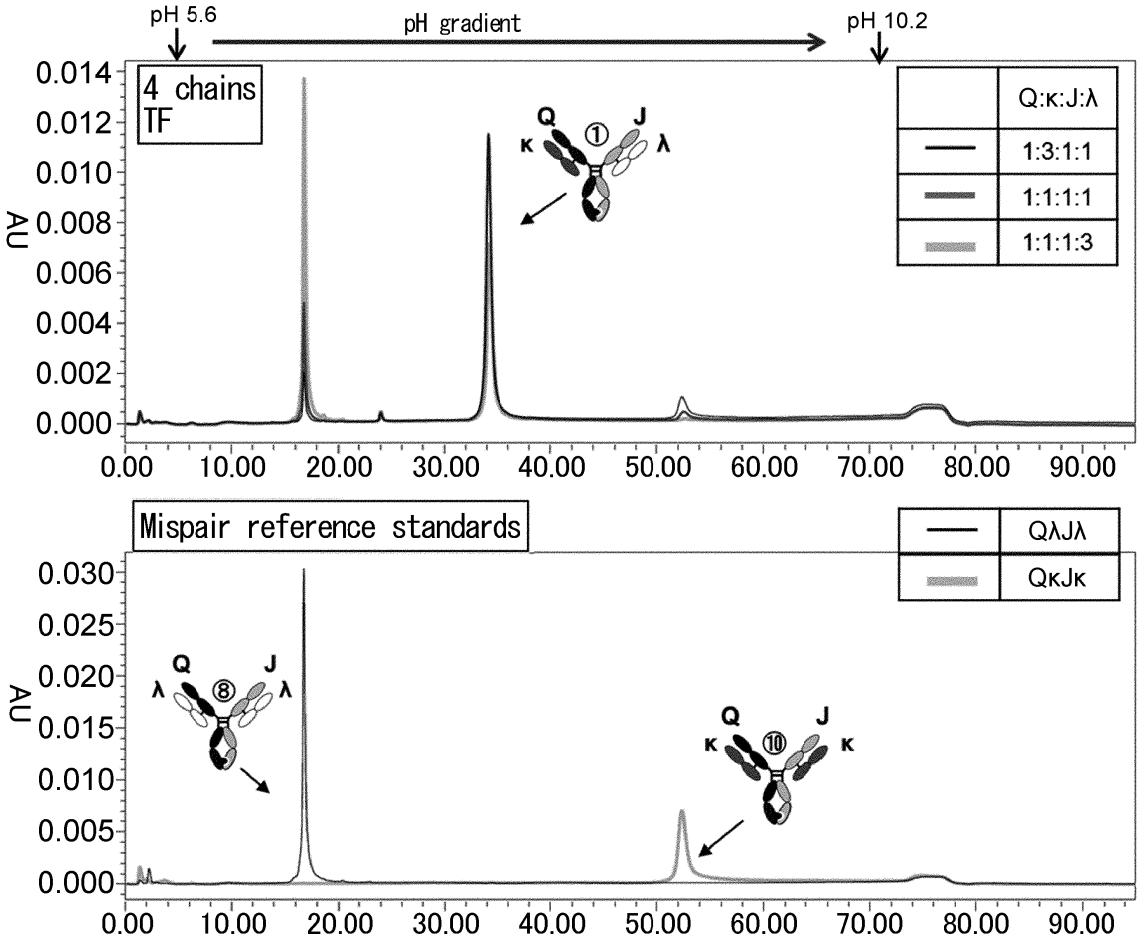


FIG. 9-14

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
15	-	-	K	-	-	-	E	-	-	-	-	E	-	-	-	K	-	-	KiH	84.05	87.10	79.13

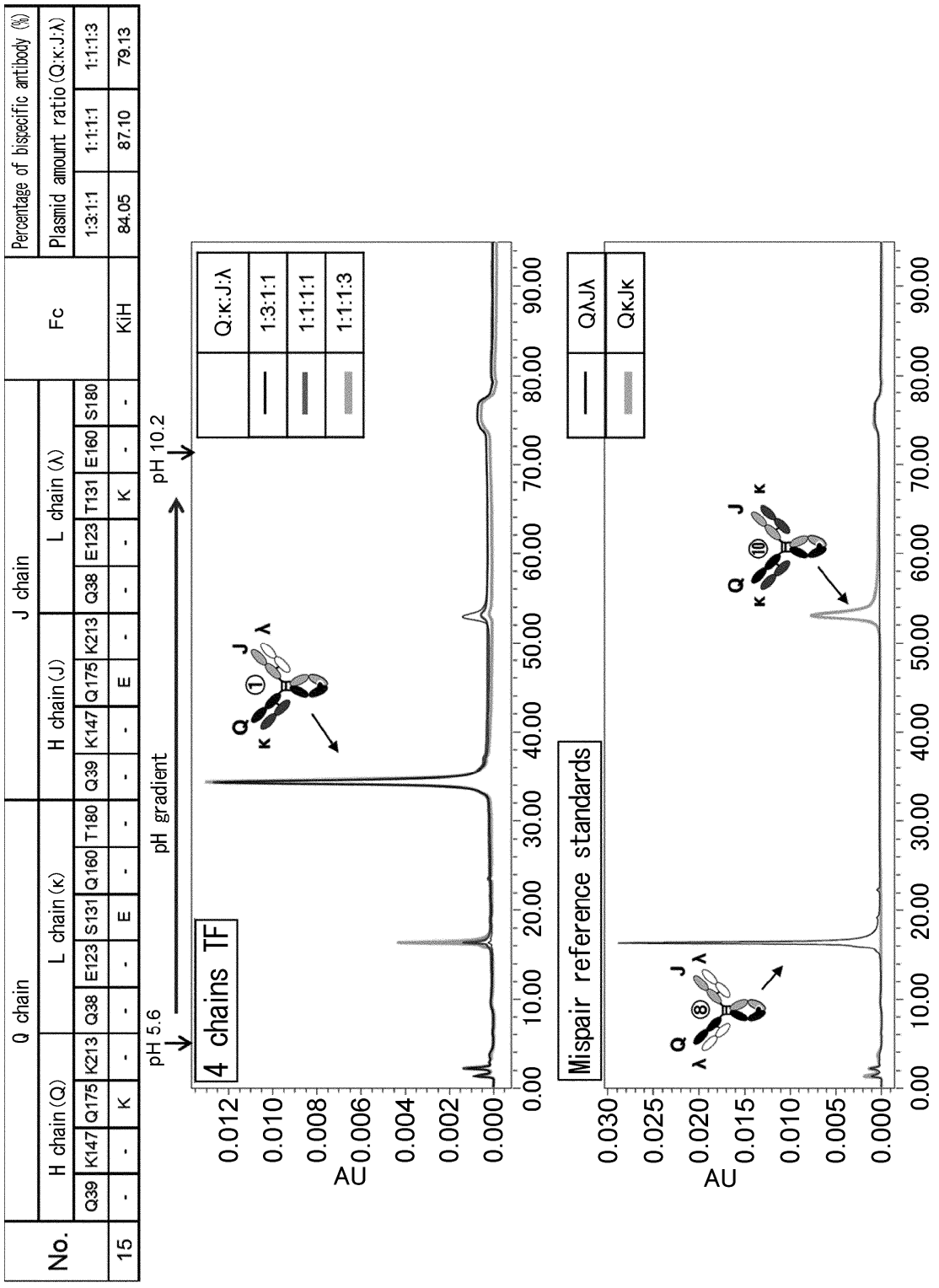


FIG. 9-15

No.	Q chain									J chain									Fc	Percentage of bispecific antibody (%)		
	H chain (Q)				L chain (κ)					H chain (J)				L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
16	-	E	E	-	-	-	K	-	K	-	-	K	-	-	-	E	-	E	KiH	98.30	95.98	81.09

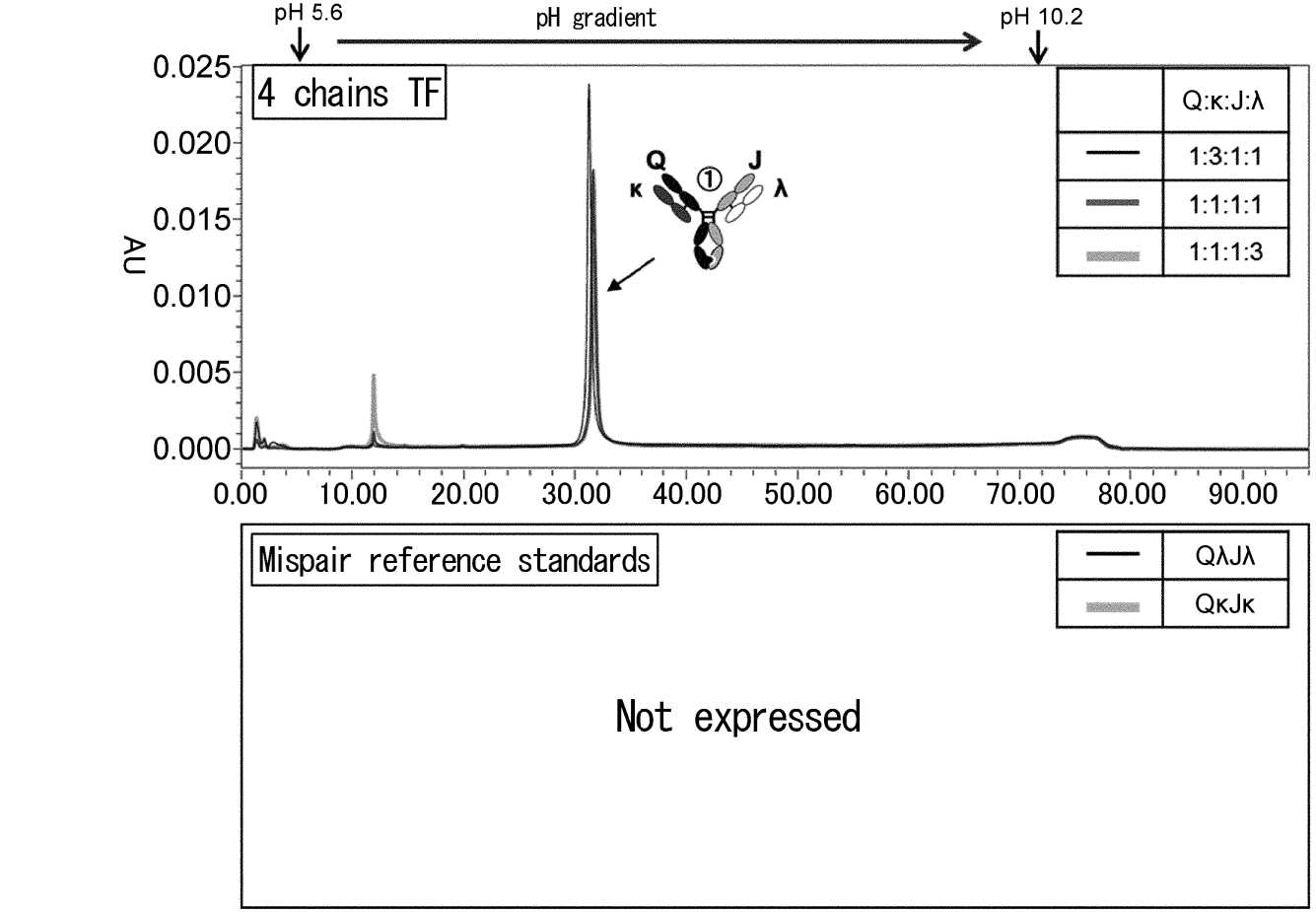


FIG. 9-16

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180			
17	-	E	E	-	-	-	K	K	-	-	-	K	-	-	-	E	-	-	KiH	1:3:1:1	1:1:1:1	1:1:1:3
																				93.48	83.38	50.66

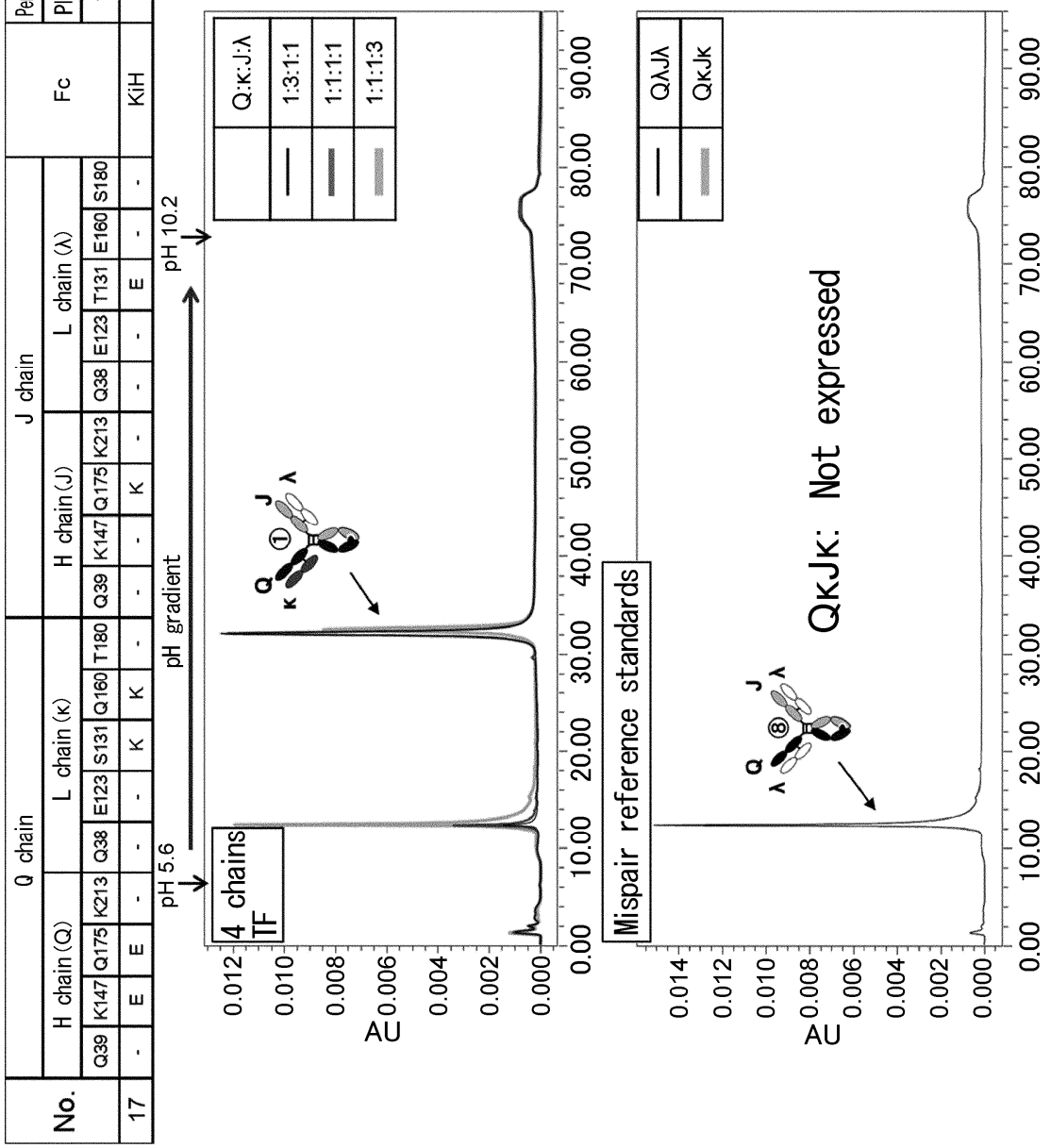


FIG. 9-17

No.	Q chain							J chain							Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)				H chain (J)			L chain (λ)					Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
18	E	-	K	-	K	-	E	-	E	K	E	E	-	E	-	K	-	K	KiH	96.50	99.24	99.85

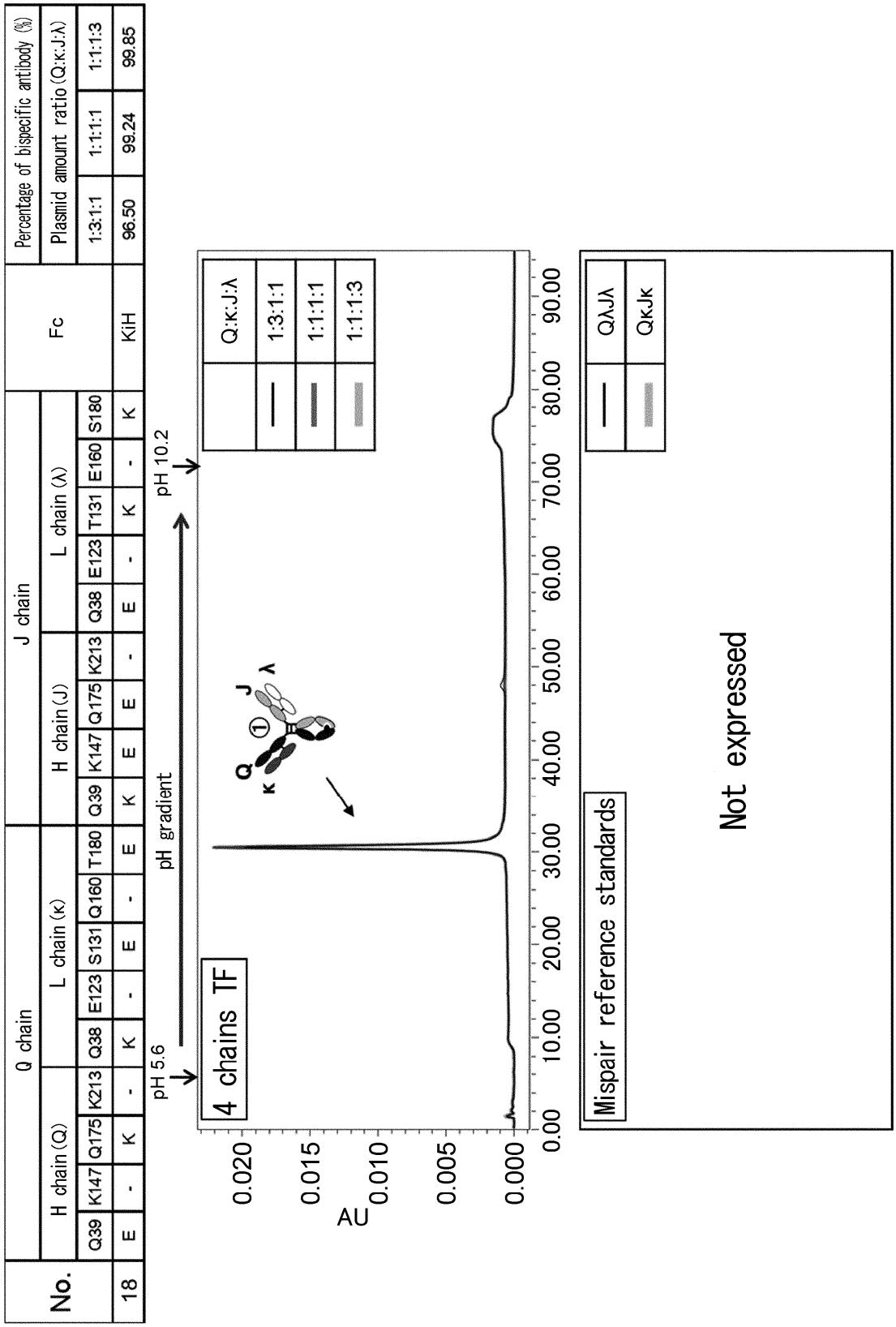


FIG. 9-18

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
19	K	-	K	-	E	-	E	-	E	E	E	E	-	K	-	K	-	K	KiH	99.59	98.42	97.75

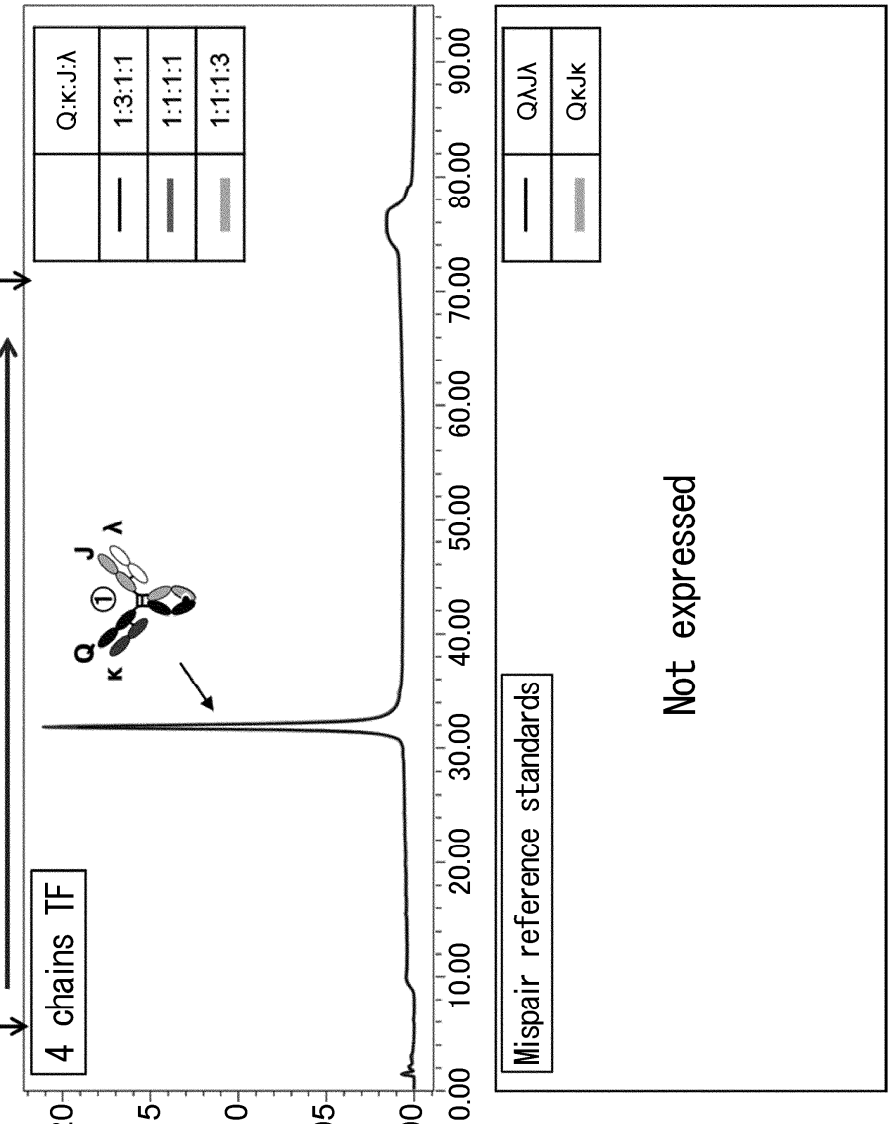


FIG. 9-19

No.	Q chain								J chain								Fc	Percentage of bispecific antibody (%)		
	H chain (Q)			L chain (κ)					H chain (J)			L chain (λ)						Plasmid amount ratio (Q:κ:J:λ)		
	Q39	K147	Q175	K213	Q38	E123	S131	Q160	T180	Q39	K147	Q175	K213	Q38	E123	T131	E160	S180		1:3:1:1	1:1:1:1	1:1:1:3
20	E	-	K	-	K	-	E	-	E	K	E	E	-	E	-	K	-	K	E356/K439E	79.51	85.59	82.39

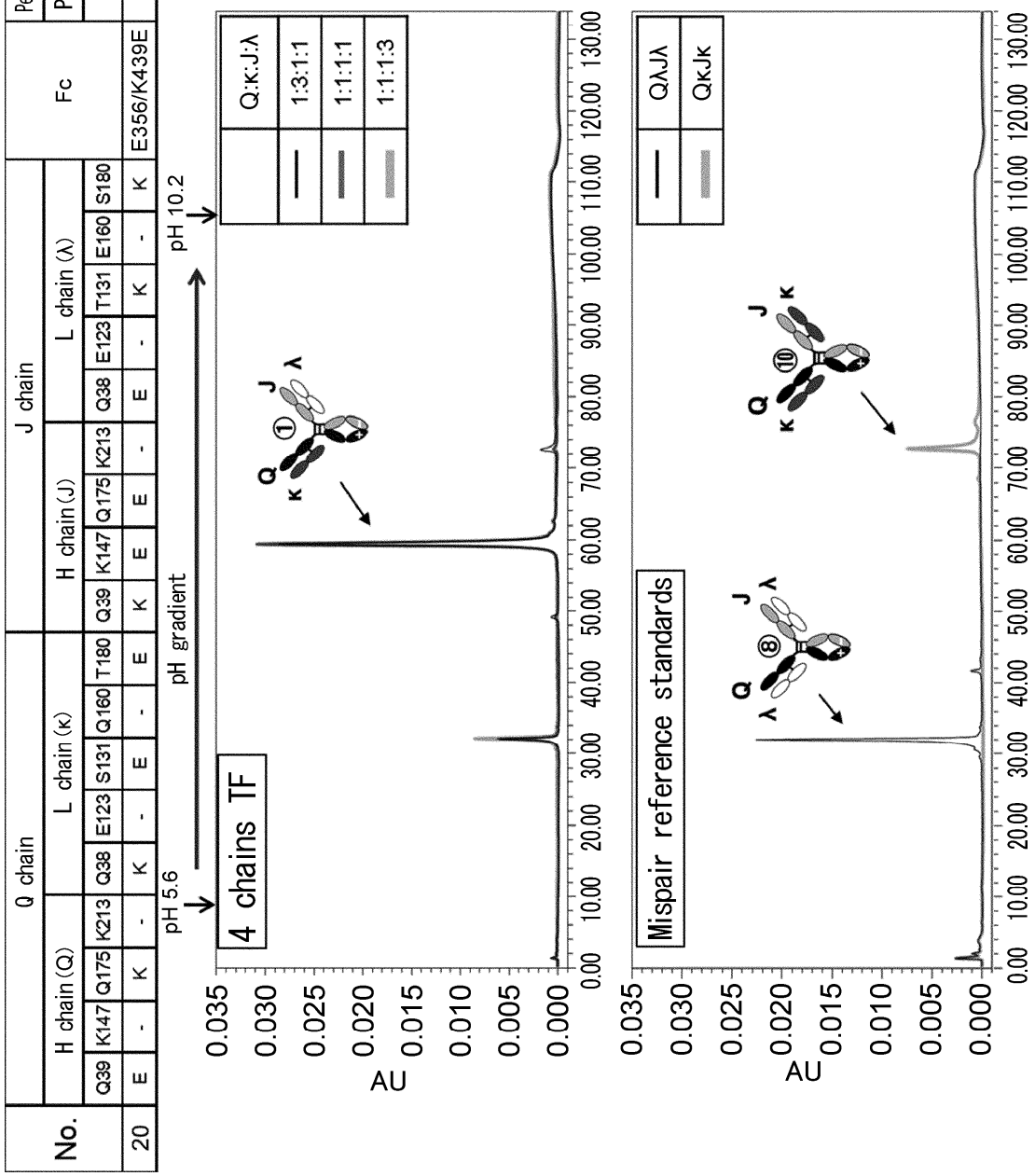


FIG. 9-20

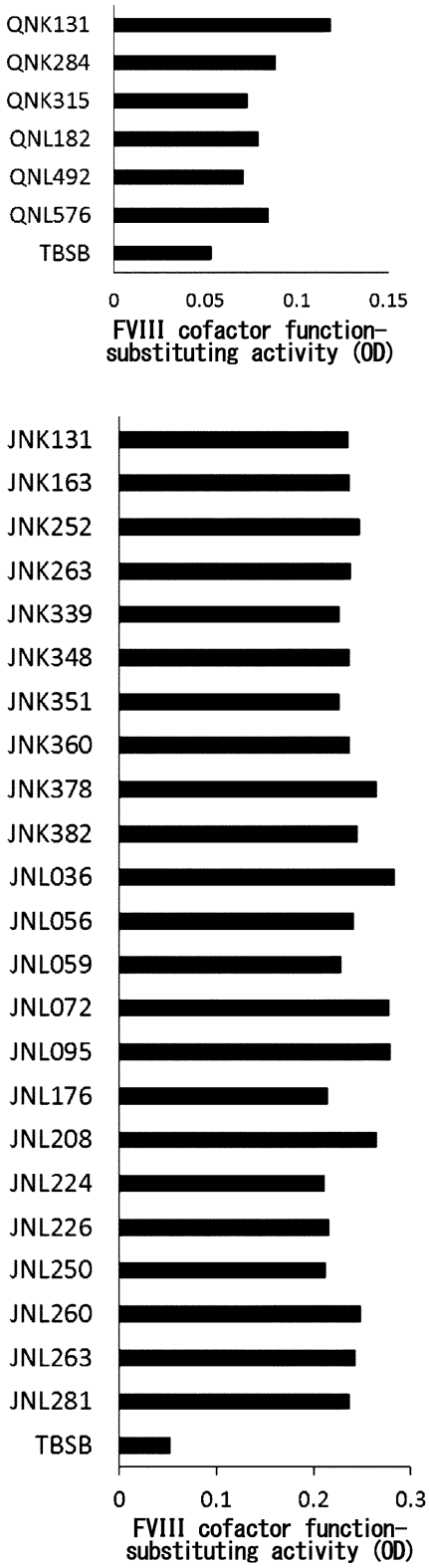

QNK131
QNK284
QNK315
QNL182
QNL492
QNL576
TBSB
0
0.05
0.1
0.15
FVIII cofactor function-substituting activity (OD)
JNK131
JNK163
JNK252
JNK263
JNK339
JNK348
JNK351
JNK360
JNK378
JNK382
JNL036
JNL056
JNL059
JNL072
JNL095
JNL176
JNL208
JNL224
JNL226
JNL250
JNL260
JNL263
JNL281
TBSB
0
0.1
0.2
0.3
FVIII cofactor function-substituting activity (OD)

FIG. 10