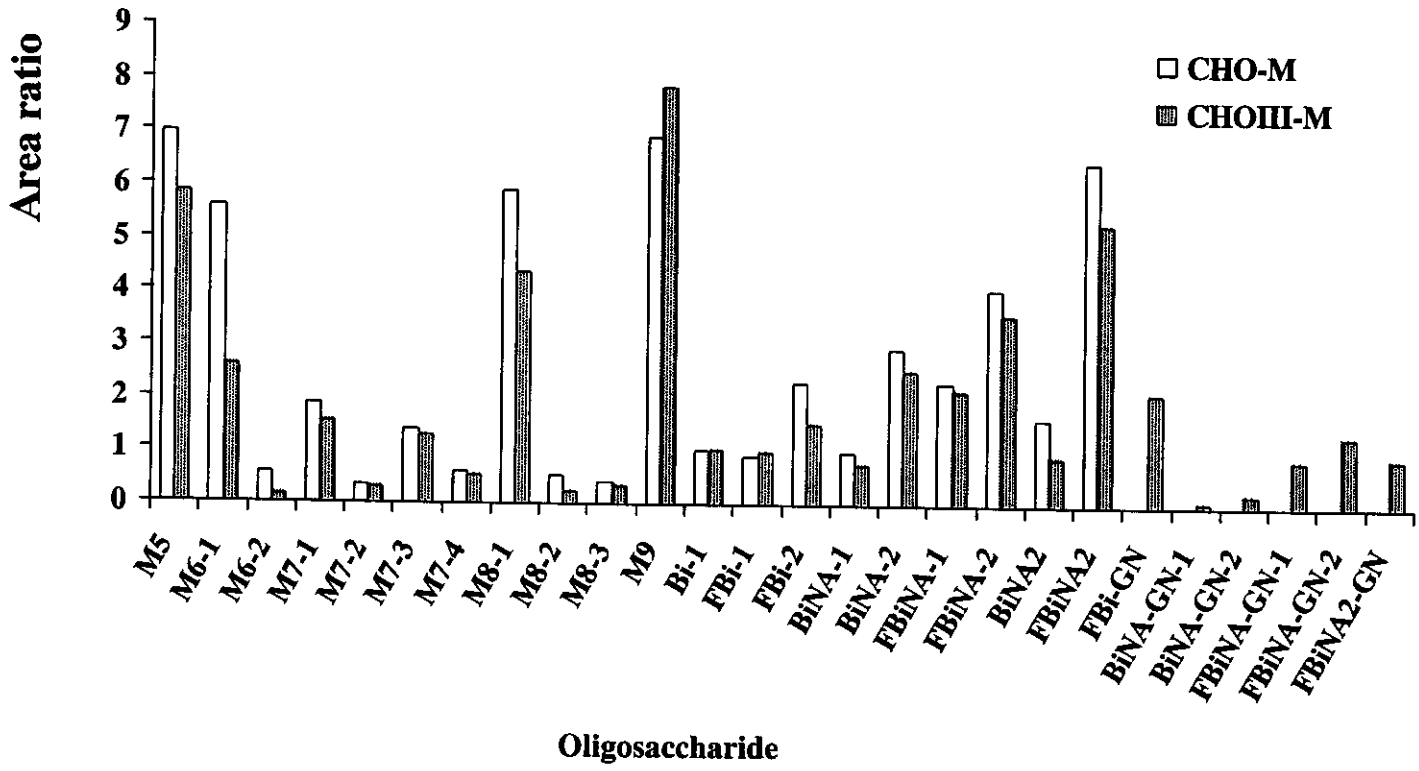
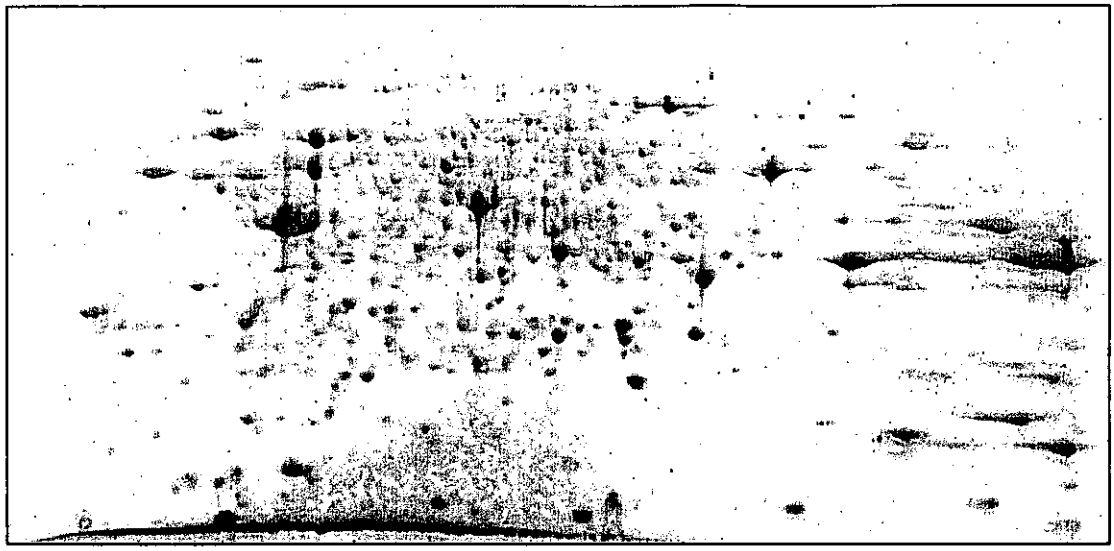


Fig. 19 Peak area ratio of oligosaccharides from CHO and CHOIII membrane

(A) 2D-PAGE



(B) Lectin blot

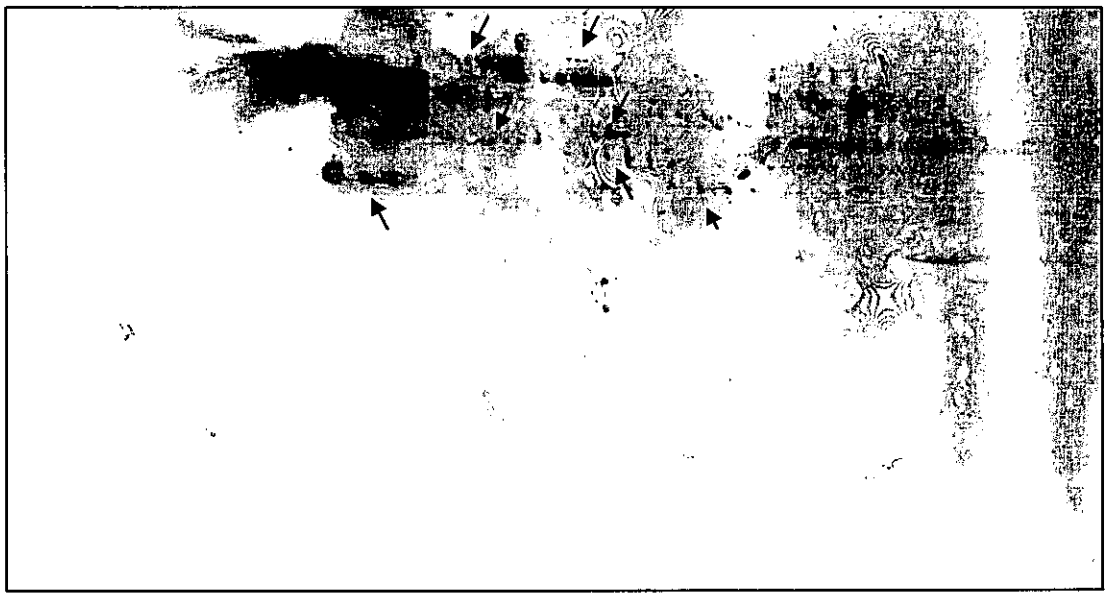


Fig. 20 2D-GE of CHO-III membrane proteins

(A) Sypro Orange, (B) Lectin blot

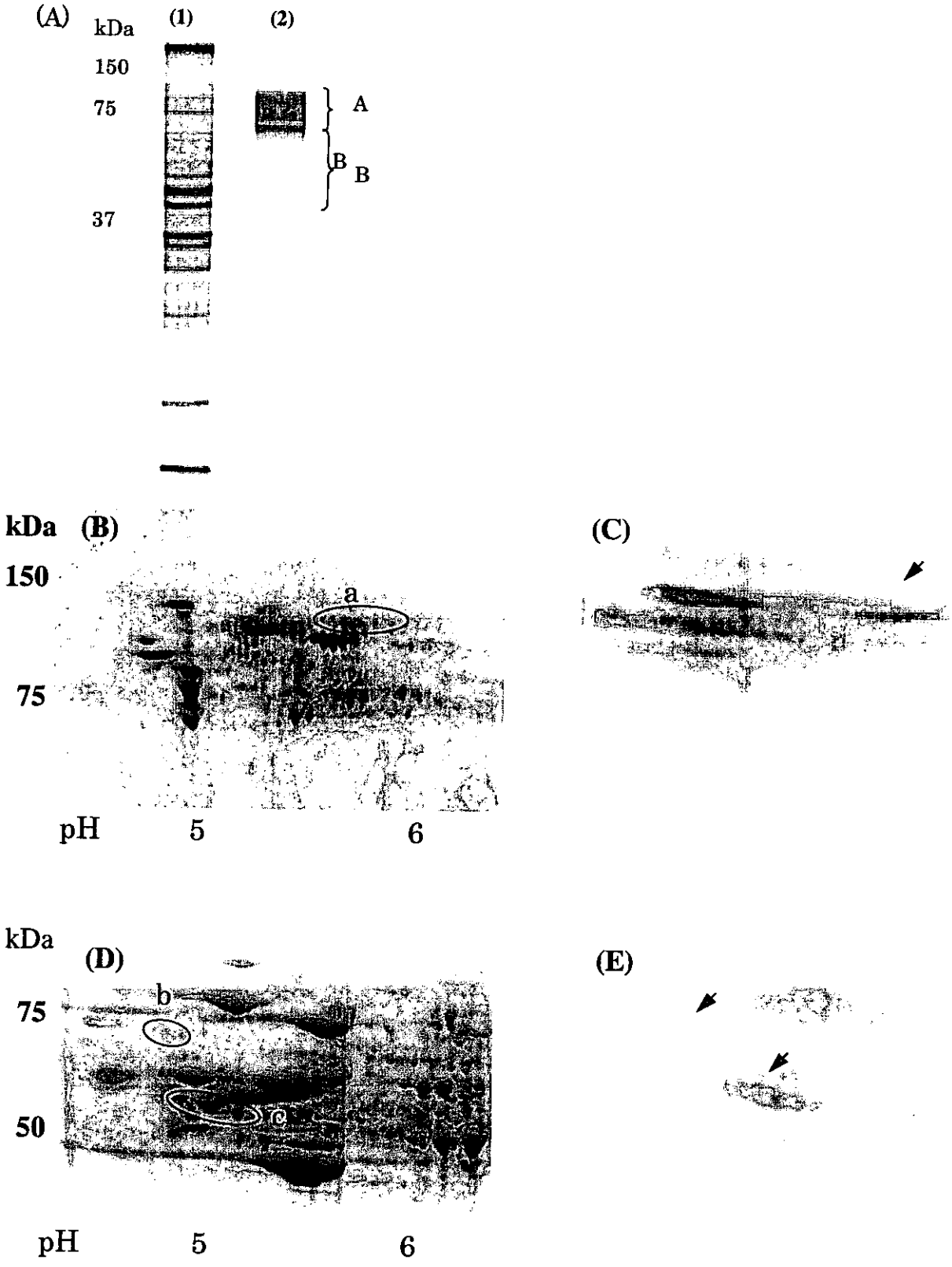
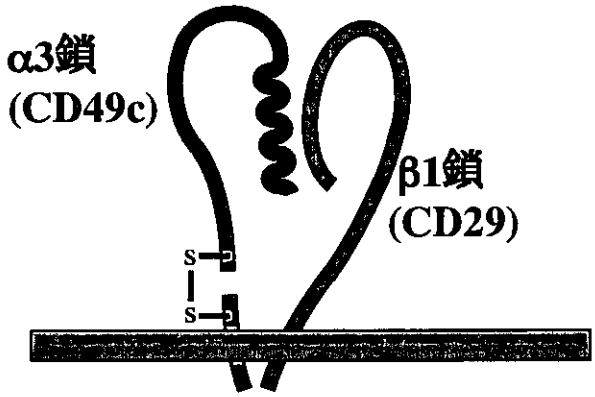


Fig. 21 (A) SDS-PAGE of CHO-III membrane
(B) 2D-GE of band A, (c) Lectin blot of Band A
(D) 2D-GE of band B, (D) Lectin blot of Band B

分布：上皮細胞
リガンド： ラミニン，フィブロネクチン，
コラーゲン
臨床：腫瘍細胞での発現異常

細胞接着の制御

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  61 qterqqryll lagaprdlsv adgytNrtga vylcpltalk ddcermdise ksdpdhhiie
 121 dmwlgvtvas qgpagrvlvc ahrytqvlws gmedqrrmvg kcyvrgndlq ldpgddwqty
 181 hnemcnsntd ylqtgmcqlg tsggftqntv yfgapgaynw kgnsymiqrk dwdlseysyk
 241 gsedqgnlyi gytvqvgsav lhptyitvva gaprhqhmga vfllsqesgg dlkrkqvleg
 301 tqvgayfgsa ialadlnndg wqdilvgapy yferkeevgg avyvfmnqag tsfpdqpsll
 361 lhgpsrsafg isiasigdin qdgfqdiavg apfeglgkvy iyhssspgll rqpqqivhgd
 421 klglpglstf gyslsgkmdv ddnsypdllv gslsdhivll rarpvinilq rtlvarpavl
 481 dpslctptsc vqvelcfayN qsagnpsyrr Nitlaytlea drdrrppprlr farsqsavfh
 541 gflsmpethc qtlelllmdn vrdklrpivi amNyslplrm pdrlklgmrs ldaypvlnqa
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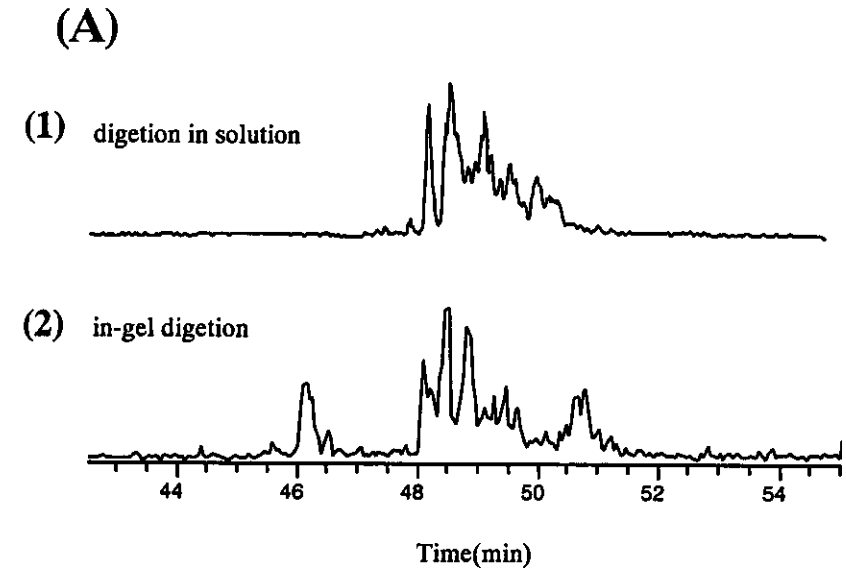
 661 psrerageda healltlevp palllssvrp sgtcqaNeti lcelgnpfkr nqrmelliaf
 721 evigvtlhtr dlkaqlqlst sshqdnlqpm tliiqvdytl qaslslmthr lqsffggtvm
 781 geagmktved vgsplkyefq vspvgdglaa lgtivlglew pyevtngkwl lyptciiihs
 841 Neswpcqppg nlvnplnlil sdpgdkphsp qrrrr
                                      qldpg gdqgsppvtl aaakkakset
 901 vltcasgrar cvwlecpipd tsNvtNvtvk arvwNstfie dyrdfdrvrv dgwatlflrt
 961 siptinmeNk ttwfsvdids elveelpaei elwlvlvavs aglllglii illwkcgffk
1021 ptryyrimpk yhavrireee rypppgstlp tkkhwvtswq irdryy
```

Heavy chain (33~872)

Light chain (876~1066)

Under line : Calcium binding region, N: Glycosylation site

Fig. 22 Amino acid sequence and N-linked glycosylation sites of Integrin α-3 (CD49c)



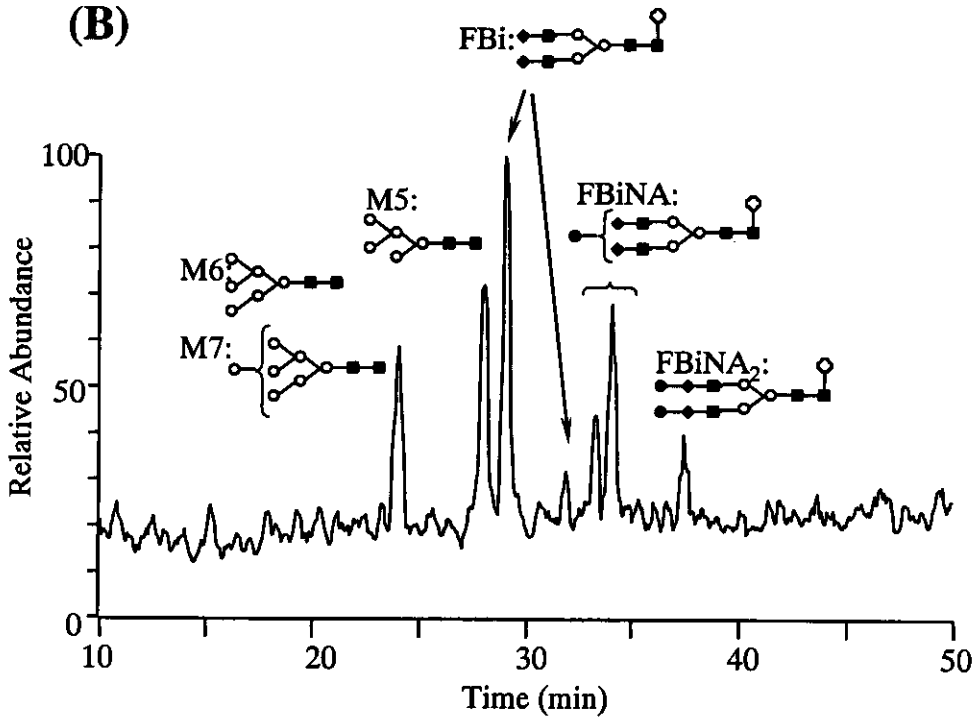


Fig. 23 Oligosaccharide profiling of gel separated proteins (A) Erythropoietin, (B) t-PA

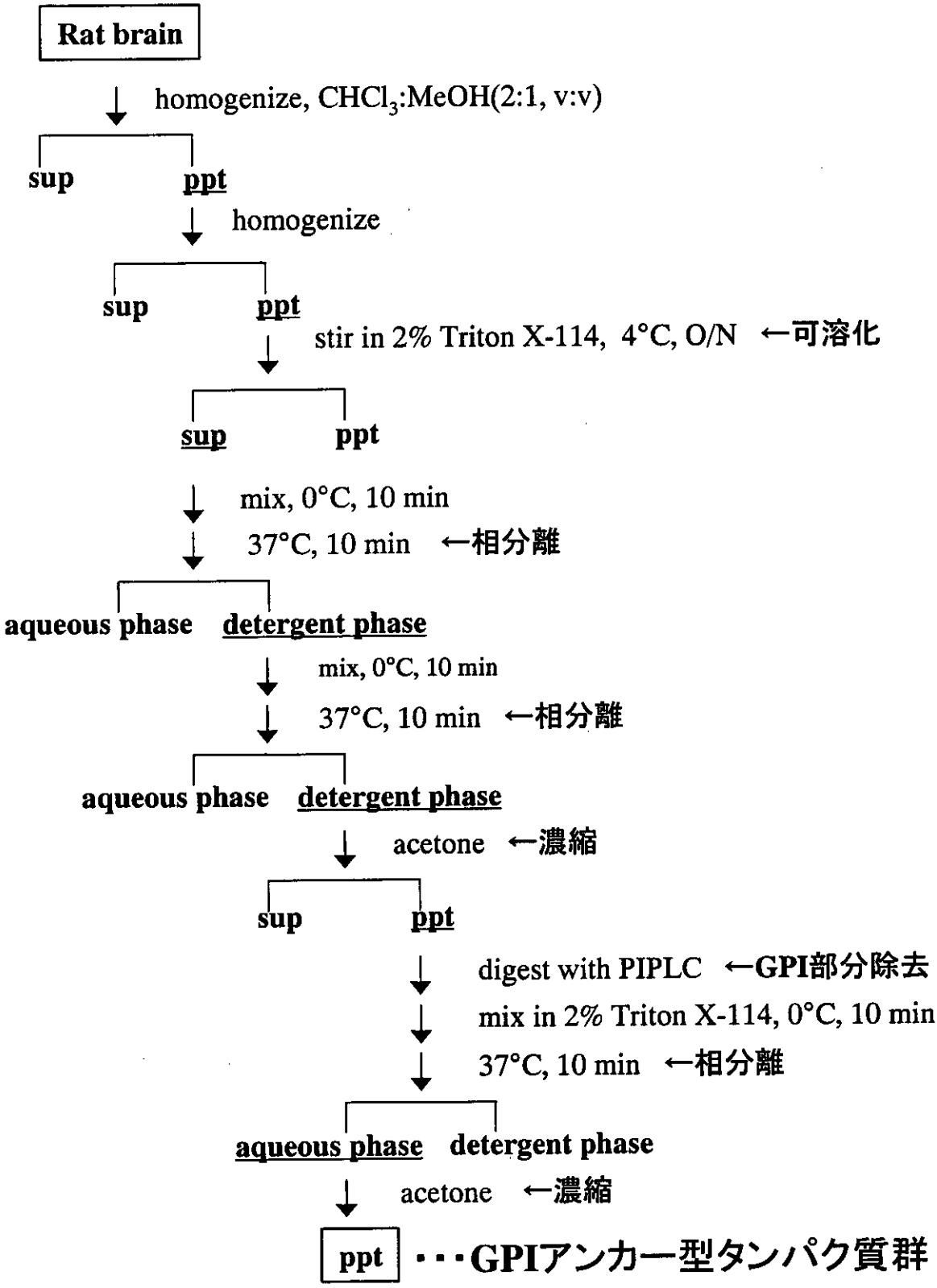


Fig. 24 Preparation of lipid-free GPI-anchored proteins from rat brain

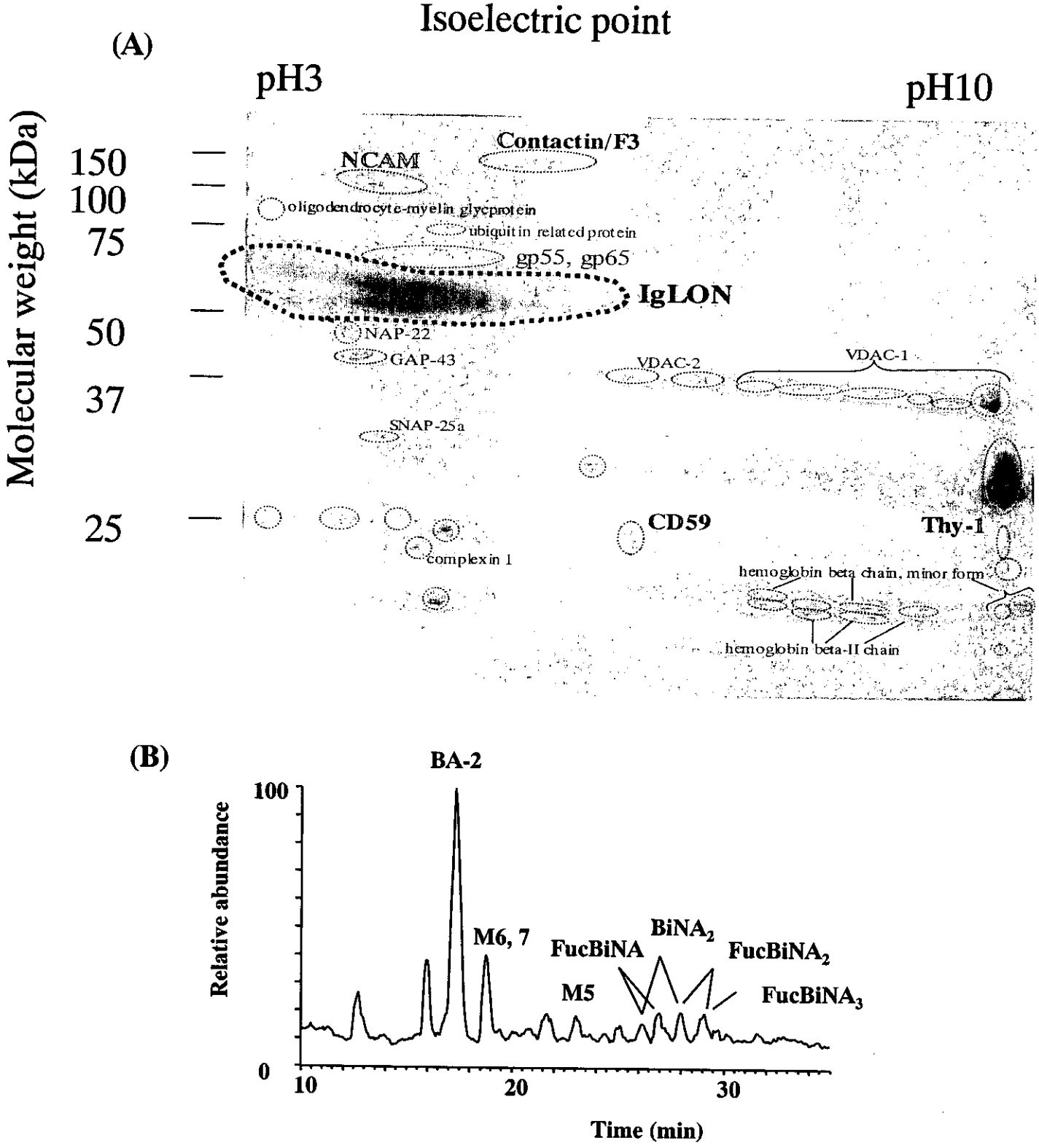


Fig. 25 (A)2D-GE of lipid-free GPI-anchored proteins from 3-week old rat brain, (B) Oligosaccharide profiling of gel separated Kilon

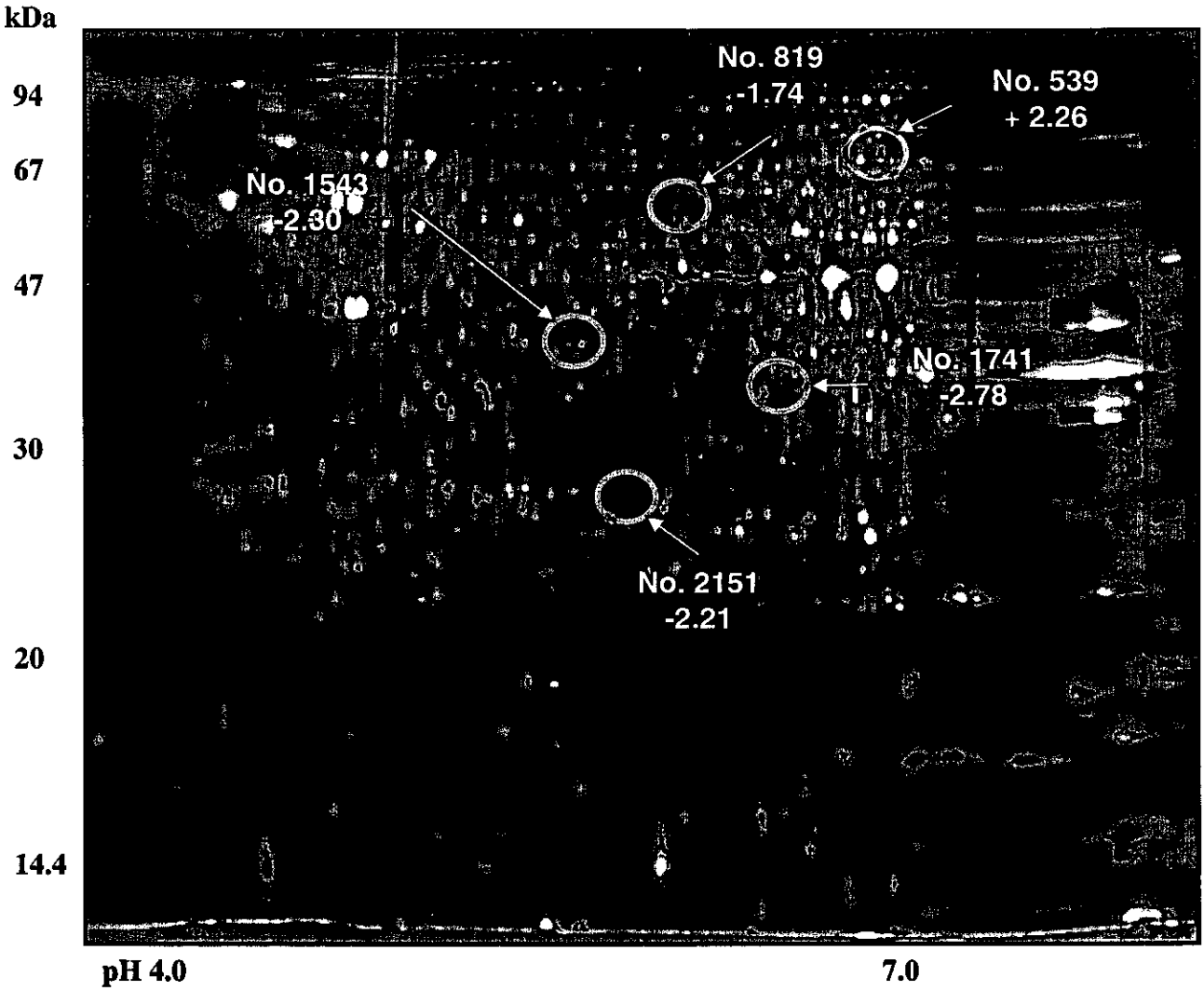


Fig.5 2D-DIGE images of Cy3 labeled HepG2 (A) and Cy5 labeled HepG2III (B) membrane fraction.

Fig. 26 2D-DIGE images of Cy3 labeled HepG2 and Cy5 labeled HepG2III membrane fraction

Table 4 Glycosylation analysis of alpha-fetoprotein

Retention time (min)	*m/z*	Charge	M.W.	**Peptide** Sequences	M.W.	**Oligosaccharide** Structures	M.W.
23	1013.43	+3	3037.3	k/**vnfteiqk**/l	977.5	[HexNAc]4[Hex]5[Neu5Ac]1[Fuc]1	2077.8
23	1519.67	+2	3037.3				
23	1081.29	+3	3240.9	k/**vnfteiqk**/l	977.5	[HexNAc]5[Hex]5[Neu5Ac]1[Fuc]1	2281.4
23	1621.44	+2	3240.9				
23	1446.62	+2	2891.2	k/**vnfteiqk**/l	977.5	[HexNAc]4[Hex]5[Neu5Ac]1	1931.8
23	1118.67	+2	2235.3	k/**vnfteiqk**/l	977.5	[HexNAc]3[Hex]4	1275.9
23	1264.05	2	2526.1	k/**vnfteiqk**/l	977.5	[HexNAc]3[Hex]4[Neu5Ac]1	1566.6
	1110.48	3	2526.1				
24	1665.44	+2	3328.9	k/**vnfteiqk**/l	977.5	[HexNAc]4[Hex]5Neu5Ac]2[Fuc]1	2369.4
24	1178.33	+3	3532.0	k/**vnfteiqk**/l	977.5	[HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1	2572.5
24	1061.78	3	3182.3	k/**vnfteiqk**/l	977.5	[HexNAc]4[Hex]5[Neu5Ac]2	2222.9
25	1592.17	2	3182.3				
27	1236.04	+3	3705.1	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2369.4
27	1853.29	+2	3704.6				
27	1187.17	+3	3558.5	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]4[Hex]5[Neu5Ac]2	2222.8
28	1780.23	+2	3558.5				
28	1303.56	+3	3907.7	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1	2572.0
28	1138.84	+3	3413.5	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]4[Hex]5[Neu5Ac]1[Fuc]1	2077.8
28	1206.52	+3	3616.6	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]5[Hex]5[Neu5Ac]1[Fuc]1	2280.9
28	1090.14	+3	3267.4	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]4[Hex]5[Neu5Ac]1	1931.7
28	1322.75	+3	3965.3	k/**ftkvnfteiqk**/l	1353.7	[HexNAc]6[Hex]5[Neu5Ac]2	2629.6

Table 5 Glycosylation analysis of ceruloplasmin

Retention time (min)	*m/z*	Charge	M.W.	**Peptide** Sequences	M.W.	**Oligosaccharide** Structures	M.W.
24	1025.17	+4	4096.7	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2	2222.9
24	1366.54	+3	4096.6				
24	1373.88	+3	4118.6	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.9
24	1061.67	+4	4242.7	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2368.9
25	1415.23	+3	4242.7				
25	1262.24	+4	5045.0	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]5[Hex]5[Neu5Ac]3[Fuc]2	3171.2
25	1682.65	+3	5045.0				
25	1189.21	+4	4752.8	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3	2879.1
25	1585.27	+3	4752.8				
25	1225.72	+4	4898.9	EHEGAIYPDNTTDFQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3[Fuc]1	3025.1
25	1633.96	+3	4898.9				
27	1093.95	+4	4371.8	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2368.8
27	1458.27	+3	4371.8				
27	1057.43	+4	4225.7	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
27	1409.57	+3	4225.7				
28	1257.99	+4	5028.0	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3[Fuc]1	3025.0
28	1677.01	+3	5028.0				
28	1221.48	+4	4881.9	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3	2878.9
28	1628.30	+3	4881.9				
33	1347.54	+3	4039.6	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]1	1931.6
34	1450.24	+3	4347.7	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2+NH3	2239.7
34	1083.68	+4	4330.7	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
34	1444.56	+3	4330.7	ENLTAPGSDSAVFFEQGTTR			
34	1493.28	+3	4476.8	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2368.8
34	1089.17	+4	4352.7	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.7
35	1668.97	+3	5003.9	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]5[Hex]6[Neu5Ac]3+NH3	2895.9
35	1663.29	+3	4986.9	ENLTAPGSDSAVFFEQGTTR	2126.0	[HexNAc]5[Hex]6[Neu5Ac]3	2878.9
35	1247.72	+4	4986.9				
37	1282.48	+3	3844.4	AGLQAFFQVQECNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
37	1923.24	+2	3844.5	AGLQAFFQVQECNK			
38	1331.17	+3	3990.5	AGLQAFFQVQECNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2368.8
38	1289.81	+3	3866.4	AGLQAFFQVQECNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.7

Table 6 Ad vectors used in this study

Ad vector	Foreign gene					
	E1 deletion region		E3 deletion region		Region between E4 and 3'ITR	
	Promoter	GOI	Promoter	GOI	Promoter	GOI
AdOn-L4	TRE/CMV	Luciferase	CMV	rtTA	—	—
AdBI-rtTA-L	TRE/CMV	Luciferase+rtTA	—	—	—	—
Ad-rtTA-IRES-tTS-L	TRE/CMV	Luciferase	CMV	rtTA+tTS	—	—
Ad-tTS-BI-rtTA-L	TRE/CMV	Luciferase+rtTA	—	—	EF-1α	tTS
Ad-rtTA-tTS-L	TRE/CMV	Luciferase	CMV	rtTA	EF-1α	tTS
Ad-L2	CMV	Luciferase	—	—	—	—
AdOn-SEAP4	TRE/CMV	SEAP	CMV	rtTA	—	—
Ad-M2-SEAP4	TRE/CMV	SEAP	CMV	$rtTA2^S$-M2	—	—
Ad-S2-SEAP4	TRE/CMV	SEAP	CMV	$rtTA2^S$-S2	—	—
Ad-rtTA-tTS-SEAP	TRE/CMV	SEAP	CMV	rtTA	EF-1α	tTS
Ad-M2-tTS-SEAP	TRE/CMV	SEAP	CMV	$rtTA2^S$-M2	EF-1α	tTS
Ad-S2-tTS-SEAP	TRE/CMV	SEAP	CMV	$rtTA2^S$-S2	EF-1α	tTS
Ad-SEAP2	CMV	SEAP	—	—	—	—

CMV = CMV intermediate-early promoter / enhancer
TRE/CMV = tet-responsive promoter containing minimal CMV promoter
EF-1α = human elongation factor-1α promoter
Ad-rtTA-IRES-tTS-L contains CMV promoter / intron A / rtTA / IRES / tTS / P(A) cassette in the E3 deletion region.
AdBI-rtTA-L and Ad-tTS-BI-rtTA-L express luciferase and rtTA from one bidirectional tet-responsive promoter cloned in the E1 deletion region.

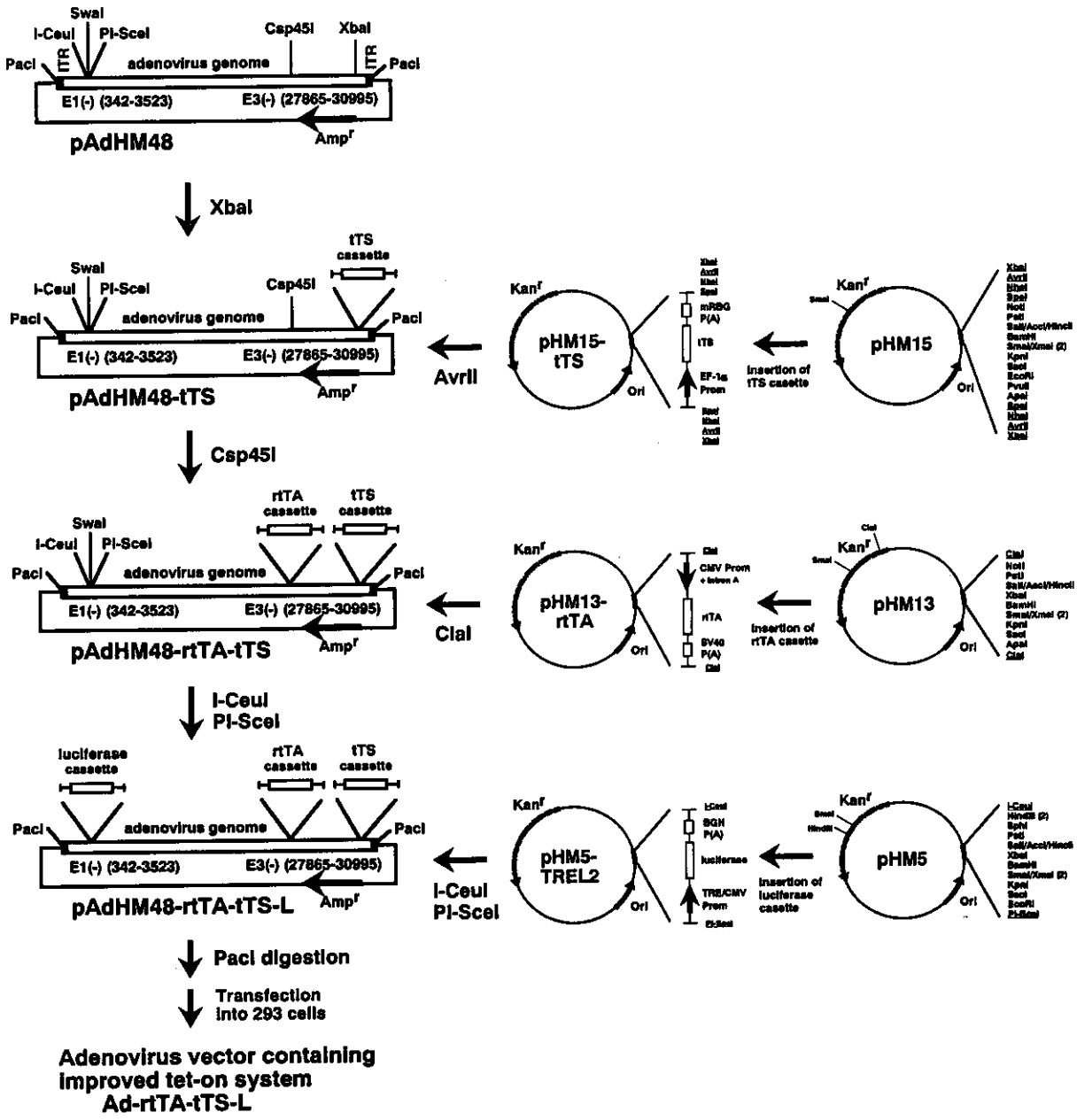


Fig.27 The construction strategy of Ad vectors containing the triple gene expression system.

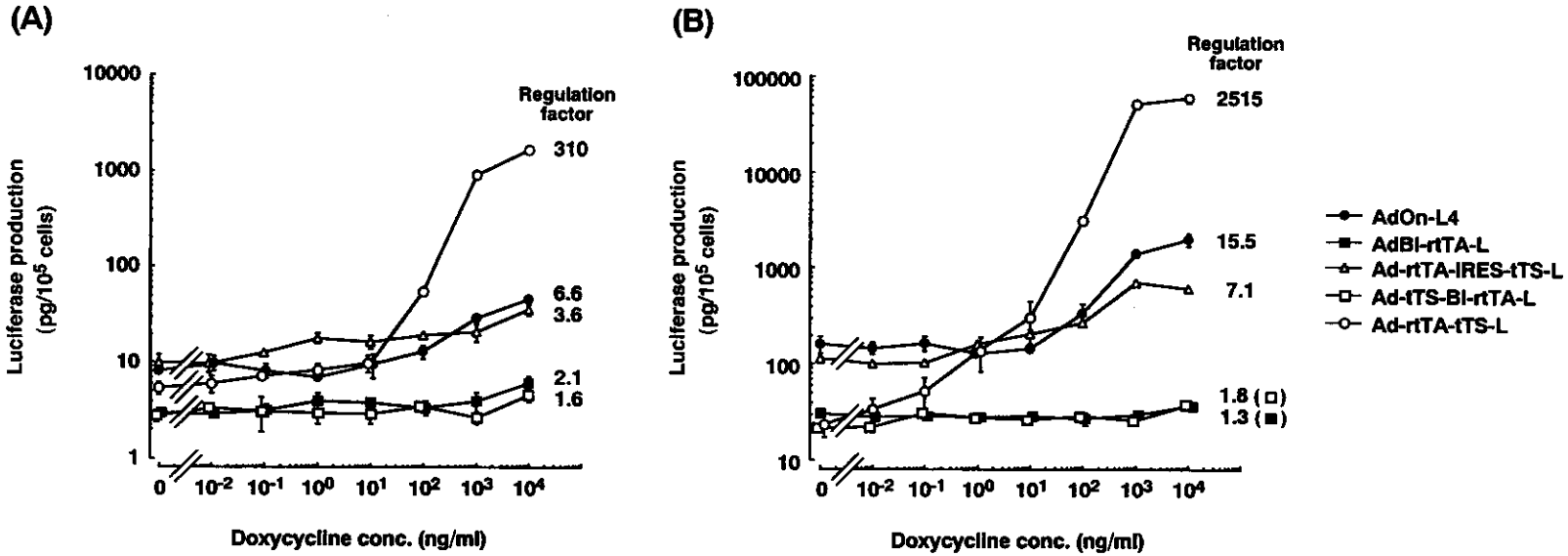


Fig.28 Regulated luciferase expression in SK HEP-1 cells transduced with various Ad-mediated tet-on systems.

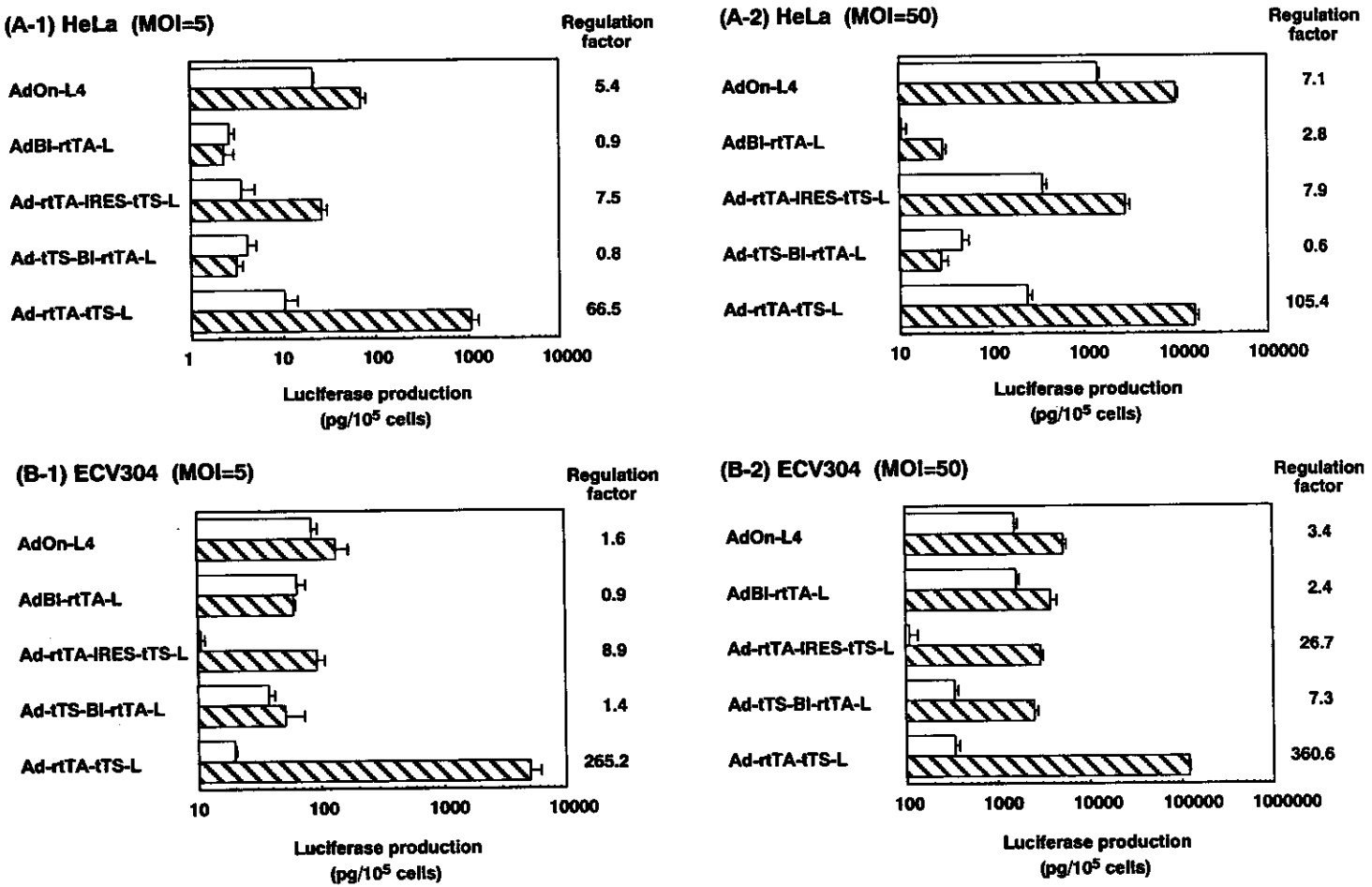


Fig.29 Regulated luciferase expression in HeLa and ECV304 cells transduced with various Ad-mediated tet-on systems.

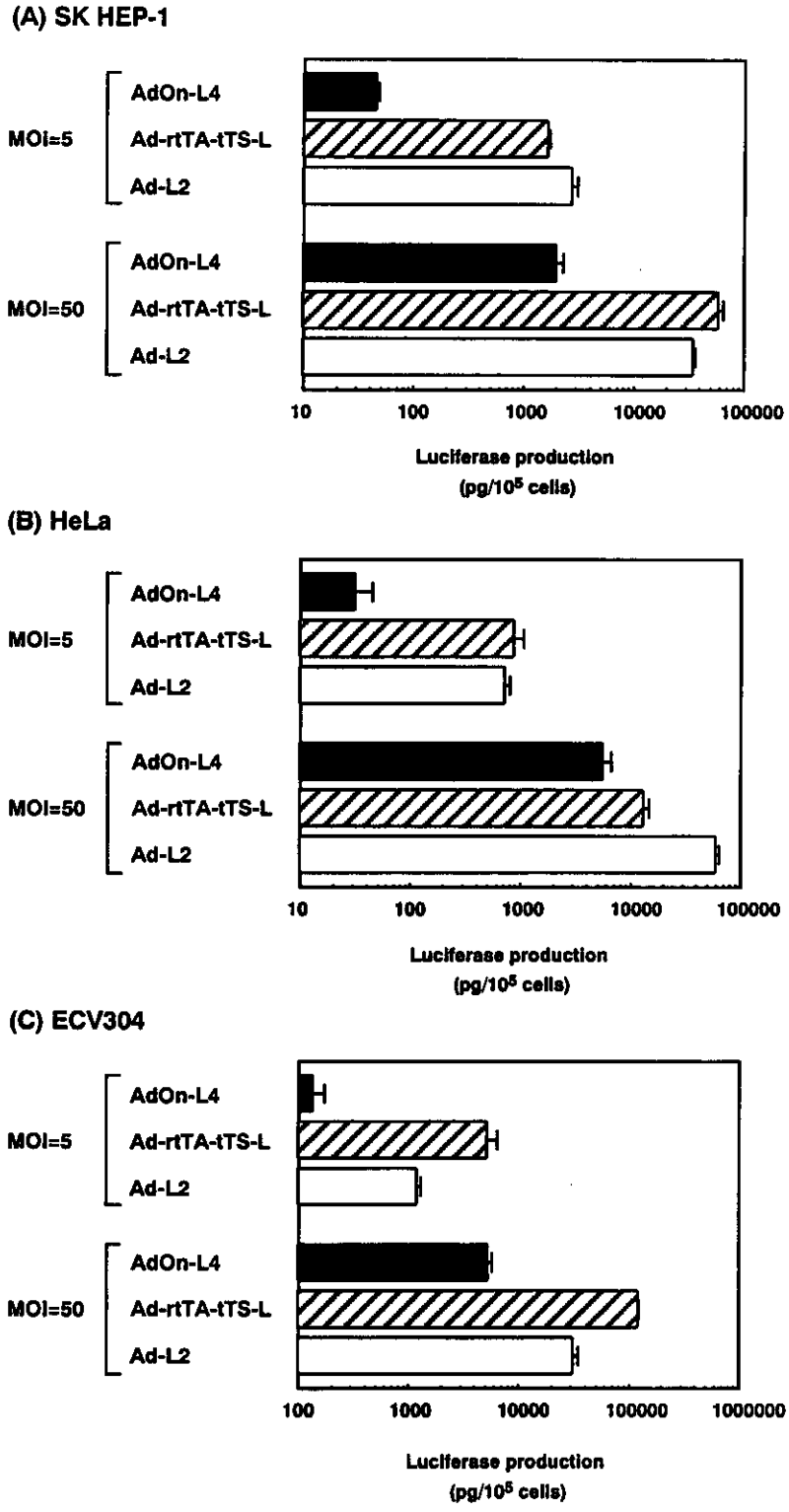


Fig.30 Comparison of induced (maximum) luciferase production in cells transduced with AdOn-L4, Ad-rtTA-tTS-L, or Ad-L2.

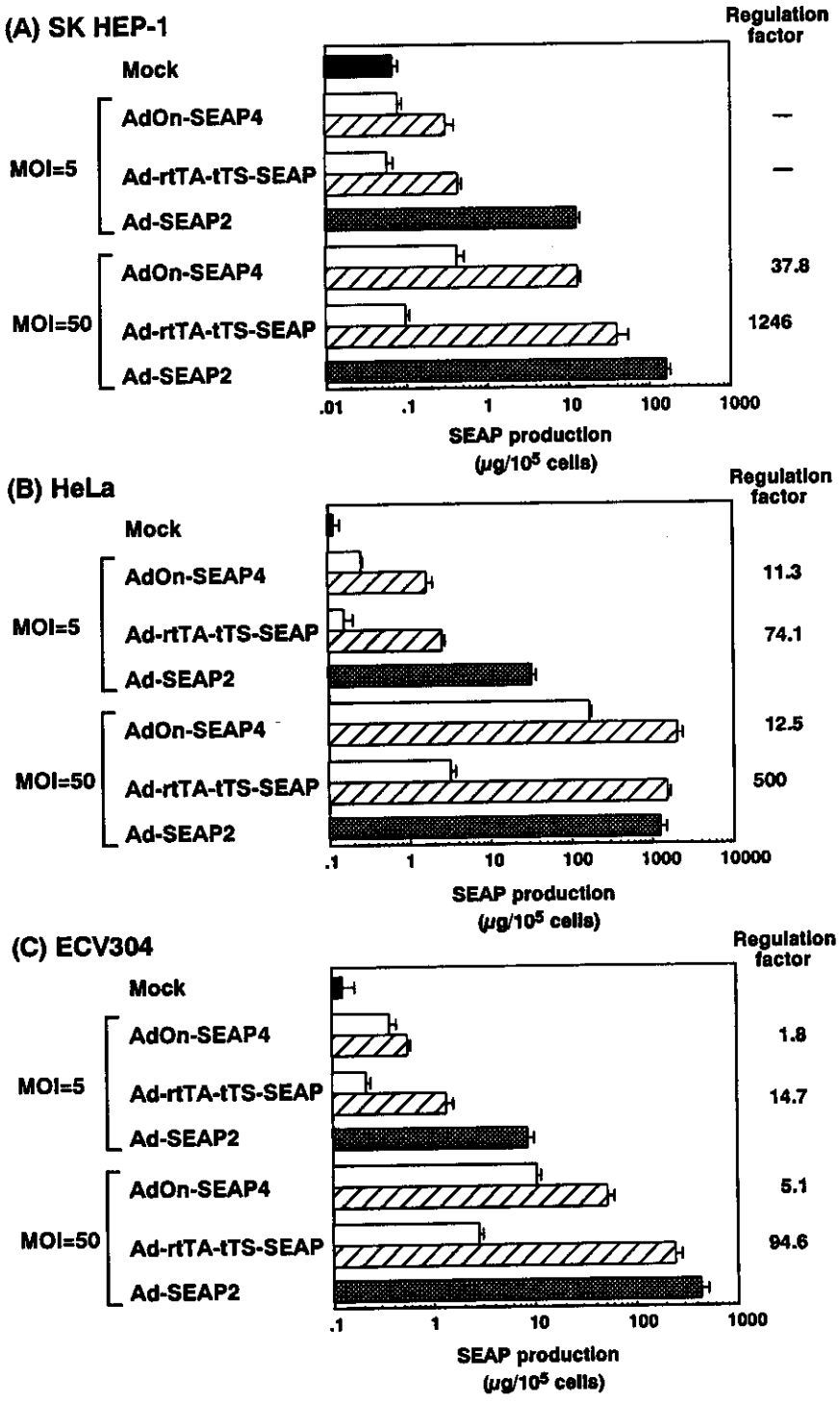


Fig.31 Regulated SEAP expression in SK HEP-1, HeLa, and ECV304 cells transduced with AdOn-SEAP4 or Ad-rtTA-tTS-SEAP.

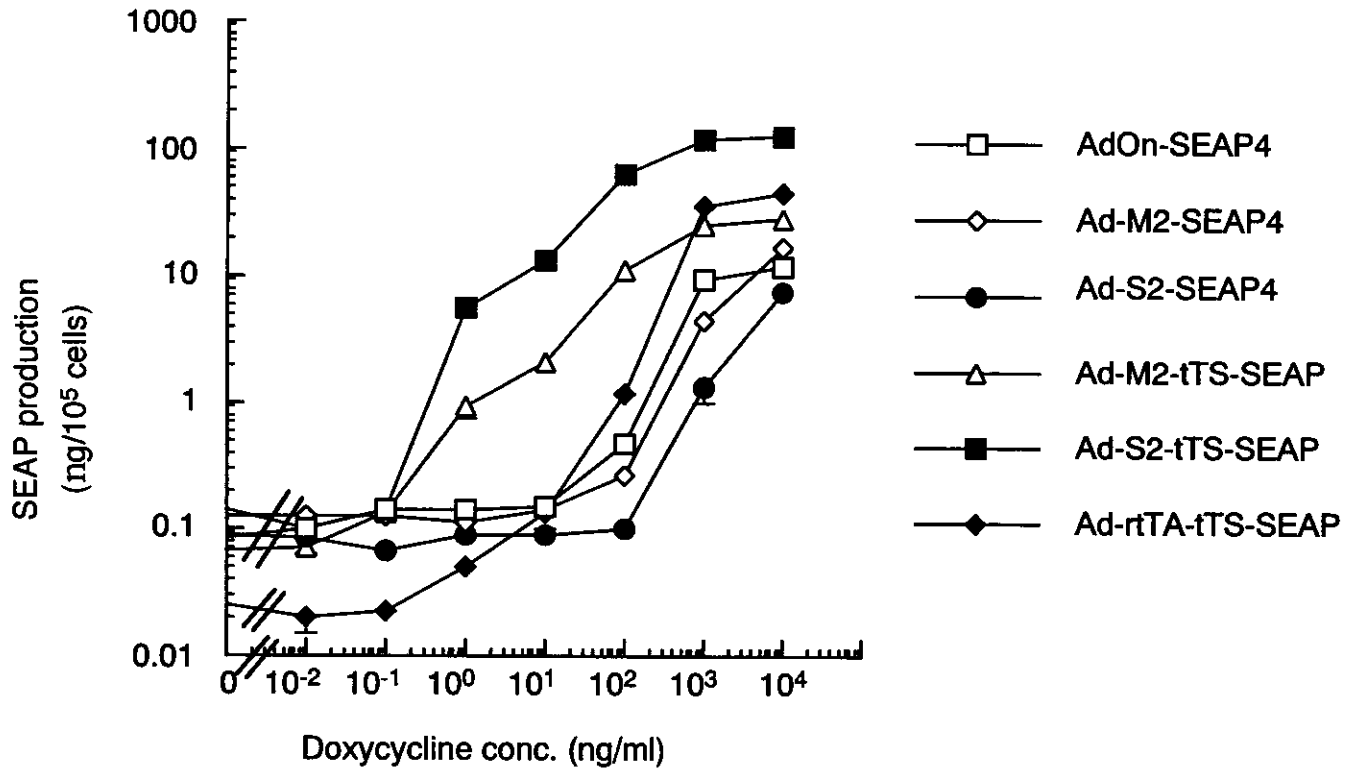


Fig.32 Regulated SEAP expression in SK HEP-1 cells transduced with various Ad-mediated tet-on systems.

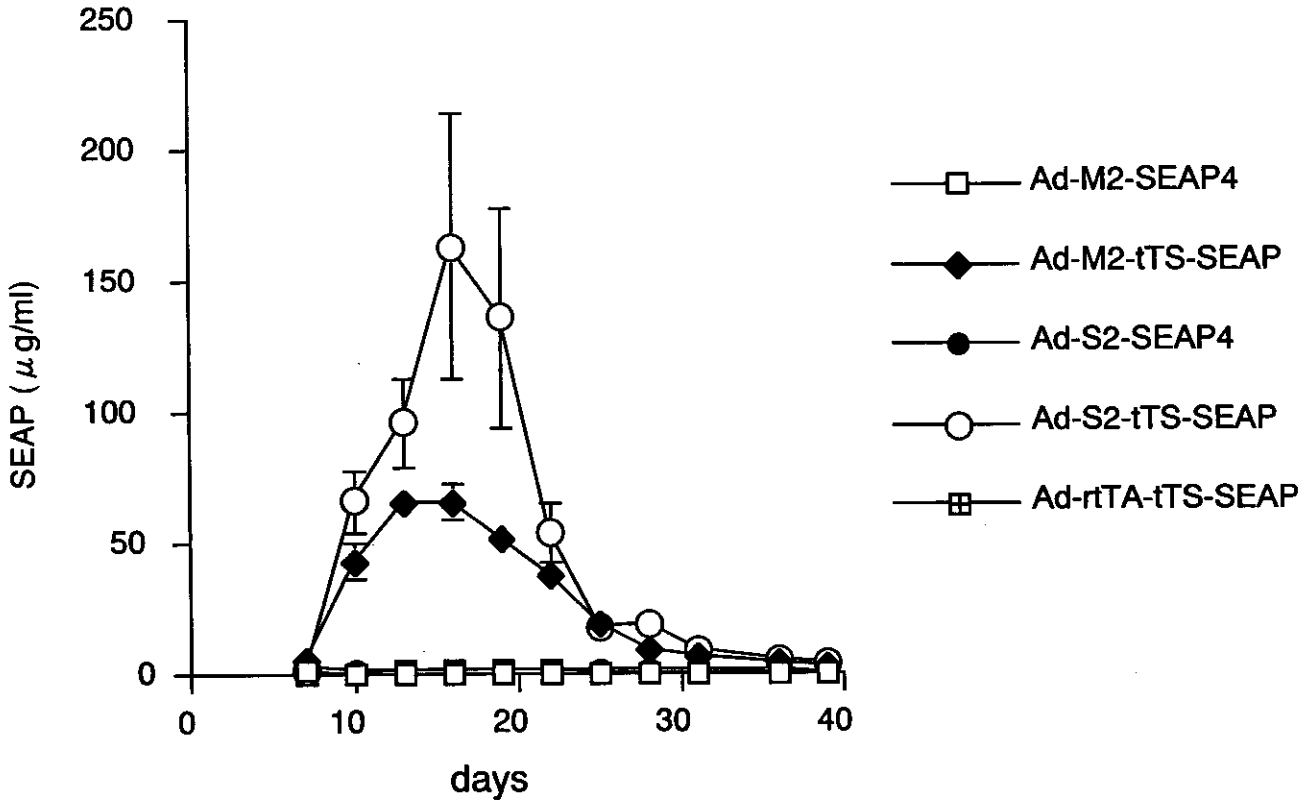


Fig. 33 Regulated SEAP expression in Balb/c nude mice.

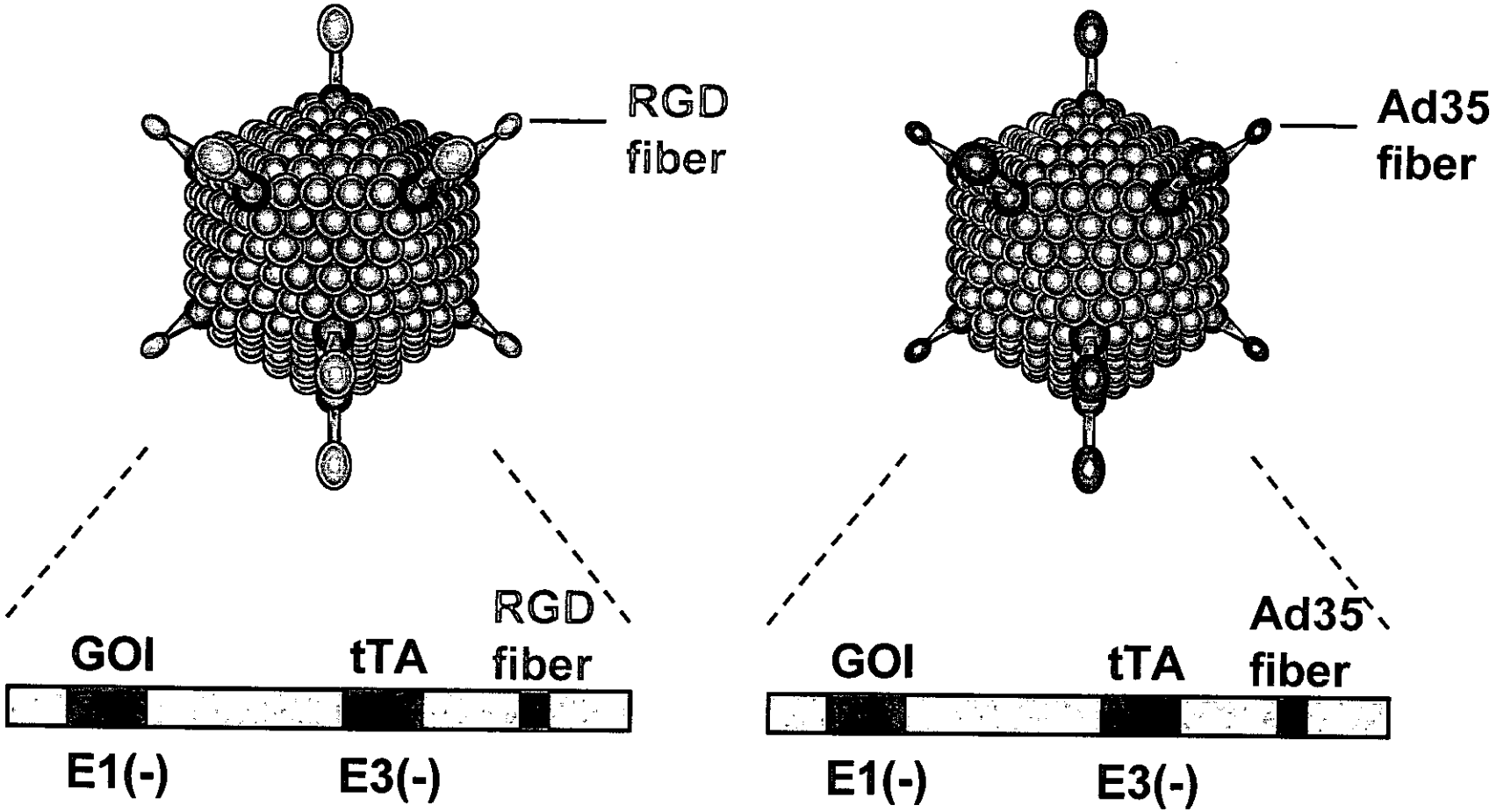


Fig.34 Capsid-modified single adenovirus vectors containing tet-off system

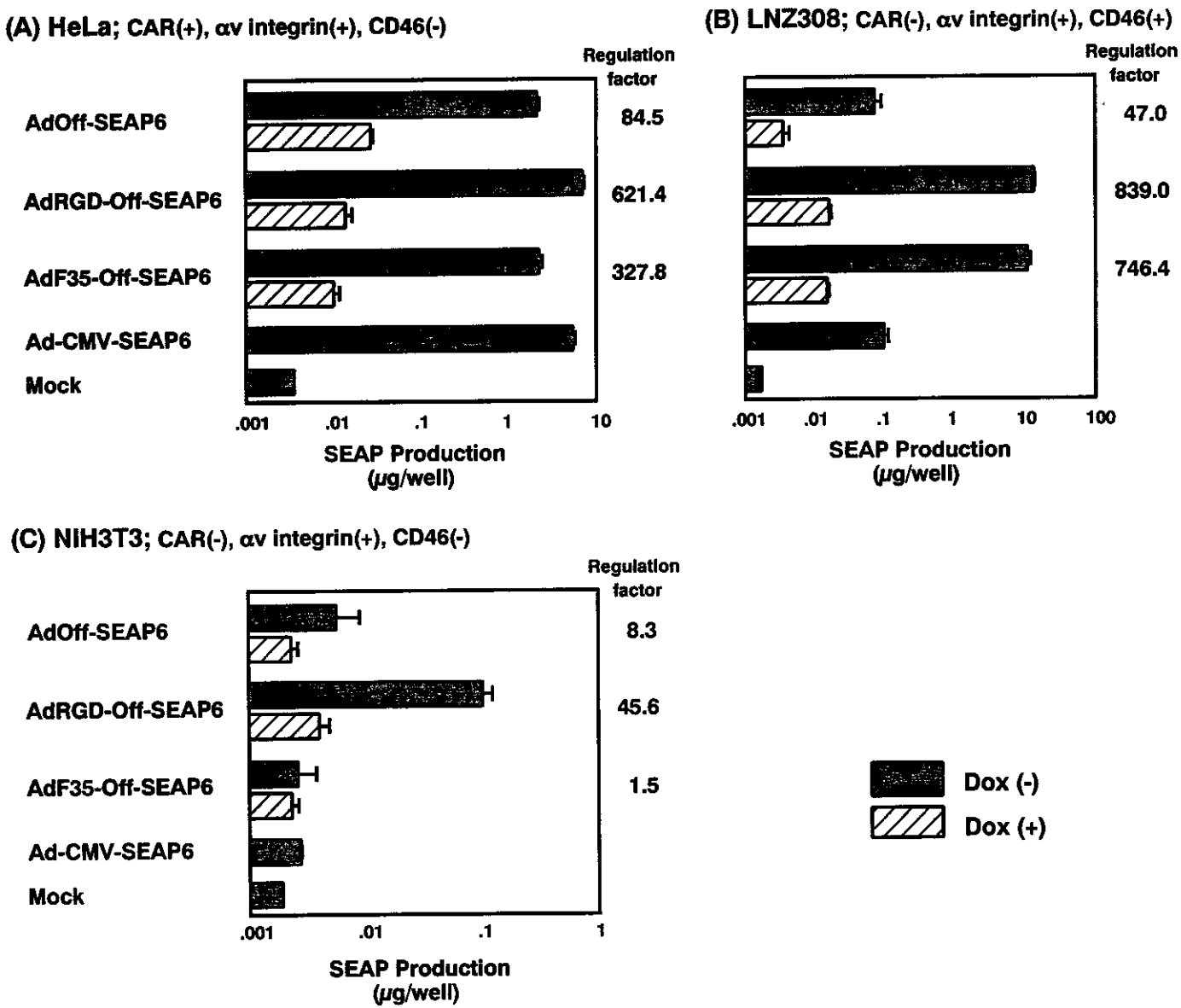


Fig. 35 Capsid-modified single adenovirus vectors containing tet-off system