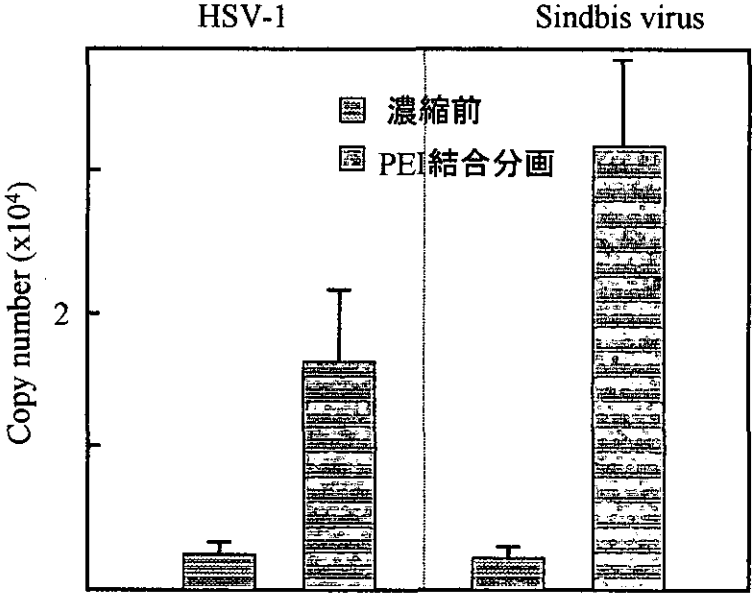


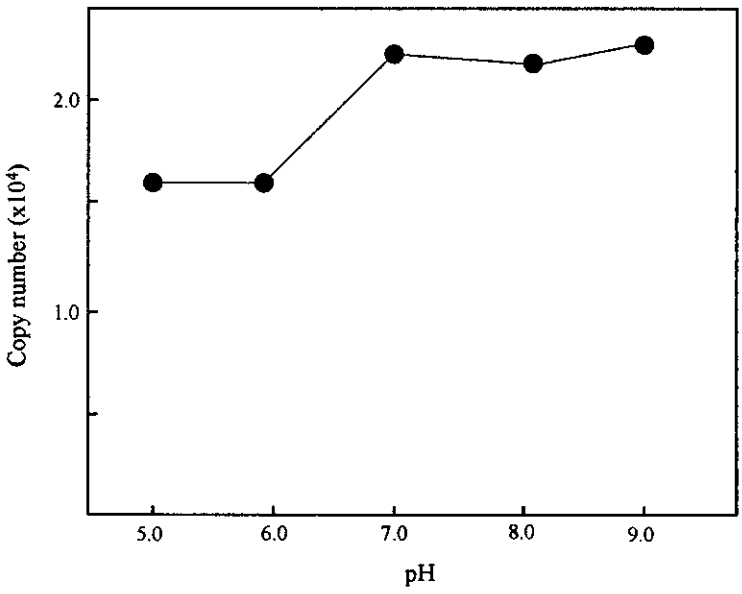
図 1 HSV-1 及び sindbis virus の PEI 磁気ビーズによる濃縮

表5. PEIの分子量の違いによる濃縮効率の差異
(copy number x10⁴)

	MW70,000	MW 1,800
–serum	4.26 ± 0.48	1.95± 0.67
+serum	3.46 ± 0.24	1.0 ± 0.1

5 x 10⁴コピー/mlのウイルス液1mlをEPI磁気ビーズに結合させた。

図2. Effect of pH on the concentration of HSV-1 by PEI-beads



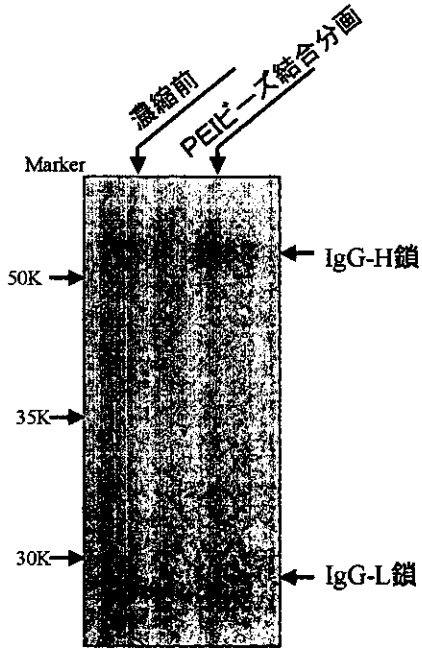
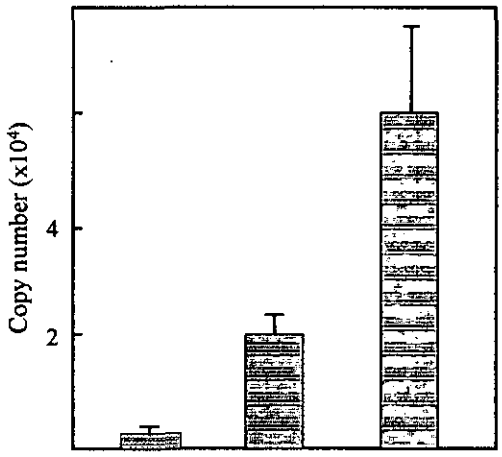


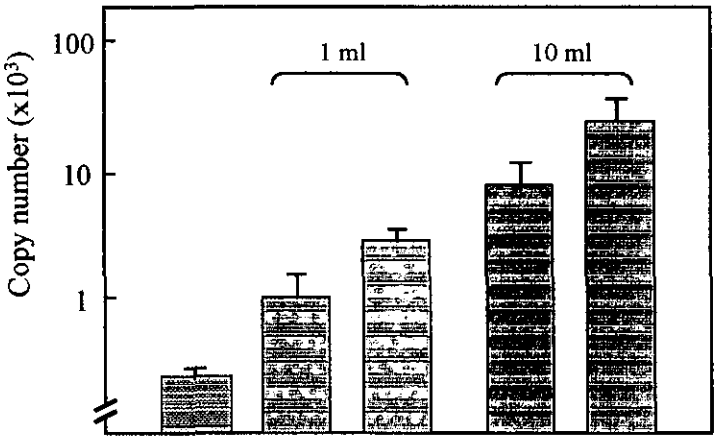
図３．PEI 磁気ビーズによる IgG の濃縮

図4. 抗HSV-1抗体のPEI磁気ビーズによるウイルス濃縮への影響



PEI-bead	–	+	+
Anti-HSV-1	–	–	+

図5. PEI磁気ビーズによるHBVの濃縮と抗HBV抗体の影響



PEI-bead	–	+	+	+	+
Anti-HBV	–	–	+	–	+

図6. PEI磁気ビーズによるHCVの濃縮

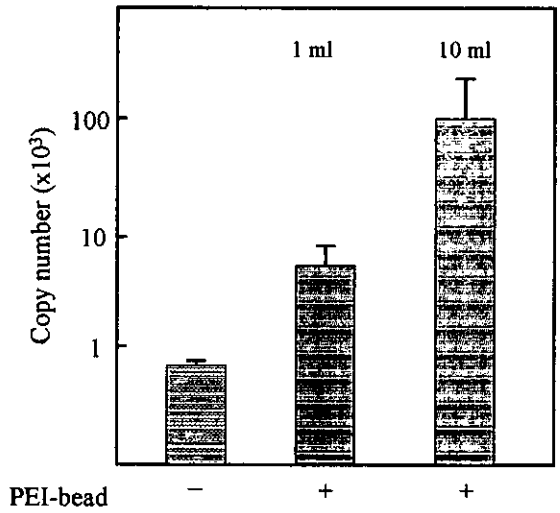


表6 PowerPlex16を用いた各種細胞株のSTRマーカーリピート数の解析結果

A. The fluorescein-labeled allele designations

	D3S1358	TH01	D21S11	D18S51	PentaE
HL60-1	16	7,8	29**, 30	14, 15**	13, 14
HL60-2	16	7,8	29**, 30	14, 15**	13, 14
HL60-RG-1	16	8	29**, 30	14, 15	13, 14
FLC-4	15**, 16	6**,8*,9	31.2, 32.2	13	12
WTK1	16	8, 9.3	29	11, 15	5, 7
TK6	16	8, 9.3	29	11, 16	5, 7

* smaller peak than **
** a half of allele peak

B. The JOE-labeled allele designations

	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D
HL60-1	12, 13	8**, 11	11, 12	11	13, 14	10, 12
HL60-2	12, 13	8**, 11	11, 12	11	13, 14	10, 12
HL60-RG-1	12, 13	8**, 11	11, 12	11	13, 14	10, 12
FLC-4	9**, 13	11*,12	10*, 12	12**, 13	11, 12*	9, 12*
WTK1	12, 13	11	9, 12	11, 12	11, 13	11, 12
TK6	12, 13	11	9, 11	11, 12	11, 12	11, 12

* smaller peak than **
** a half of allele peak
JOE = 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

C. The TMR-labeled allele designations

	Amelogenin	vWA	D8S1179	TPOX	FGA
HL60-1	X	16	12, 13	8, 11	22, 24
HL60-2	X	16	12, 13	8, 11	22, 24
HL60-RG-1	X	16	12, 13	8, 11	21*, 22, 24
FLC-4	X	14, 16**, 17	17	8, 11	22
WTK1	X, Y	16**, 17, 20	10, 13	8, 11	22, 24
TK6	X, Y	16**, 17, 20	10, 13	8, 11	22, 24

* smaller peak than **
** a half of allele peak
TMR = carboxy-tetramethylrhodamine

表5 17番染色体上のSTRマーカーを用いた解析結果

STRマーカー	label	サイズ範囲		TK6		HL60/HL60-RG	
		min	max	allele-1	allele-2	allele-1	allele-2
115B3	6FAM	179	209	189-190	198-200	189	-
THRA1	6FAM	158	176	165-166	173-176	165	169
CHLC	HEX	200		195	199	196	200
AFM248yg9	TET	143	155	143	147	148	154
42D6	6FAM	154	174	138	155	138	-
AFM234xc9	HEX	114	138	109	130	116	-
AFM107yb8	TET	154	170	151-153	157-161	156	164
AFM049xc1	TET	181	207	183-185	187-189	-	192
AFM210xa5	HEX	166	188	172	180	-	-
AFM044xg3	6FAM	226	238	225	235	236	238 (RG 237)

（表中の数字はバンドサイズ）

- ; no peak

図7 STRマーカーを使ったDNA多型の検出法

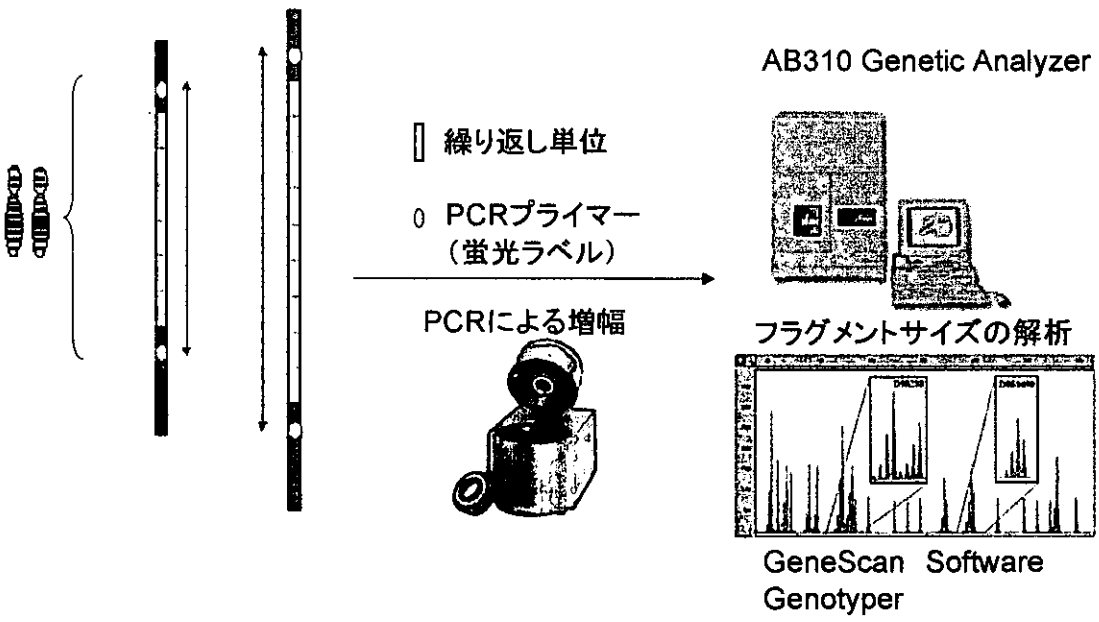


図8 STRマーカー解析例

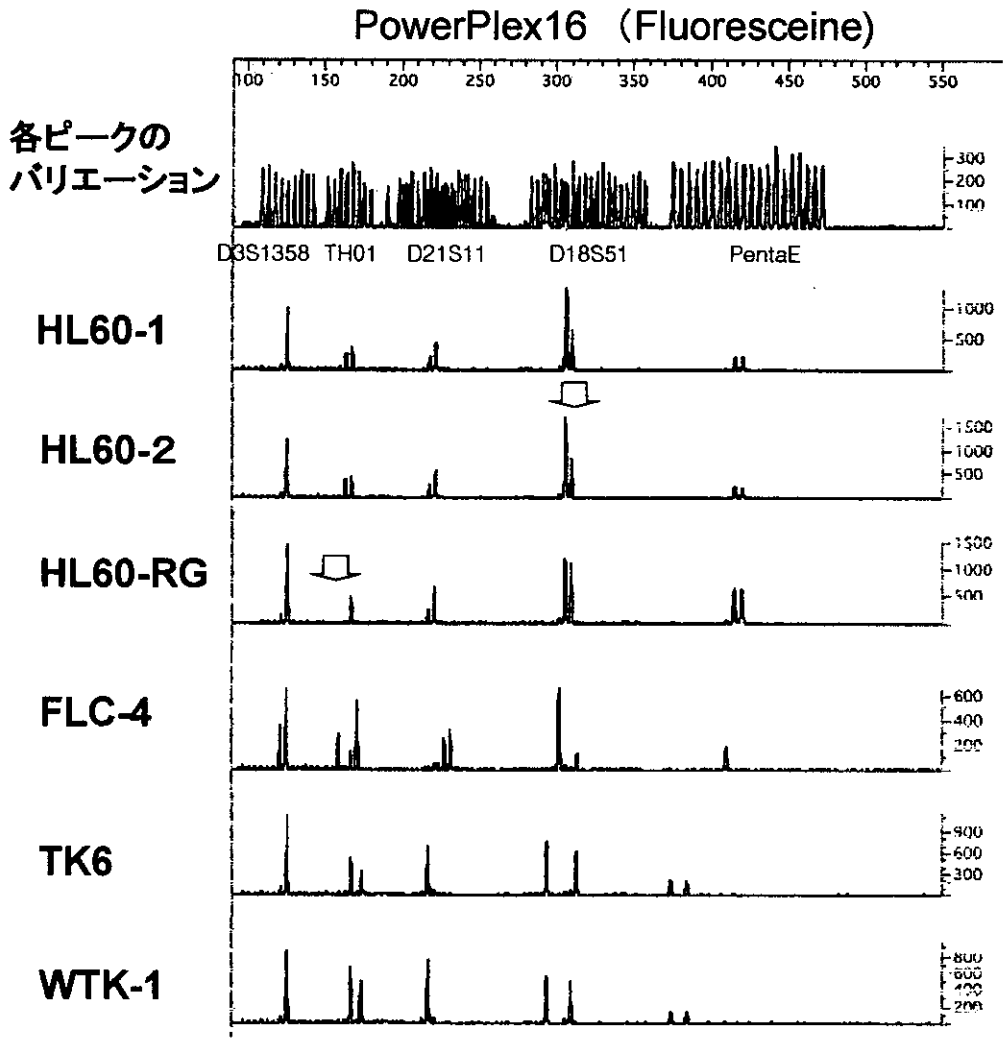


表８．アレイ上のサイトカインの位置と名称

position		cytokines	
1	A	Pos	positive control
1	B	Pos	positive control
1	C	Pos	positive control
1	D	Pos	positive control
1	E	Neg	negative control
1	F	Neg	negative control
1	G	ENA-78	Epithelial neutrophil-activating protein 78
1	H	GCSF	Granulocyte-colony stimulating factor
1	I	GM-CSF	Granulocyte-macrophage colony stimulating factor
1	J	GRO	Growth Related Oncogene
1	K	GRO-α	Growth Related Oncogene-Alpha
2	A	I-309	I-309
2	B	IL-1α	Interleukin 1 Alpha
2	C	IL-1β	Interleukin 1 Beta
2	D	IL-2	Interleukin 2
2	E	IL-3	Interleukin 3
2	F	IL-4	Interleukin 4
2	G	IL-5	Interleukin 5
2	H	IL-6	Interleukin 6
2	I	IL-7	Interleukin 7
2	J	IL-8	Interleukin 8
2	K	IL-10	Interleukin 10
3	A	IL-12	Interleukin 12
3	B	IL-13	Interleukin 13
3	C	IL-15	Interleukin 15
3	D	IFN-γ	Interferon gamma
3	E	MCP-1	Monocyte Chemoattractant Protein 1
3	F	MCP-2	Monocyte Chemoattractant Protein 2
3	G	MCP-3	Monocyte Chemoattractant Protein 3
3	H	MCSF	Macrophage-colony Stimulating Factor
3	I	MDC	Macrophage-derived Chemokine
3	J	MIG	Monokine induced by gamma interferon
3	K	MIP-1β	Macrophage Inflammatory Protein 1 Beta

4	A	MIP-1 δ	Macrophage Inflammatory Protein 1 Delta
4	B	RANTES	regulated upon activation, normal T-cell expressed, and presumably secreted
4	C	SCF	Stem Cell Factor
4	D	SDF-1	Stromal cell-drived factor
4	E	TARC	Thymus and Activation-Regulated Chemokine
4	F	TGF-β 1	Transforming growth factor-beta 1
4	G	TNF-α	Tumor necrosis factor-alpha
4	H	TNF-β	Tumor necrosis factor-beta
4	I	EGF	Epidermal growth factor
4	J	IGF-I	Insulin-like growth factor-1
4	K	Ang	Angiogenin
5	A	OSM	Oncostatin M
5	B	TPO	Thrombopoietin
5	C	VEGF	Vascular Endothelial Growth Factor
5	D	PDGF-B	Platelet-derived Growth Factor-B
5	E	Leptin	Leptin
5	F	BDNF	Brein-derived neurotrophic factor
5	G	BLC	B-lymphocyte chemoattractant
5	H	Ck β 8-1	Chemokine-beta-8
5	I	Eotaxin	Eotaxin
5	J	Eotaxin-2	MPIF-2 (Myeloid progenitor inhibitory factor-2)
5	K	Eotaxin-3	MIP-4-alpha (macrophage inflammmatory protein-4-alpha)
6	A	FGF-4	Fibroblast growth factor-4
6	B	FGF-6	Fibroblast growth factor-6
6	C	FGF-7	Fibroblast growth factor-7
6	D	FGF-9	Fibroblast growth factor-9
6	E	Flt-3 Ligand	fms-like tyrosine kinase-3 ligand
6	F	Fractalkine	Fractalkine
6	G	GCP-2	Granulocyte Chemotactic Protein-2
6	H	GDNF	Glial-derived Neurotrophic Factor
6	I	HGF	hepatocyte growth factor
6	J	IGFBP-1	Insulin-like growth factor binding proteins-1
6	K	IGFBP-2	Insulin-like growth factor binding proteins-2
7	A	IGFBP-3	Insulin-like growth factor binding proteins-3

7	B	IGFBP-4	Insulin-like growth factor binding proteins-4
7	C	IL-16	Interleukin 16
7	D	IP-10	Interferon gamma inducible protein-10
7	E	LIF	Leukemia Inhibitory Factor
7	F	LIGHT	LIGHT
7	G	MCP-4	Monocyte Chemoattractant Protein 4
7	H	MIF	mesoderm inducing factor
7	I	MIP-3 α	Macrophage Inflammatory Protein-3 aipha
7	J	NAP-2	Neutrophil Activating Peptide 2
7	K	NT-3	neurotrophin-3
8	A	NT-4	neurotrophin-4
8	B	Osteoprotegerin	Osteoprotegerin
8	C	PARC	Pulmonary and Activation-Regulated Chemokine
8	D	PIGF	Placenta growth factor
8	E	TGF- β 2	Transforming growth factor-beta 2
8	F	TGF- β 3	Transforming growth factor-beta 3
8	G	TIMP-1	tussue inhibitor of metalloproteinases-1
8	H	TIMP-2	tussue inhibitor of metalloproteinases-2
8	I	Neg	negative control
8	J	Pos	positive control
8	K	Pos	positive control

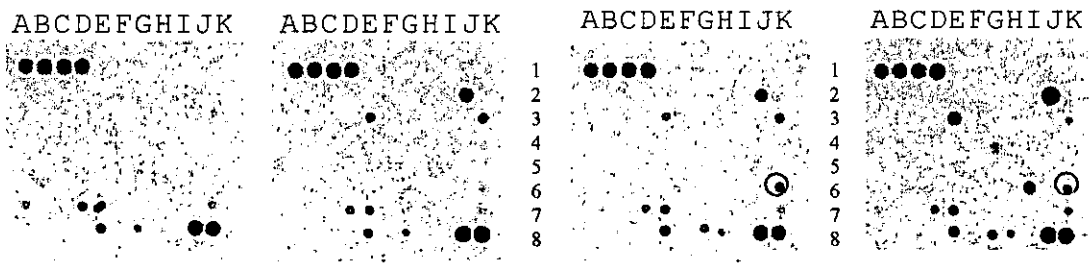


図９．HL-60 と HL-60RG の培養上清のサイトカインアレイによる解析

表4．ELISAによるHL-60細胞及びHL-60RG細胞の産生する各種サイトカインの定量

	IL-2	IL-4	IL-5	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10	HGF (pg/ml)	VEGF (pg/ml)	TNF-α	IFN-γ	M-CSF	GM-CSF
HL-60RG細胞	ND	ND	ND	15.8±3.2	139±30	ND	4560±672	389±26	ND	ND	ND	ND
HL-60細胞	ND	ND	ND	ND	286±12	ND	ND	185±6	ND	ND	ND	ND

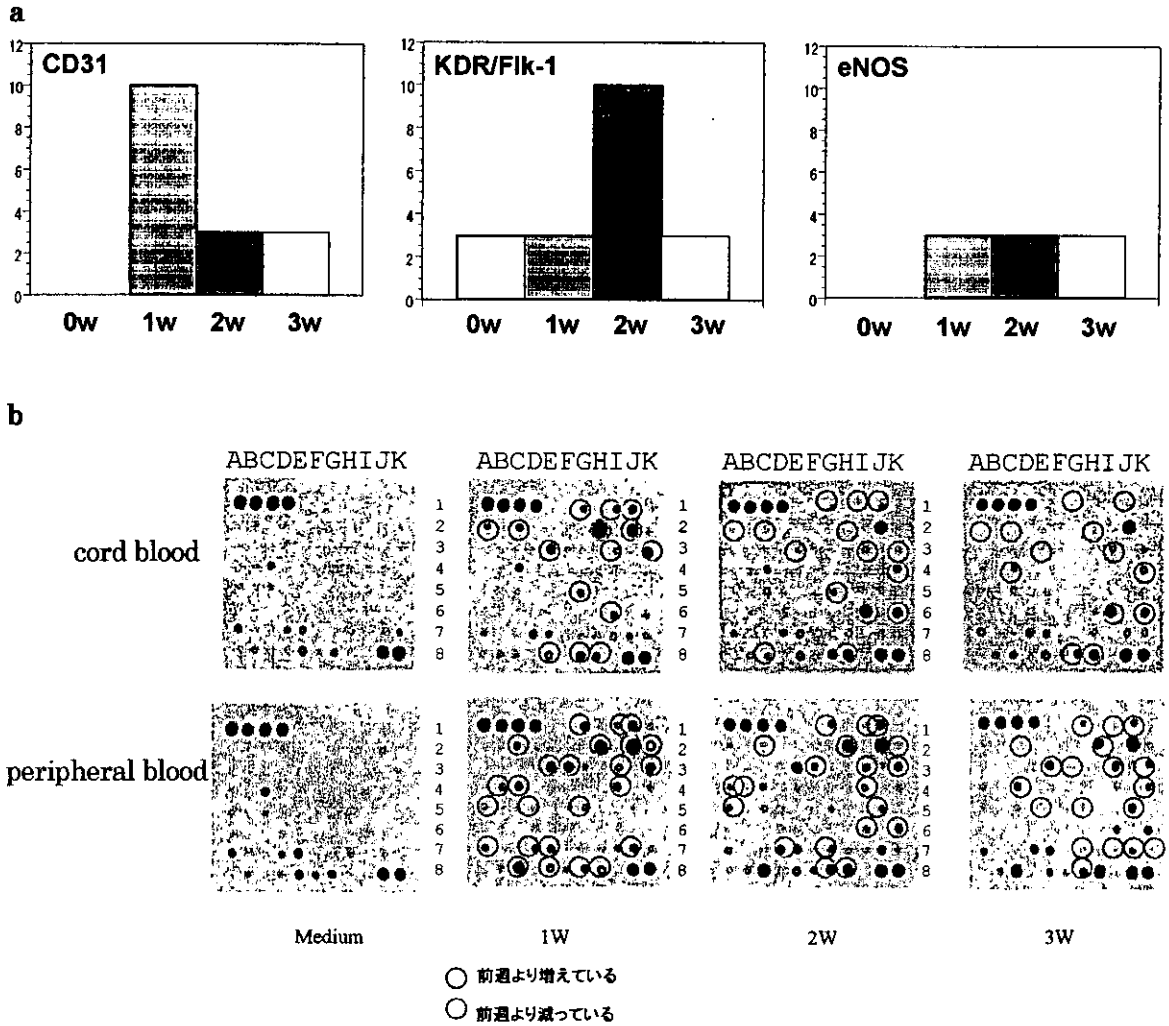


図１０．臍帯血、末梢血由来の AC133 陽性細胞の培養上清のサイトカインアレイのよる解析

mkwvesifli fllnftesrt lhrneygias ildsyqctae isladlatif

faqfvqeaty kevskmvkda ltaiekptgd eqssgclenq lpafleelch

ekeilekygh sdccsqseeg rhncflahkk ptpasiplfq vpepvtscea

yeedretfmn kfiyeiarrh pflyaptill waarydkiip scckaenave

cfqtkaatvt kelresslln qhacavmknf gtrtfqaitv tklsqkftkv

nfteiqklvl dvahvhehcc rgdvldclqd gekimsyics qqdtlsnkit

eccklttler gqciihaend ekpeglspnl nrflgdrdfn qfssgeknif

lasfvheysr rhpqlavsvi lrvakgyqel lekcfqtenp lecqdkgeee

lqkyiqesqa lakrscglfq klgeyylqna flvaytkkap qltsselmai

trkmaataat ccqlsedkll acgegaadii ighlcirhem tpvnpgvgqc

ctssyanrrp cfsslvvdet yvppafsddk fifhkdlcqa qgvalqtmkq

eflinlvkqk pqiteeqlea viadfsglle kccqgqeqev cfaeegqkli

sktraalgv

Fig. 11 Amino acid sequence and N-glycosylation sites of alpha-Fetoprotein
n: N-glycosylation site

mkililgifl flcstpawak ekhyyigiie ttwdyasdhg ekklisvdte
hsniylqngp drigrlykka lylqytdetf rttiekpvwl gflgpiikae
tgdkvyvhlk nlasrpytfh shgityykeh egaiypdⓝtt dfqraddkvy
pgeqytymll ateeqspgeg dgncvtriyh shidapkdia sgligpliic
kkdsldkeke khidrefvvm fsvvdeⓝfsw yledniktyc sepekvdkdn
edfqesnrmy svngytfgsl pglsmcaedr vkwylfgmgn evdvhaaffh
gqaltnknyr idtinlfpat lfdaymvaqn pgewmlscqn lnhlkaglqa
ffqvqecⓝks sskdnirgkh vrhyyiaaee iiwnyapsgi diftkeⓝlta
pgsdsavffe qgttriggsy kklvyreytd asftnrkerg peeehlgilg
pviwaevgdt irvtfhnkga yplsiepigv rfnknnegty yspnynpqsr
svppsashva ptetftyewt vpkevgptna dpvclakmyy savdptkdif
tgligpmkic kkgslhangr qkdvdkefyl fptvfdeⓝes llllednirmf
ttapdqvdke dedfqesnkm hsmngfmygn qpgltmckgd svvwylfsag
neadvhgiyf sgntylwrge rrdtanlfpq tsltlhmwpd tegtfnvecl
ttdhytggmk qkytvnqcrr qsedstfylg ertyyiaave vewdyspqre
wekelhhlqe qⓝvsnafldk gefyigskyk kvvyrqytds tfrvpverka
eeehlgilgp qlhadvgdkv kiifknmatr pysihahgvq tesstvtptl
pgetltyvwk ipersgagte dsacipwayy stvdqvkdly sgligplivc
rrpylkvfnp rrklefallf lvfdeⓝeswy lddniktysd hpekvnkdde
efiesnkmha ingrmfgnlq gltmhvgdev nwylmgmgne idlhtvhfhg
hsfqykhrgv yssdvfdifp gtyqtlemfp rtpgiwllhc hvtdhihagm
ettytvlqne dtksg

Fig. 12 Amino acid sequence and N-glycosylation sites of ceruloplasmin (CP)
ⓝ: Potential N-glycosylation sites
ⓝ: N-glycosylation sites

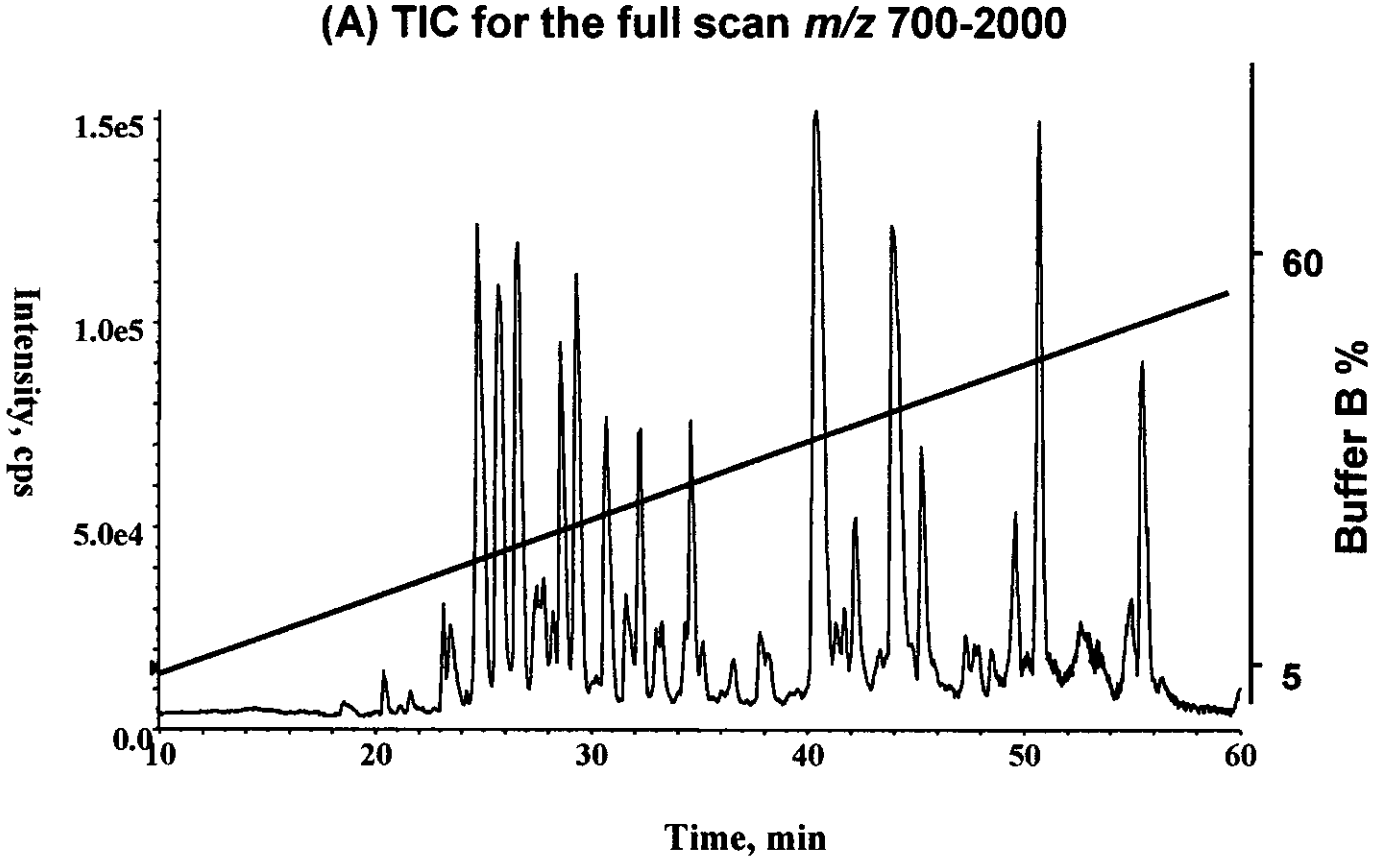


Fig. 13 LC-MS/MS of tryptic digest of α-fetoprotein

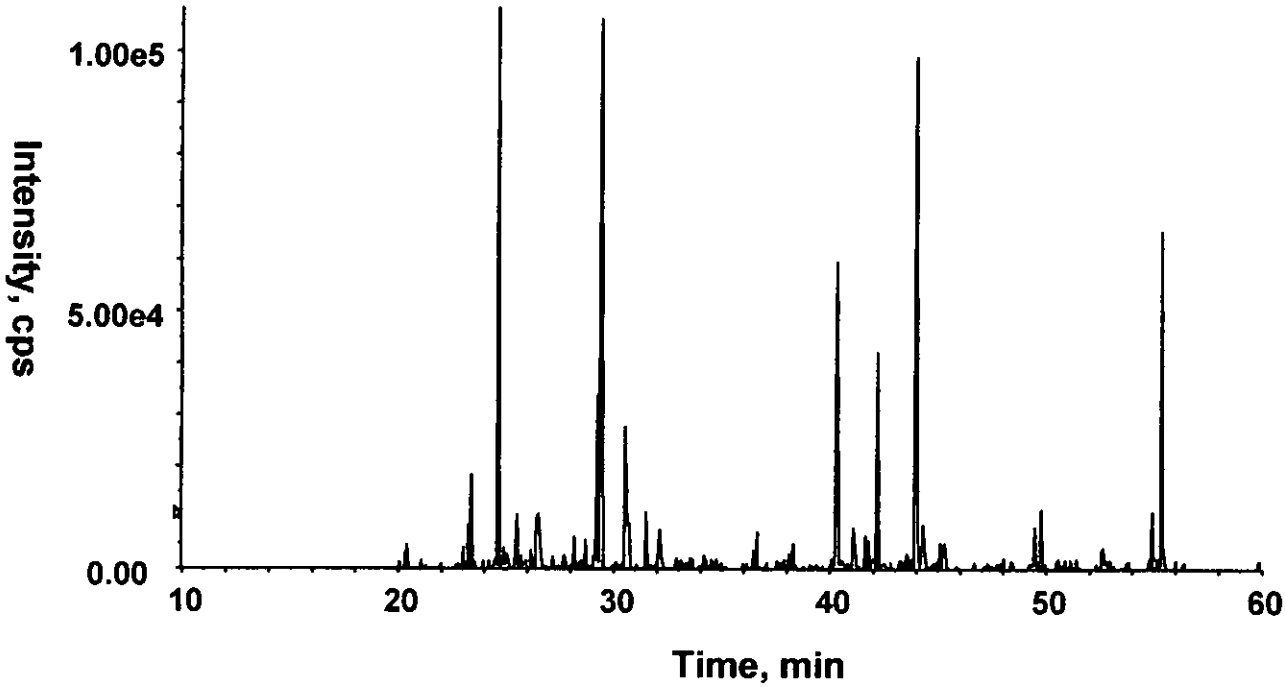
Column Magic C18

3 u, 0.2*50 mm, 2 ul/min

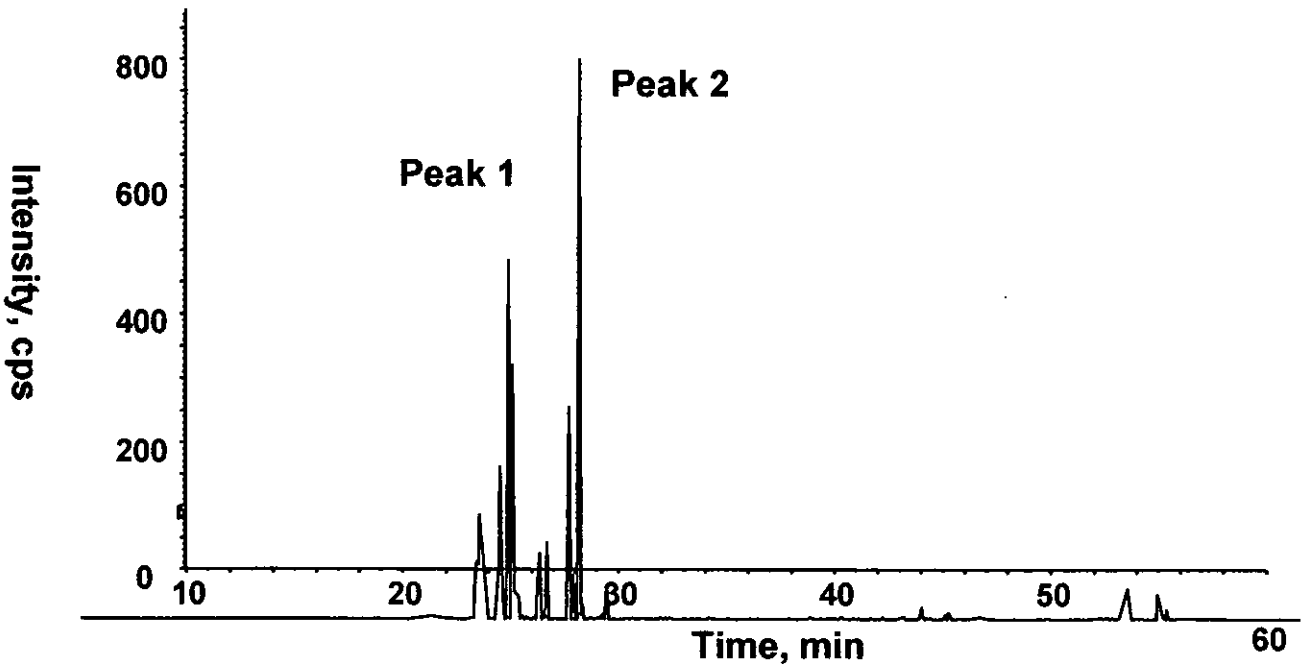
A: 2 % CH_3CN + 0.1 % formic acid

B: 90 % CH_3CN + 0.1 % formic acid

(B) TIC for the product ion scan at *m/z* 100 - 2000



(C) Product ion scan at *m/z* 204



Residue	Mass	b	b-NH3	y	y-NH3
V	99.07	100.08	83.05	978.53	961.50
N	114.04	214.12	197.09	879.46	862.43
F	147.07	361.19	344.16	765.41	748.39
T	101.05	462.23	445.21	618.35	601.32
E	129.04	591.28	574.25	517.30	500.27
I	113.08	704.36	687.33	388.26	371.23
Q	128.06	832.42	815.39	275.17	258.14
K	128.10	960.51	943.49	147.11	130.09

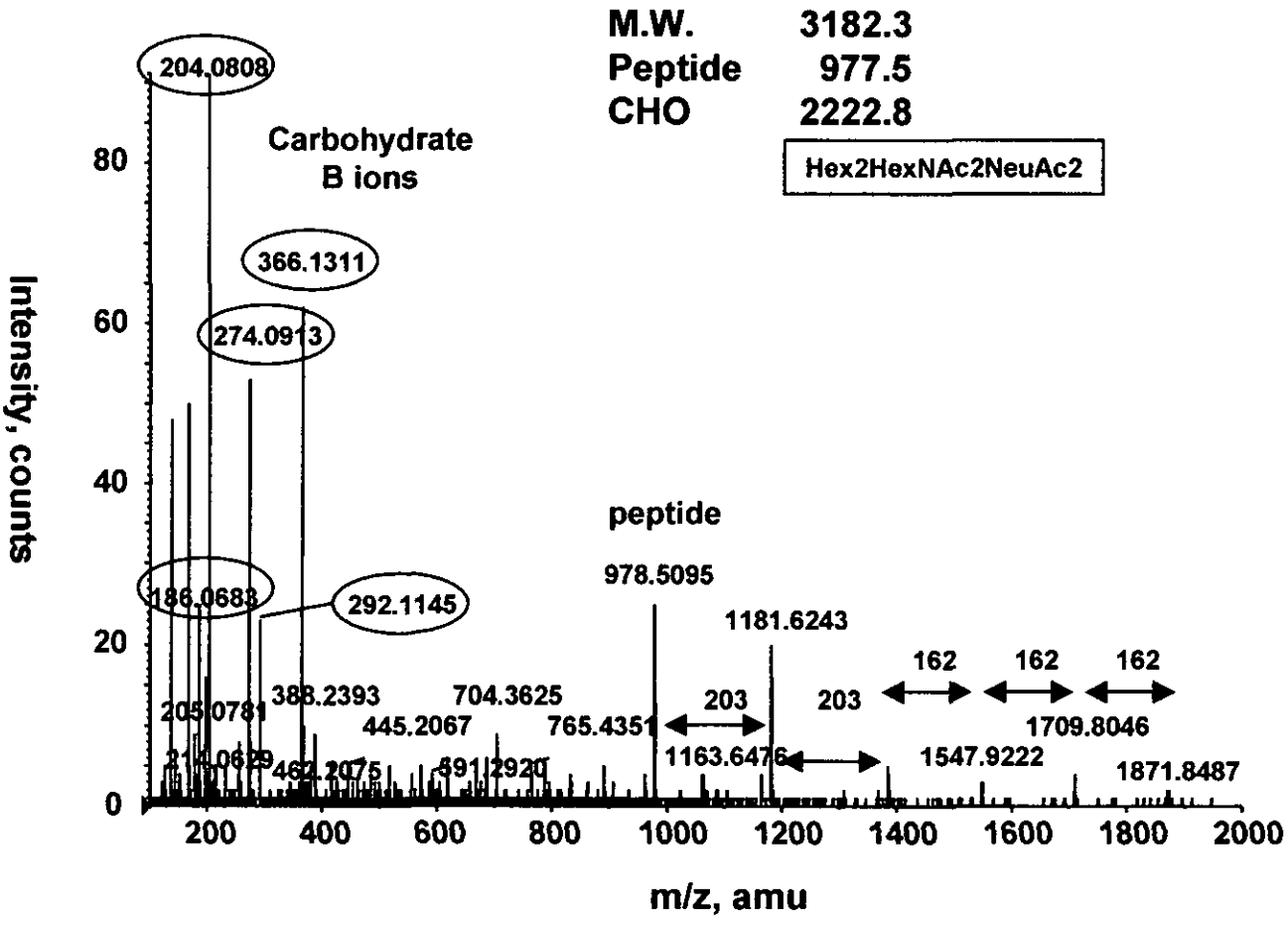


Fig. 14 Product ion spectrum of M^+(m/z1061.8^{3+}) at 25 min

Table 10 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

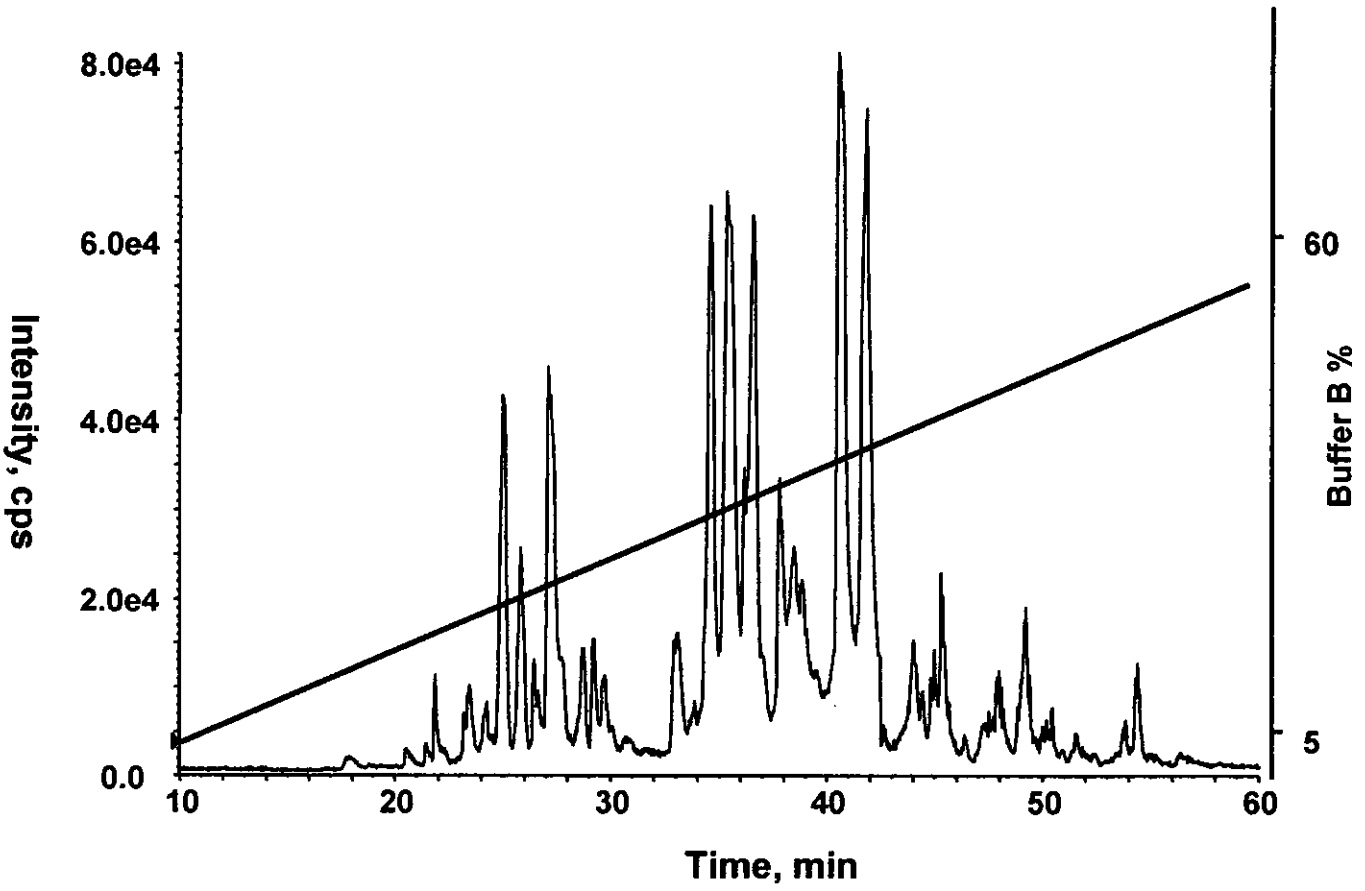


Fig. 15 LC-MS/MS of tryptic digest of ceruloplasmin

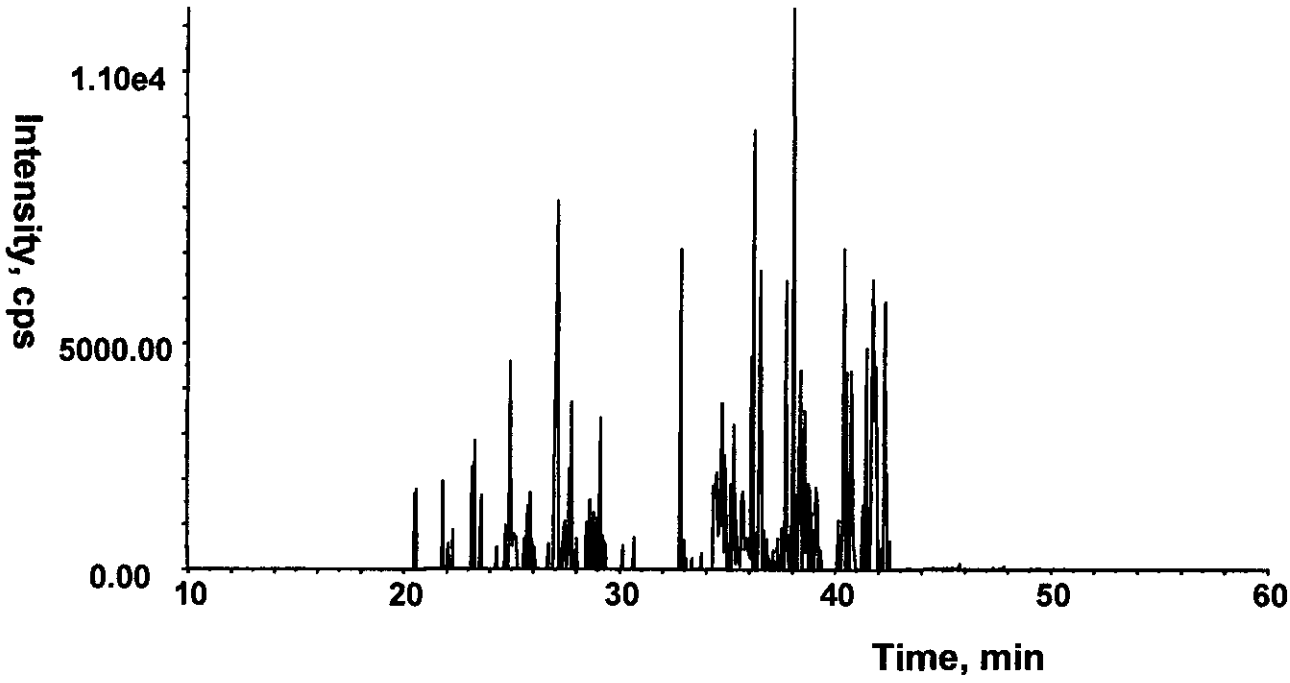
Column Magic C18

3 u, 0.2*50 mm, 2 ul/min

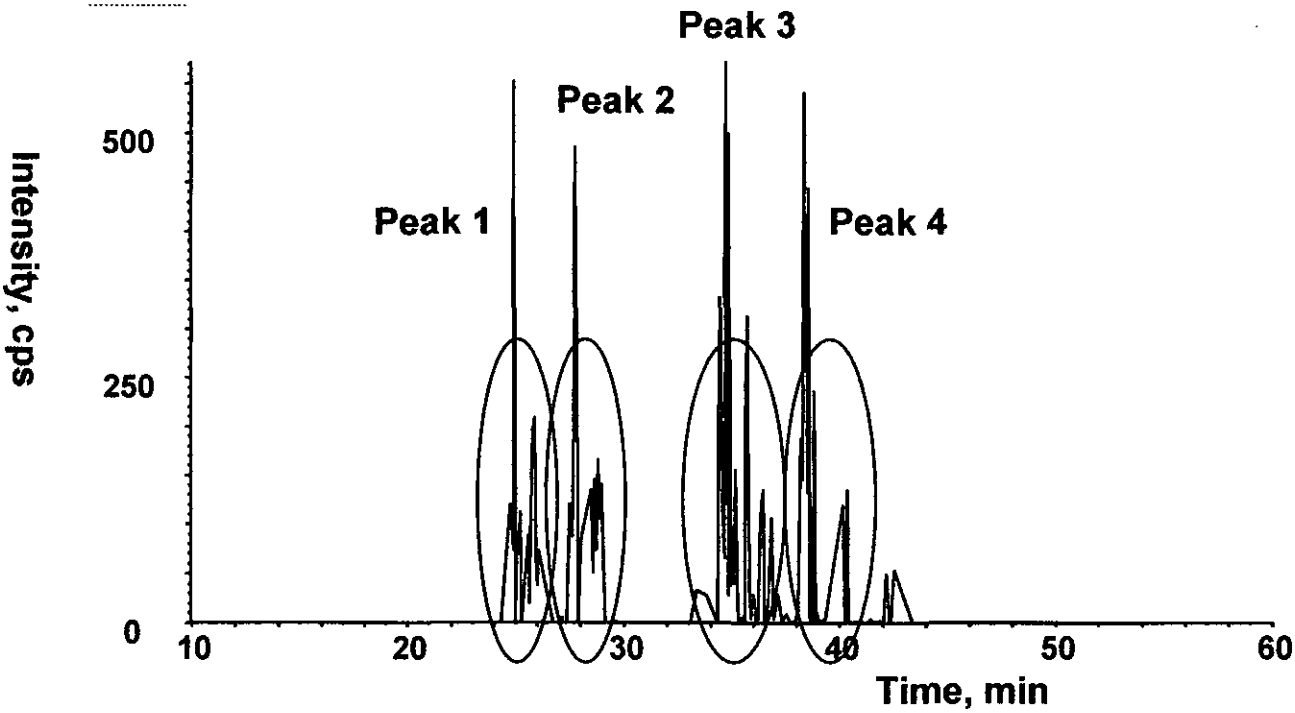
A: 2 % CH_3CN + 0.1 % formic acid

B: 90 % CH_3CN + 0.1 % formic acid

(B) TIC for the product ion scan at *m/z* 100 - 2000



(C) Product ion scan at *m/z* 204



Residue	Mass	b	b–NH3	y	y–NH3
E	129.04	130.05	113.02	1892.84	1875.81
H	137.06	267.11	250.08	1763.80	1746.77
E	129.04	396.15	379.12	1626.74	1609.71
G	57.02	453.17	436.15	1497.70	1480.67
A	71.04	524.21	507.18	1440.68	1423.65
I	113.08	637.29	620.27	1369.64	1352.61
Y	163.06	800.36	783.33	1256.55	1239.53
P	97.05	897.41	880.38	1093.49	1076.46
D	115.03	1012.44	995.41	996.44	979.41
N	114.04	1126.48	1109.45	881.41	864.38
T	101.05	1227.53	1210.50	767.37	750.34
T	101.05	1328.58	1311.55	666.32	649.29
D	115.03	1443.60	1426.58	565.27	548.25
F	147.07	1590.67	1573.64	450.25	433.22
Q	128.06	1718.73	1701.70	303.18	286.15
R	156.10	1874.83	1857.80	175.12	158.09

M.W. 4096.6
Peptide 1891.8
CHO 2222.8

$Hex_2HexNAc_2NeuAc_2$

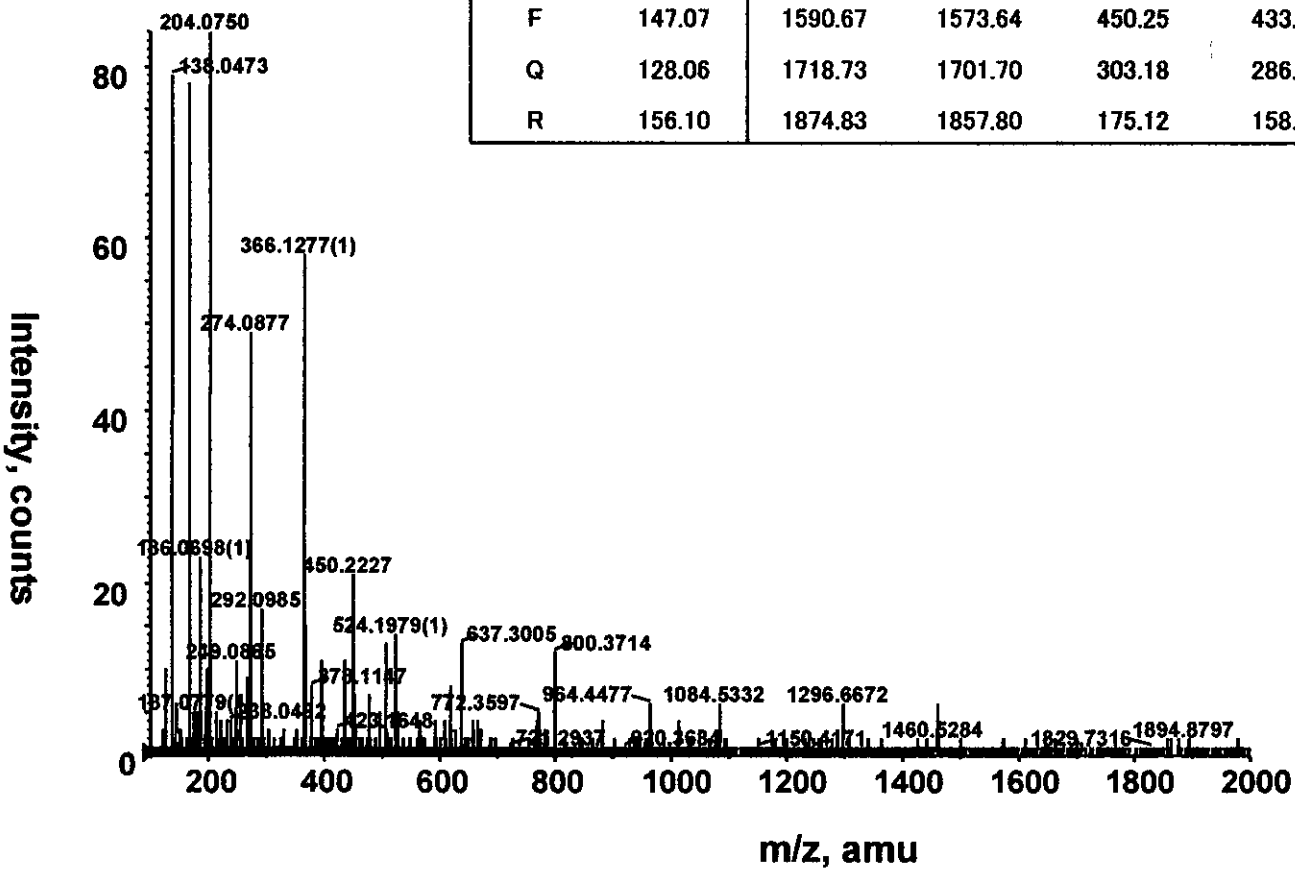


Fig. 16 Product ion spectrum of M^+(m/z1366.6^{3+}) at 25 min