

図 2 2 HL60 と TK6 細胞の染色体ごとの詳細比較

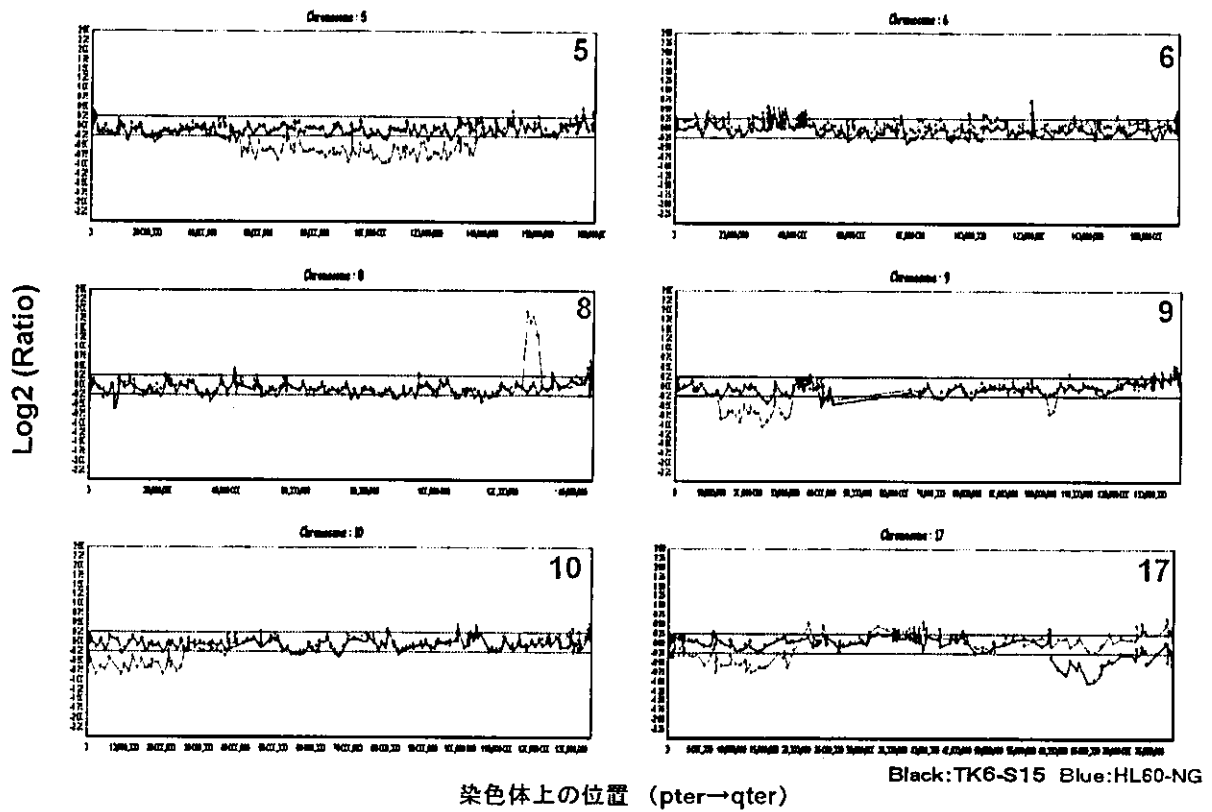


図 2 3 STR マーカーを用いた解析より予想された欠失領域と CGH アレイ解析結果の比較

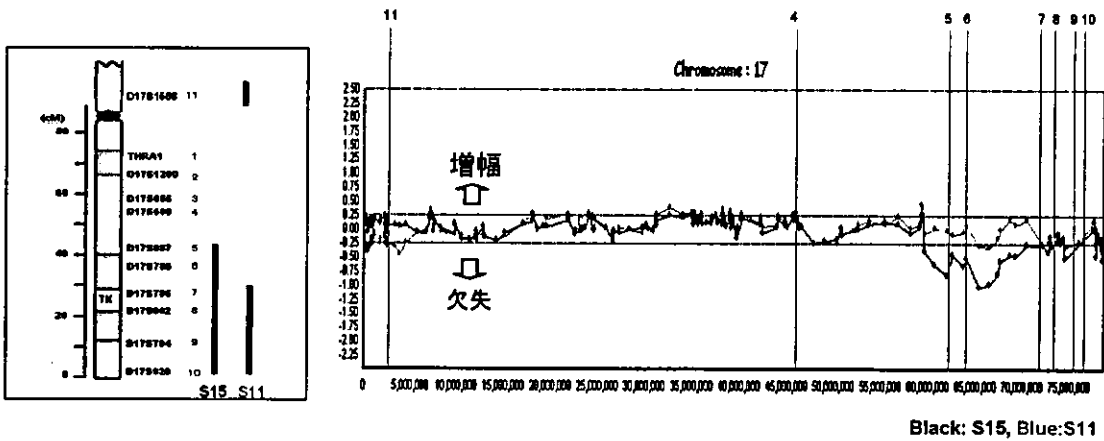


図 2 4 TK6-S15 クローンにおける X 染色体上の増幅領域

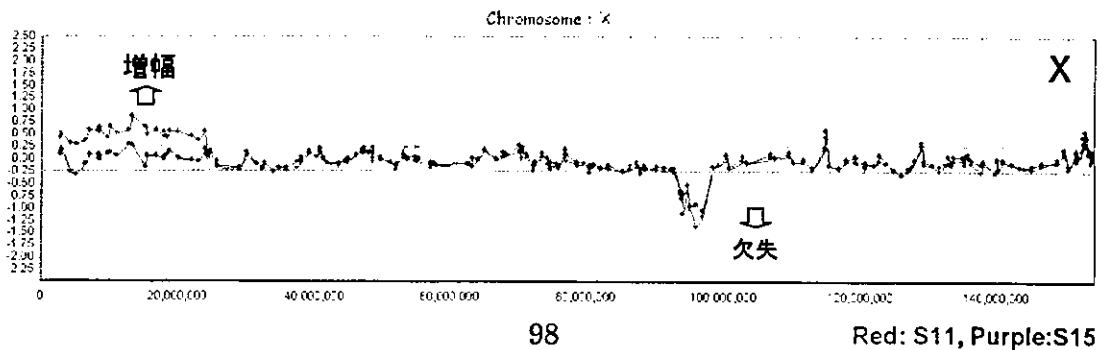


図 2 5 TK6 細胞の SKY 法による核型分析と転座遺伝子における遺伝子の増減

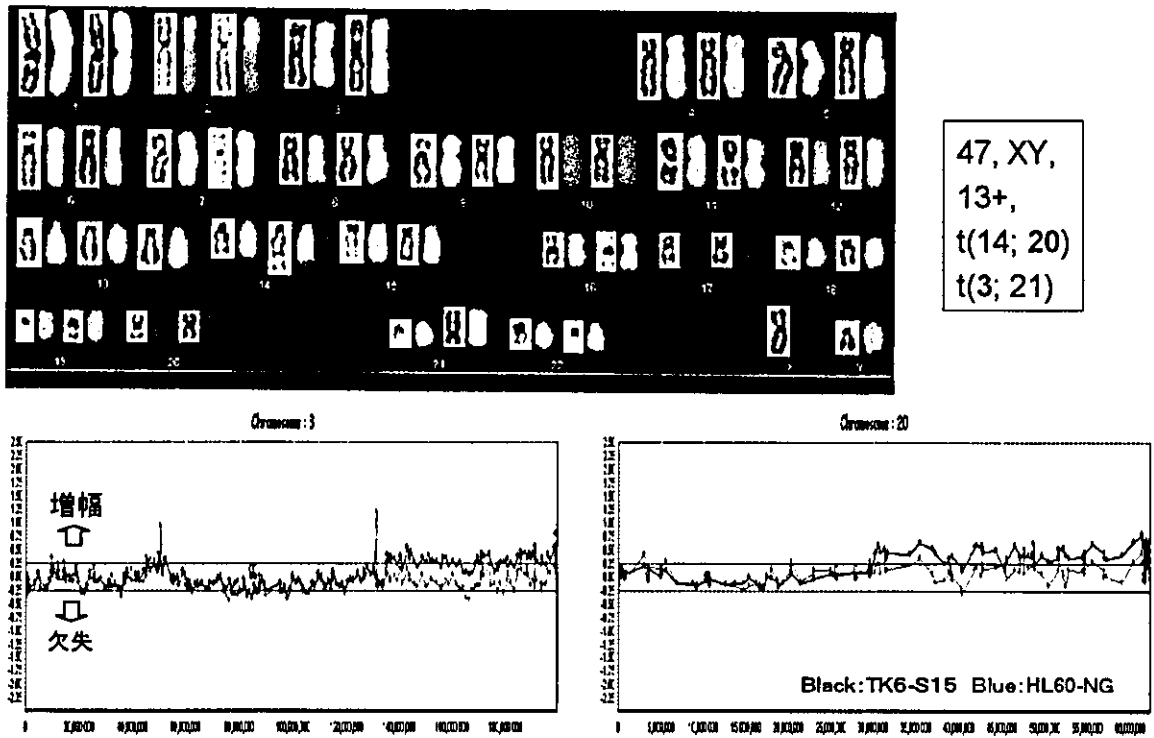


図 2 6 HL60 および HL60-RG 株の CGH と SKY 法による核型分析

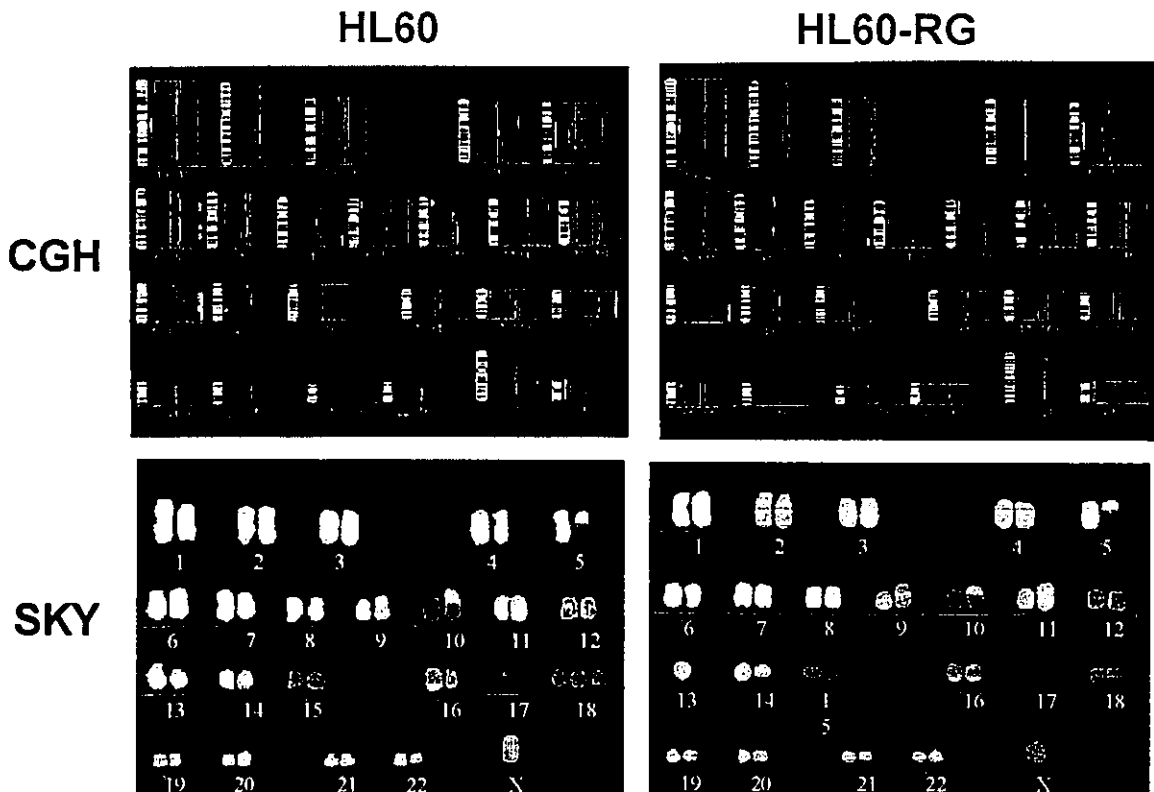


図 2 7 HL60 と HL60-RG 細胞の染色体ごとの詳細比較

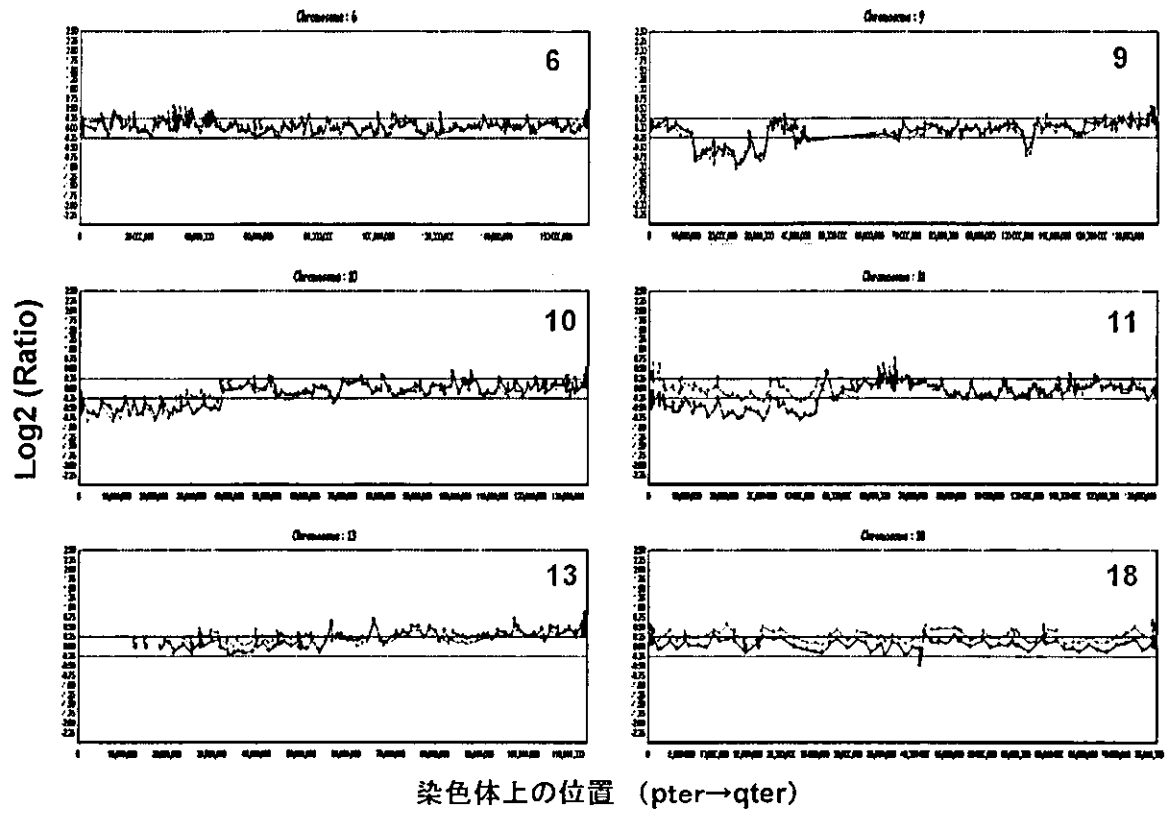


表 11. アレイ上のサイトカインの位置と名称

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

| | | secreted |
|----|----------------|---|
| 4C | SCF | Stem Cell Factor |
| 4D | SDF-1 | Stromal cell-derived factor |
| 4E | TARC | Thymus and Activation-Regulated Chemokine |
| 4F | TGF- β 1 | Transforming growth factor-beta 1 |
| 4G | TNF- α | Tumor necrosis factor-alpha |
| 4H | TNF- β | Tumor necrosis factor-beta |
| 4I | EGF | Epidermal growth factor |
| 4J | IGF-I | Insulin-like growth factor-1 |
| 4K | Ang | Angiogenin |
| 5A | OSM | Oncostatin M |
| 5B | TPO | Thrombopoietin |
| 5C | VEGF | Vascular Endothelial Growth Factor |
| 5D | PDGF-B | Platelet-derived Growth Factor-B |
| 5E | Leptin | Leptin |
| 5F | BDNF | Brain-derived neurotrophic factor |
| 5G | BLC | B-lymphocyte chemoattractant |
| 5H | Ck β 8-1 | Chemokine-beta-8 |
| 5I | Eotaxin | Eotaxin |
| 5J | Eotaxin-2 | MIP-2 (Myeloid progenitor inhibitory factor-2) |
| 5K | Eotaxin-3 | MIP-4-alpha (macrophage inflammatory protein-4-alpha) |
| 6A | FGF-4 | Fibroblast growth factor-4 |
| 6B | FGF-6 | Fibroblast growth factor-6 |
| 6C | FGF-7 | Fibroblast growth factor-7 |
| 6D | FGF-9 | Fibroblast growth factor-9 |
| 6E | Flt-3 Ligand | fms-like tyrosine kinase-3 ligand |
| 6F | Fractalkine | Fractalkine |
| 6G | GCP-2 | Granulocyte Chemotactic Protein-2 |
| 6H | GDNF | Glial-derived Neurotrophic Factor |
| 6I | HGF | hepatocyte growth factor |
| 6J | IGFBP-1 | Insulin-like growth factor binding proteins-1 |
| 6K | IGFBP-2 | Insulin-like growth factor binding proteins-2 |
| 7A | IGFBP-3 | Insulin-like growth factor binding proteins-3 |
| 7B | IGFBP-4 | Insulin-like growth factor binding proteins-4 |
| 7C | IL-16 | Interleukin 16 |
| 7D | IP-10 | Interferon gamma inducible protein-10 |
| 7E | LIF | Leukemia Inhibitory Factor |

| | | |
|----|-----------------|--|
| 7F | LIGHT | LIGHT |
| 7G | MCP-4 | Monocyte Chemoattractant Protein 4 |
| 7H | MIF | mesoderm inducing factor |
| 7I | MIP-3 α | Macrophage Inflammatory Protein-3 alpha |
| 7J | NAP-2 | Neutrophil Activating Peptide 2 |
| 7K | NT-3 | neurotrophin-3 |
| 8A | NT-4 | neurotrophin-4 |
| 8B | Osteoprotegerin | Osteoprotegerin |
| 8C | PARC | Pulmonary and Activation-Regulated Chemokine |
| 8D | PIGF | Placenta growth factor |
| 8E | TGF- β 2 | Transforming growth factor-beta 2 |
| 8F | TGF- β 3 | Transforming growth factor-beta 3 |
| 8G | TIMP-1 | tussue inhibitor of metalloproteinases-1 |
| 8H | TIMP-2 | tussue inhibitor of metalloproteinases-2 |
| 8I | Neg | negative control |
| 8J | Pos | positive control |
| 8K | Pos | positive control |

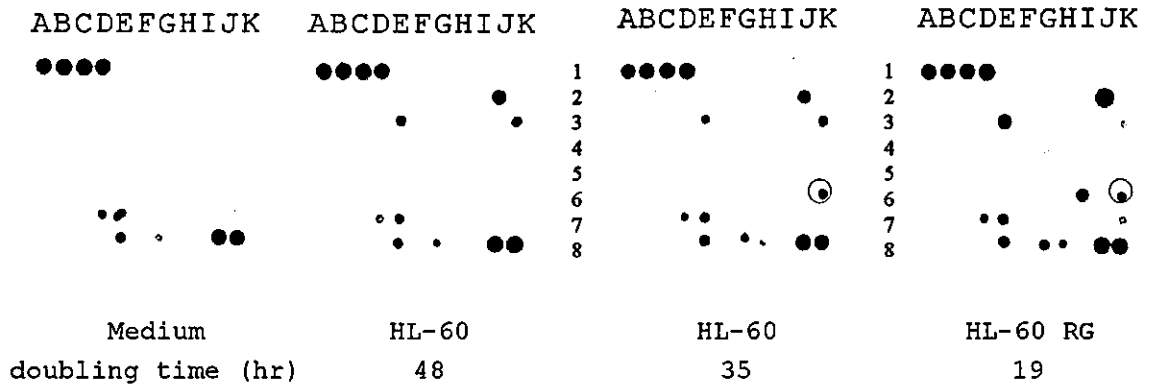


図 28. HL-60 と HL-60RG の培養上清のサイトカインアレイによる解析

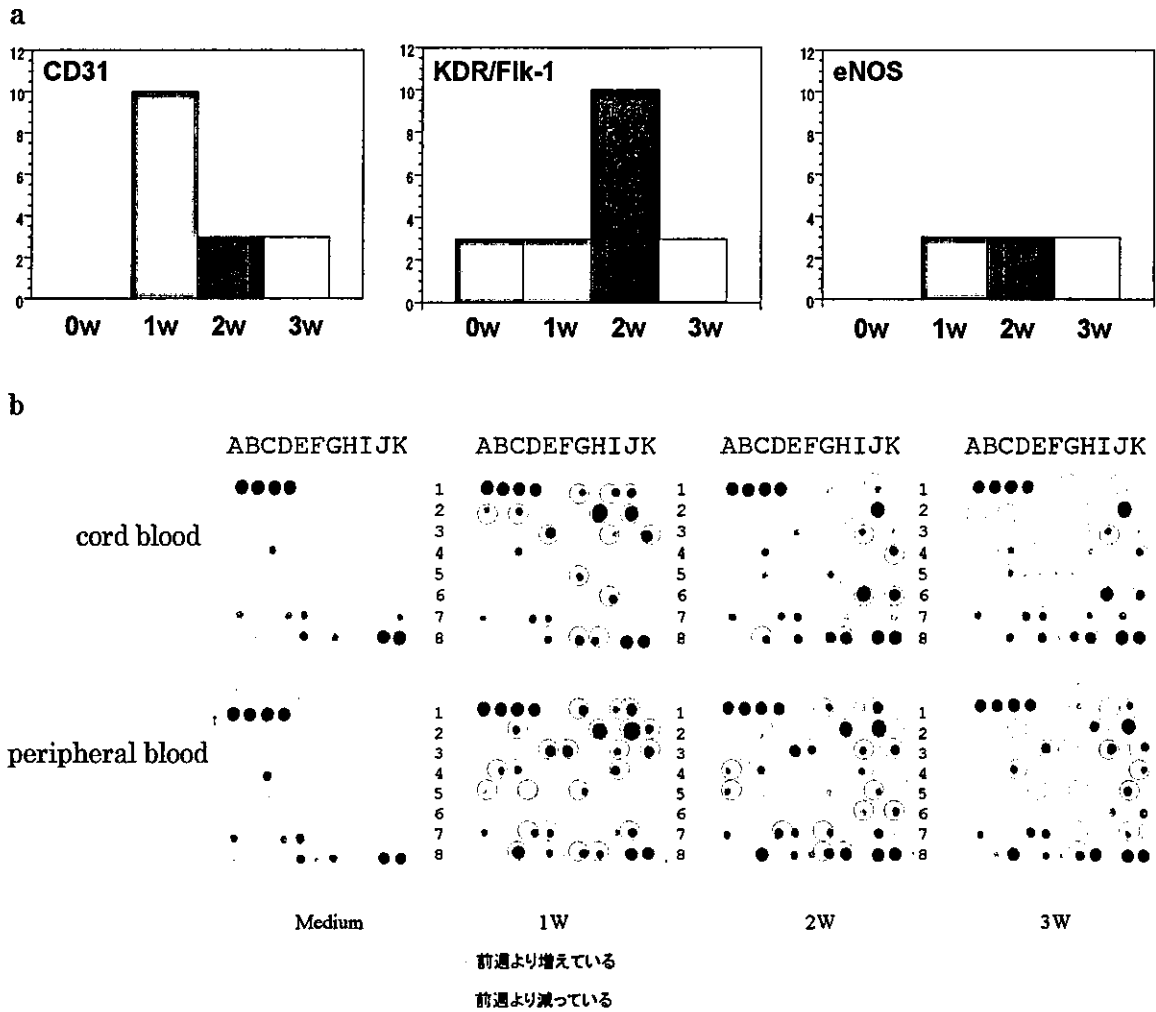


図 29. 臍帯血、末梢血由来 AC133 陽性細胞の培養上清のサイトカインアレイによる解析

表 1 2. 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 \pm 3.2 | 139 \pm 30 | ND | 4560 \pm 672 | 389 \pm 26 | ND | ND | ND | ND |
| HL-60 細胞 | ND | ND | ND | ND | 286 \pm 12 | ND | ND | 185 \pm 6 | ND | ND | ND | ND |

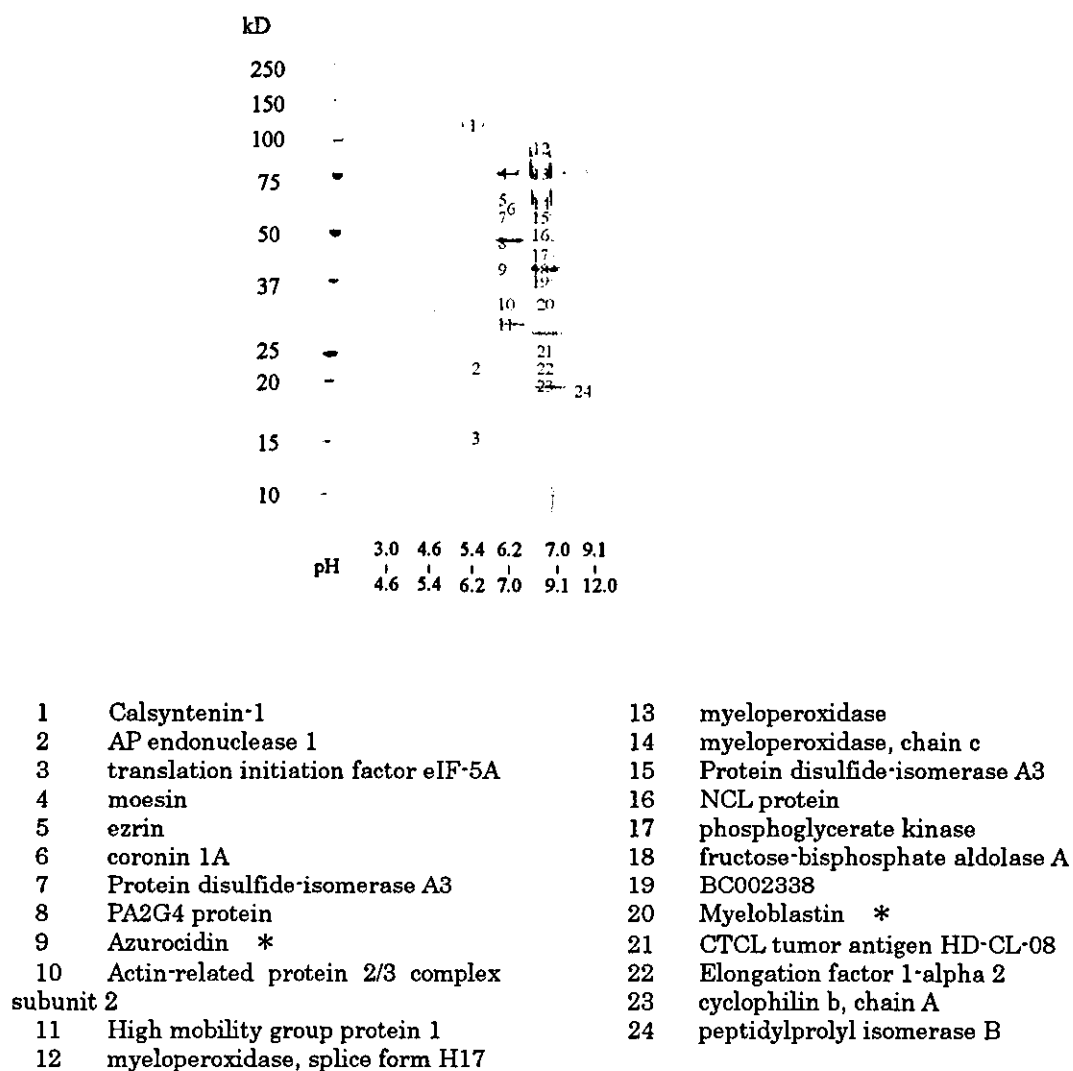


図 30. 無担体等電点電気泳動/SDS-PAGE による細胞由来タンパク質の分離と同定

表13 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/vnftelqk/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/vnftelqk/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/vnftelqk/l | 977.5 | [HexNAc]4[Hex]5[Neu5Ac]1 | 1931.8 |
| 23 | 1118.67 | +2 | 2235.3 | k/vnftelqk/l | 977.5 | [HexNAc]3[Hex]4 | 1275.9 |
| 23 | 1264.05 | 2 | 2526.1 | k/vnftelqk/l | 977.5 | [HexNAc]3[Hex]4[Neu5Ac]1 | 1566.6 |
| | 1110.48 | 3 | 2526.1 | | | | |
| 24 | 1665.44 | +2 | 3328.9 | k/vnftelqk/l | 977.5 | [HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1 | 2369.4 |
| 24 | 1178.33 | +3 | 3532.0 | k/vnftelqk/l | 977.5 | [HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1 | 2572.5 |
| 24 | 1061.78 | 3 | 3182.3 | k/vnftelqk/l | 977.5 | [HexNAc]4[Hex]5[Neu5Ac]2 | 2222.9 |
| 25 | 1592.17 | 2 | 3182.3 | | | | |
| 27 | 1236.04 | +3 | 3705.1 | k/ftkvnftelqk/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/ftkvnftelqk/l | 1353.7 | [HexNAc]4[Hex]5[Neu5Ac]2 | 2222.8 |
| 28 | 1780.23 | +2 | 3558.5 | | | | |
| 28 | 1303.56 | +3 | 3907.7 | k/ftkvnftelqk/l | 1353.7 | [HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1 | 2572.0 |
| 28 | 1138.84 | +3 | 3413.5 | k/ftkvnftelqk/l | 1353.7 | [HexNAc]4[Hex]5[Neu5Ac]1[Fuc]1 | 2077.8 |
| 28 | 1206.52 | +3 | 3616.6 | k/ftkvnftelqk/l | 1353.7 | [HexNAc]5[Hex]5[Neu5Ac]1[Fuc]1 | 2280.9 |
| 28 | 1090.14 | +3 | 3267.4 | k/ftkvnftelqk/l | 1353.7 | [HexNAc]4[Hex]5[Neu5Ac]1 | 1931.7 |
| 28 | 1322.75 | +3 | 3965.3 | k/ftkvnftelqk/l | 1353.7 | [HexNAc]6[Hex]5[Neu5Ac]2 | 2629.6 |

表14 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 | EHEGAIYPDNTDFQR | 1891.8 | [HexNAc]4[Hex]5[Neu5Ac]2 | 2222.9 | | | | |
| 24 | 1366.54 | +3 | 4096.6 | | | | | | | | |
| 24 | 1373.88 | +3 | 4118.6 | | | | | | | | |
| 24 | 1061.67 | +4 | 4242.7 | | | | | | | | |
| 25 | 1415.23 | +3 | 4242.7 | | | | | | | | |
| 25 | 1262.24 | +4 | 5045.0 | | | | | | | | |
| 25 | 1682.65 | +3 | 5045.0 | | | | | | | | |
| 25 | 1189.21 | +4 | 4752.8 | | | | | | | | |
| 25 | 1585.27 | +3 | 4752.8 | | | | | | | | |
| 25 | 1225.72 | +4 | 4898.9 | | | | | | | | |
| 25 | 1633.96 | +3 | 4898.9 | | | | | | | | |
| 27 | 1093.95 | +4 | 4371.8 | | | | | ELHHLQEQNVNAFLDK | 2021.0 | [HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1 | 2368.8 |
| 27 | 1458.27 | +3 | 4371.8 | | | | | | | | |
| 27 | 1057.43 | +4 | 4225.7 | | | | | | | | |
| 27 | 1409.57 | +3 | 4225.7 | | | | | | | | |
| 28 | 1257.99 | +4 | 5028.0 | | | | | | | | |
| 28 | 1677.01 | +3 | 5028.0 | | | | | | | | |
| 28 | 1221.48 | +4 | 4881.9 | | | | | | | | |
| 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 | | | | | | | | |
| 34 | 1083.68 | +4 | 4330.7 | | | | | | | | |
| 34 | 1444.56 | +3 | 4330.7 | | | | | | | | |
| 34 | 1493.28 | +3 | 4476.8 | | | | | | | | |
| 34 | 1089.17 | +4 | 4352.7 | | | | | | | | |
| 35 | 1668.97 | +3 | 5003.9 | | | | | | | | |
| 35 | 1663.29 | +3 | 4986.9 | | | | | | | | |
| 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 | | | | | | | | |
| 38 | 1331.17 | +3 | 3990.5 | | | | | | | | |
| 38 | 1289.81 | +3 | 3866.4 | | | | | | | | |
| | | | | | | [HexNAc]4[Hex]5[Neu5Ac]2[Na] | 2244.7 | | | | |

表15 Glycosylation analysis of rat Thy-1

| Glycosylation site | Carbohydrate composition | | | | Theoretical carbohydrate mass | Tryptic | | Asp-N | | | | | | |
|--------------------|--------------------------|--------|-------|--------------|-------------------------------|---------|----------|--------------------|--------------------|--------------|--------|--------------------|--------------------|-----------------|
| | Hex | HexNAc | NeuAc | Observed m/z | | Charge | Peak No. | Amino acid residue | Theoretical m/z | Observed m/z | Charge | Peak No. | Amino acid residue | Theoretical m/z |
| Asn23 | 0 | 6 | 2 | 0 | 1234.43 | 845.33 | 3 | T3 | H21-H31 (1315.63) | 845.03 | 3 | A3 | E22-H31 (1178.57) | 769.34 |
| | | | | | | 897.27 | 3 | T4 | H21-F33 (1591.74) | 897.06 | 2 | A3 | E22-H31 (1178.57) | 1198.50 |
| | | | | | | 1405.81 | 2 | T4 | H21-F33 (1591.74) | 1405.09 | 3 | A3 | N23-T36 (1628.80) | 946.75 |
| | | | | | | | | | | 1423.40 | 2 | A3 | N23-T36 (1628.80) | 1422.62 |
| | 0 | 6 | 2 | 0 | 1396.49 | 899.49 | 3 | T3 | H21-H31 (1315.63) | 899.04 | 3 | A3 | E22-H31 (1178.57) | 853.36 |
| | | | | | | 1349.35 | 2 | T3 | H21-H31 (1315.63) | 1349.06 | 2 | A3 | E22-H31 (1178.57) | 1279.53 |
| | | | | | | 942.16 | 3 | T3 | H21-E32 (1444.87) | 942.08 | 2 | A5 | E22-Q30 (1041.51) | 1211.00 |
| | | | | | | 901.37 | 3 | T5 | H21-F33 (1591.74) | 901.08 | | | | |
| | | | | | | 1486.40 | 2 | T5 | H21-F33 (1591.74) | 1486.12 | | | | |
| | 0 | 3 | 5 | 0 | 1519.57 | | | | | 1044.43 | 3 | A3 | N23-T36 (1628.80) | 1043.79 |
| | 0 | 7 | 2 | 0 | 1558.54 | 880.41 | 3 | T3 | H21-E32 (1444.87) | 880.07 | 3 | A3 | E22-H31 (1178.57) | 907.37 |
| | | | | | | | | | | 1361.03 | 2 | A3 | E22-H31 (1178.57) | 1360.56 |
| 1 | 3 | 5 | 0 | 1695.62 | | | | | 1083.32 | 3 | A3 | N23-T36 (1628.80) | 1092.48 | |
| 1 | 5 | 4 | 0 | 1736.65 | | | | | 1133.90 | 3 | A3 | N23-T36 (1628.80) | 1132.82 | |
| 0 | 6 | 3 | 1 | 1890.66 | | | | | 1169.51 | 3 | A3 | N23-T36 (1628.80) | 1167.49 | |
| 1 | 8 | 4 | 0 | 1948.70 | | | | | 1187.21 | 3 | A3 | N23-T36 (1628.80) | 1186.84 | |
| Asn74 | 1 | 2 | 3 | 0 | 1405.52 | 1026.26 | 3 | T6 | V50-F75 (1896.12) | 1026.18 | | | | |
| | 0 | 6 | 2 | 0 | 1234.43 | 949.52 | 2 | T2 | A73-K78 (880.35) | 949.39 | 3 | A7 | D64-K78 (1768.01) | 995.15 |
| | | | | | | 1182.72 | 2 | T6 | V69-K78 (1106.62) | 1182.53 | | | | |
| | 1 | 3 | 3 | 0 | 1259.47 | | | | | 1195.68 | 3 | A4 | F60-D79 (2342.26) | 1195.58 |
| | 1 | 4 | 3 | 0 | 1421.52 | 1043.45 | 2 | T2 | A73-K78 (880.35) | 1042.94 | 3 | A4 | N60-D79 (2342.26) | 1248.60 |
| | 1 | 3 | 4 | 0 | 1462.54 | 1063.68 | 2 | T2 | A73-K78 (880.35) | 1063.45 | | | | |
| | | | | | | 1276.61 | 2 | T6 | V69-K78 (1106.62) | 1276.59 | | | | |
| | 0 | 4 | 3 | 1 | 1566.56 | | | | | 1298.08 | 3 | A4 | N60-D79 (2342.26) | 1297.94 |
| | 1 | 5 | 3 | 0 | 1583.57 | 1124.18 | 2 | T2 | A73-K78 (880.35) | 1123.96 | | | | |
| | | | | | | 1337.84 | 2 | T6 | V69-K78 (1106.62) | 1337.10 | | | | |
| | 1 | 3 | 6 | 0 | 1665.62 | 1165.13 | 2 | T2 | A73-K78 (880.35) | 1164.99 | 2 | A4 | T71-K78 (894.48) | 1272.05 |
| | | | | | | 919.71 | 3 | T6 | V69-K78 (1106.62) | 919.09 | 3 | A7 | D64-K78 (1768.01) | 1138.88 |
| | | | | | | 1378.19 | 2 | T6 | V69-K78 (1106.62) | 1378.12 | | | | |
| | 0 | 3 | 6 | 0 | 1722.65 | | | | | 1106.39 | 3 | A4 | F66-D79 (1608.91) | 1105.65 |
| | 2 | 5 | 3 | 0 | 1729.63 | 1167.53 | 2 | T2 | A73-K78 (880.35) | 1166.99 | | | | |
| | 2 | 4 | 4 | 0 | 1770.66 | 1217.87 | 2 | T2 | A73-K78 (880.35) | 1217.51 | | | | |
| | | | | | | 1430.71 | 2 | T6 | V69-K78 (1106.62) | 1430.84 | | | | |
| | 1 | 5 | 4 | 0 | 1788.65 | 1228.38 | 2 | T2 | A73-K78 (880.35) | 1226.50 | | | | |
| | | | | | | 940.01 | 3 | T6 | V69-K78 (1106.62) | 940.43 | | | | |
| | | | | | | 1439.05 | 2 | T6 | V69-K78 (1106.62) | 1439.84 | | | | |
| | 1 | 4 | 5 | 0 | 1827.69 | 913.79 | 3 | T6 | V69-K78 (1106.62) | 913.10 | | | | |
| | 2 | 6 | 4 | 0 | 1932.71 | 1298.75 | 2 | T2 | A73-K78 (880.35) | 1298.53 | 2 | A7 | D64-K78 (1768.01) | 1227.91 |
| | | | | | | 1008.71 | 3 | T6 | V69-K78 (1106.62) | 1008.11 | | | | |
| | | | | | | 1511.98 | 2 | T6 | V69-K78 (1106.62) | 1511.87 | | | | |
| 1 | 5 | 4 | 0 | 1948.70 | 1308.81 | 2 | T2 | A73-K78 (880.35) | 1308.53 | 3 | A4 | F66-D79 (1608.91) | 1181.21 | |
| | | | | | 1013.98 | 3 | T6 | V69-K78 (1106.62) | 1013.45 | | | | | |
| 0 | 4 | 5 | 1 | 1972.71 | | | | | 1241.81 | 3 | A7 | D64-K78 (1768.01) | 1241.25 | |
| 2 | 4 | 6 | 0 | 1973.73 | 880.04 | 3 | T2 | A73-K78 (880.35) | 879.70 | 3 | A7 | D64-K78 (1768.01) | 1241.58 | |
| | | | | | 1319.24 | 2 | T2 | A73-K78 (880.35) | 1319.04 | 3 | A7 | D64-K78 (1768.01) | 1241.59 | |
| | | | | | 1022.43 | 3 | T6 | V69-K78 (1106.62) | 1021.79 | | | | | |
| | | | | | 1532.49 | 2 | T6 | V69-K78 (1106.62) | 1532.49 | | | | | |
| 1 | 5 | 5 | 0 | 1989.73 | | | | | 1146.70 | 3 | A4 | F68-D79 (1608.91) | 1146.20 | |
| 1 | 6 | 3 | 1 | 2036.72 | 1043.24 | 3 | T7 | V69-K78 (1106.62) | 1042.78 | | | | | |
| 1 | 5 | 4 | 1 | 2077.75 | 1057.08 | 3 | T7 | V69-K78 (1106.62) | 1056.46 | | | | | |
| 2 | 6 | 4 | 0 | 2094.76 | 1062.65 | 3 | T6 | V69-K78 (1106.62) | 1062.13 | 2 | A7 | D64-K78 (1768.01) | 1261.83 | |
| 1 | 4 | 5 | 1 | 2118.77 | 1070.38 | 3 | T7 | V69-K78 (1106.62) | 1070.14 | | | | | |
| 2 | 5 | 5 | 0 | 2135.79 | 1076.32 | 3 | T6 | V69-K78 (1106.62) | 1075.81 | | | | | |
| 1 | 6 | 4 | 1 | 2238.80 | 1110.97 | 3 | T7 | V69-K78 (1106.62) | 1110.49 | | | | | |
| 3 | 5 | 5 | 0 | 2261.82 | 1124.78 | 3 | T6 | V69-K78 (1106.62) | 1124.48 | | | | | |
| 1 | 7 | 5 | 0 | 2315.84 | 851.76 | 4 | T6 | V69-K78 (1106.62) | 851.82 | | | | | |
| 1 | 4 | 6 | 1 | 2321.85 | 1138.16 | 3 | T7 | V69-K78 (1106.62) | 1137.83 | | | | | |
| 2 | 5 | 6 | 1 | 2629.88 | 1241.13 | 3 | T7 | V69-K78 (1106.62) | 1240.53 | | | | | |
| Asn88 | 0 | 6 | 2 | 0 | 1234.43 | 1168.34 | 2 | T1 | V88-K99 (1117.54) | 1167.99 | 3 | A2 | E86-K99 (1515.76) | 911.74 |
| | | | | | | | | | | 1367.31 | 2 | A2 | E86-K99 (1515.76) | 1367.10 |
| | | | | | | | | | | 930.26 | 3 | A3 | Q92-R105 (1570.84) | 930.10 |
| | | | | | | | | | | 1021.28 | 3 | A3 | E86-N102 (1843.94) | 1021.13 |
| | | | | | | | | | | 1011.53 | 3 | A4 | T95-K110 (1815.06) | 1010.81 |
| | | | | | | | | | | 1516.43 | 2 | A4 | T95-K110 (1815.06) | 1516.75 |
| | | | | | | | | | | 1144.21 | 3 | A5 | E86-R105 (2212.18) | 1143.89 |
| | 0 | 3 | 4 | 0 | 1316.49 | 1209.79 | 2 | T1 | V88-K99 (1117.54) | 1209.02 | 3 | A2 | E86-K99 (1515.76) | 1008.78 |
| | 0 | 3 | 6 | 0 | 1519.57 | 1310.69 | 2 | T1 | V88-K99 (1117.54) | 1310.56 | 3 | A5 | E86-R105 (2212.18) | 1238.92 |
| | 0 | 4 | 3 | 1 | 1566.56 | | | | | 1230.86 | 3 | A4 | G81-K99 (2114.85) | 1231.17 |
| | 2 | 3 | 4 | 0 | 1608.60 | | | | | 961.29 | 3 | A4 | G81-N102 (1259.61) | 951.08 |
| | 1 | 4 | 4 | 0 | 1624.80 | 851.37 | 3 | T3 | S96-D108 (1245.67) | 851.76 | 2 | A4 | G81-N102 (1259.61) | 1426.11 |
| | 0 | 6 | 4 | 0 | 1640.59 | 1371.84 | 2 | T1 | V88-K99 (1117.54) | 1371.07 | 4 | A5 | E86-R105 (2212.18) | 959.70 |
| | | | | | | | | | | 1279.69 | 3 | A5 | E86-R105 (2212.18) | 1279.26 |
| | 0 | 5 | 3 | 1 | 1728.81 | | | | | 1176.27 | 3 | A4 | T95-K110 (1815.06) | 1176.23 |
| | 1 | 6 | 3 | 0 | 1745.82 | | | | | 1082.52 | 3 | A2 | E86-K99 (1515.76) | 1082.13 |
| | | | | | | | | | | 969.99 | 4 | A5 | E86-R105 (2212.18) | 965.98 |
| | | | | | | | | | | 1314.72 | 3 | A5 | E86-R105 (2212.18) | 1314.27 |
| | 1 | 5 | 4 | 0 | 1788.65 | | | | | 995.54 | 4 | A5 | E86-R105 (2212.18) | 996.21 |
| | | | | | | | | | | 1328.49 | 3 | A5 | E86-R105 (2212.18) | 1327.95 |
| | 1 | 5 | 3 | 1 | 1874.87 | | | | | 1018.89 | 4 | A6 | E86-R105 (2212.18) | 1018.22 |
| | 0 | 6 | 3 | 1 | 1890.88 | 1486.18 | 2 | T1 | V88-K99 (1117.54) | 1486.10 | 3 | A6 | E86-R105 (2212.18) | 1022.22 |
| | 2 | 5 | 4 | 0 | 1932.71 | 1517.35 | 2 | T1 | V88-K99 (1117.54) | 1517.13 | | | | |
| | 1 | 6 | 4 | 0 | 1948.70 | 1525.78 | 2 | T1 | V88-K99 (1117.54) | 1525.12 | | | | |
| | | | | | | | | | 1150.13 | 3 | A2 | E86-K99 (1515.76) | 1146.83 | |
| | | | | | | | | | 1259.45 | 3 | A3 | E86-N102 (1843.94) | 1259.22 | |
| | | | | | | | | | 1037.27 | 4 | A5 | E86-R105 (2212.18) | 1036.73 | |
| | | | | | | | | | 1382.34 | 3 | A5 | E86-R105 (2212.18) | 1381.97 | |
| 1 | 6 | 3 | 1 | 2036.72 | | | | | 1059.07 | 4 | A6 | E86-R105 (2212.18) | 1058.73 | |
| 0 | 6 | 4 | 1 | 2092.74 | | | | | 1073.37 | 4 | A6 | E86-R105 (2212.18) | 1072.99 | |

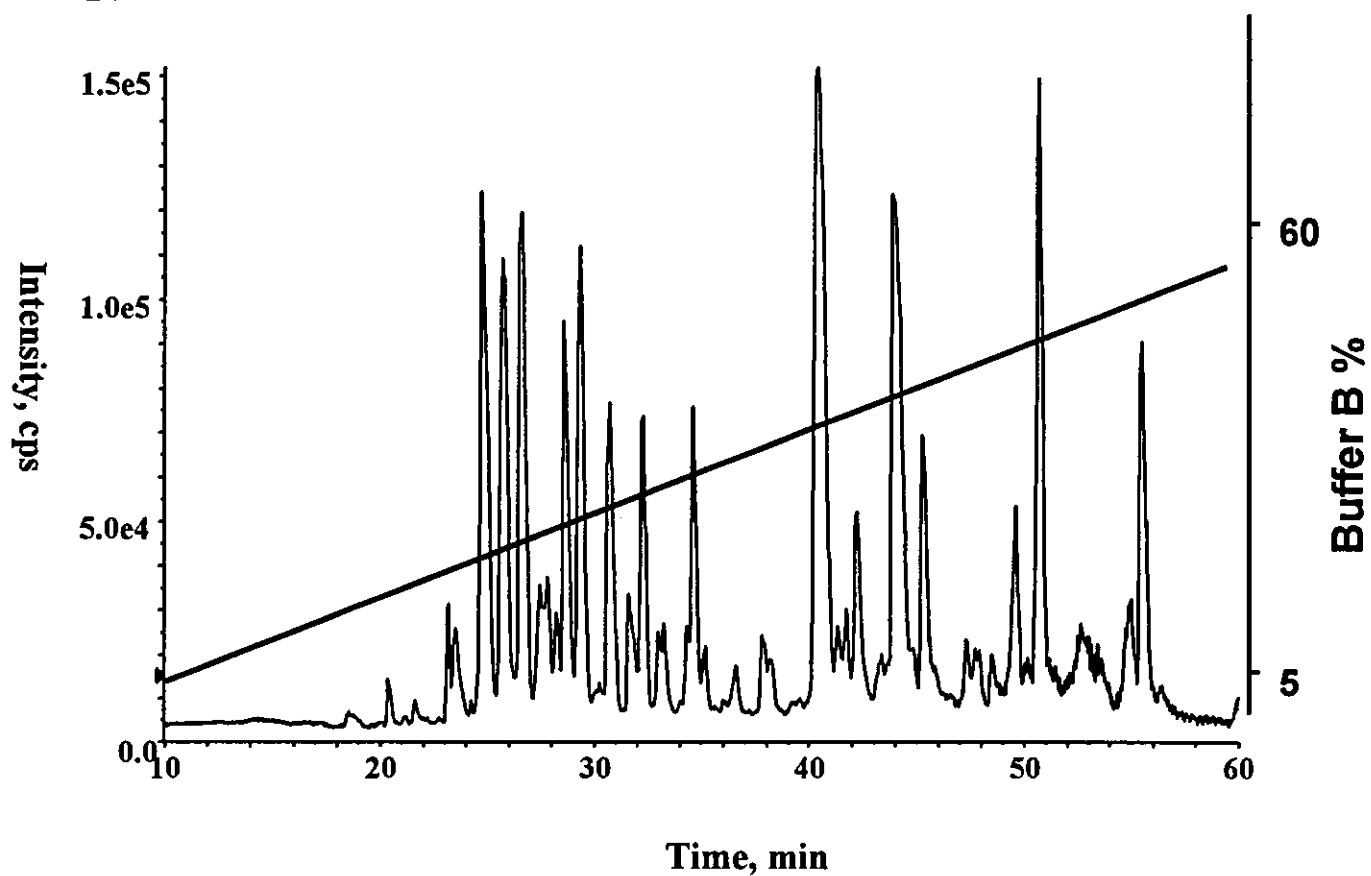
| | | | | |
|--------------------|-------------------|-------------------|--------------------|-------------------|
| mkwvesifli | fllnftesrt | lhrneygias | ildsyqctae | isladlatif |
| faqfvqeaty | kevskmvkda | ltaiekptgd | eqssgclenq | lpafleelch |
| ekeilekygh | sdccsqseeg | rhncflahkk | ptpasiplfq | vpepvtscea |
| yedretfmn | kfiyeiarrh | pflyaptill | waarydkiiip | scckaenave |
| cfqtkaatvt | kelresslln | qhacavmknf | gtrtfqaitv | tklsqkftkv |
| nfteiqklvl | dvahvhehcc | rgdvldclqd | gekimsyics | qqdtlsnkit |
| eccklttler | gqcihaend | ekpeglspnl | nrflgdrdfn | qfssgeknif |
| lasfvheysr | rhpqlavsvi | lrvakgyqel | lekcqtenp | lecqdkgeee |
| lqkyiqesqa | lakrscglfq | klgeyylqna | flvaytkkap | qлтsselmai |
| trkmaataat | ccqlsedkll | acgegaadii | ighlcihem | tpvnpvggqc |
| ctssyanrrp | cfsslvvdet | yvppafsddk | fifhkdlcqa | qgvalqtmkq |
| efflinlvkqk | pqiteeqlea | viadfsglle | kccqgqegev | cfaeegqli |
| sktraalgv | | | | |

图31 Amino acid sequence and N-glycosylation sites of alpha-Fetoprotein
n: N-glycosylation site

| | | | | |
|---|---|---|---|---|
| mkililgfl | flcstpawak | ekhyyigiie | ttwdyasdhg | ekklisvdte |
| hsniylqngp | drigrlykka | lylqytdetf | rttiekpowl | gflgpiikae |
| tgdkvyvhlk | nlasrpytfh | shgityykeh | egaiypdntt | dfqraddkvy |
| pgeqytymll | ateeqspgeg | dgnvcvtriyh | shidapkdia | sgligpliic |
| kkdsldkeke | khidrefvvm | fsvvdensfw | yledniktyc | sepekvdkdn |
| edfquesrmy | svngytfgsi | pglsmcaedr | vkwyifgmgn | evdvhaaffh |
| ggaltknknyr | idtinlfp^at | lfd^aymvaqn | pgewmlscqn | lnhlkaglqa |
| ffqvqec<u>n</u>ks | sskdnirgkh | vrhyyia^aee | iiwnyapsgi | diftke<u>n</u>lta |
| pgsdsavffe | qgt<u>t</u>riggsy | kklvyre^atd | asftnrkerg | pee^ahlgilg |
| pviwaevgdt | irvtfh^ankga | yplsiepigv | rfnkknegty | yspnynp^aqsr |
| svppsashva | ptetfty^aewt | vpkevgp^atna | dpvclakmyy | savdptk^adif |
| tgligpmkic | kkgs^alhangr | qkdvdkef^ayl | fptvfdenes | lllednirm^af |
| ttapdqvdke | dedfques^anm | hsmngfmy^agn | qpgl^atmckgd | svvwy^alfsag |
| neadvhgiyf | sgntylwrge | rrdtanlfp^aq | tsl^atlhmwpd | tegtfnve^acl |
| ttdhytggmk | qkyt^avnq^aerr | qsedstf^aylg | ertyyia^aave | vewdysp^aqre |
| wekel^ahlhqe | q<u>n</u>vsna^afldk | gefyigsky^ak | kvvyrqy^atds | tfrvp^averka |
| eeehlgilgp | qlhadvg^adkv | kiifknmat^ar | pysihah^agvq | tesstvt^aptl |
| pgetltyv^awk | ipersgag^ate | dsacipway^ay | stvdq^avk^adly | sgligpliv^ac |
| rrpyl^akvfn^ap | rrklefall^af | lvfdenes^awy | lddnikt^aysd | hpekvnk^adde |
| efies^ank^amha | ingrmf^agnl^aq | gltmh^avg^adev | nwy^almg^amg^ane | idlht^avh^afhg |
| hsfqy^akhrg^av | yssdv^afdif^ap | gtyqtlem^afp | rtpgiwll^ahc | hvt^adhihag^am |
| etty^avtl^aqne | dtksg | | | |

☒32 Amino acid sequence and N-glycosylation sites of ceruloplasmin (CP)
n: Potential N-glycosylation sites
n: N-glycosylation sites

(A) TIC for the full scan m/z 700-2000



☒ 33 LC-MS/MS of tryptic digest of α -fetoprotein

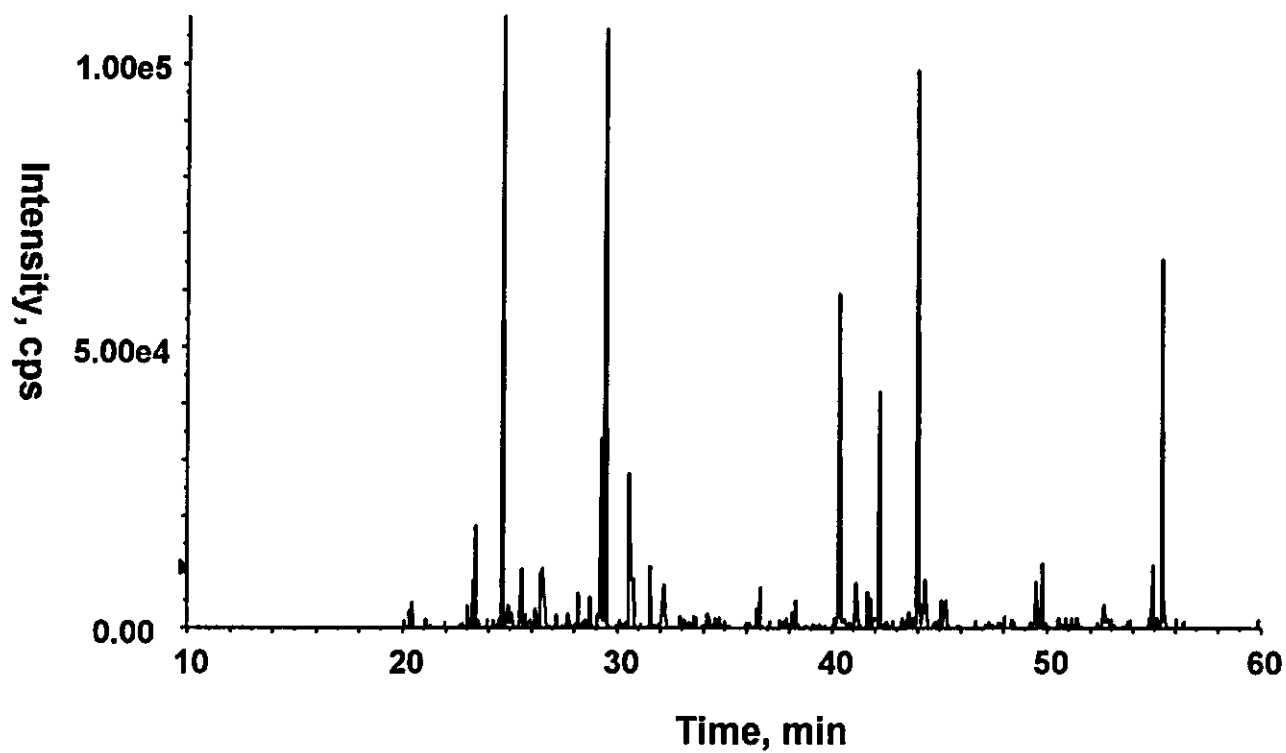
Column Magic C18

3 μ , 0.2*50 mm, 2 μ l/min

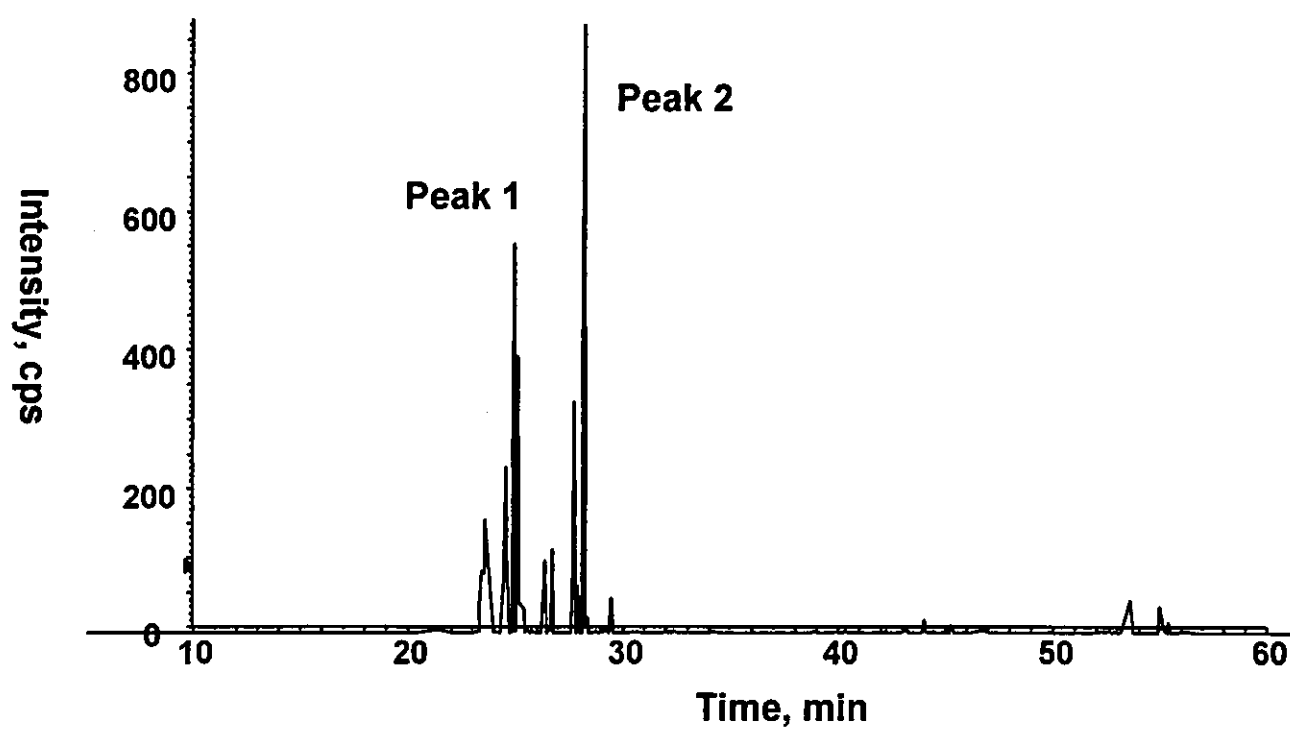
A: 2 % CH_3CN + 0.1 % formic acid

B: 90 % CH_3CN + 0.1 % formic acid

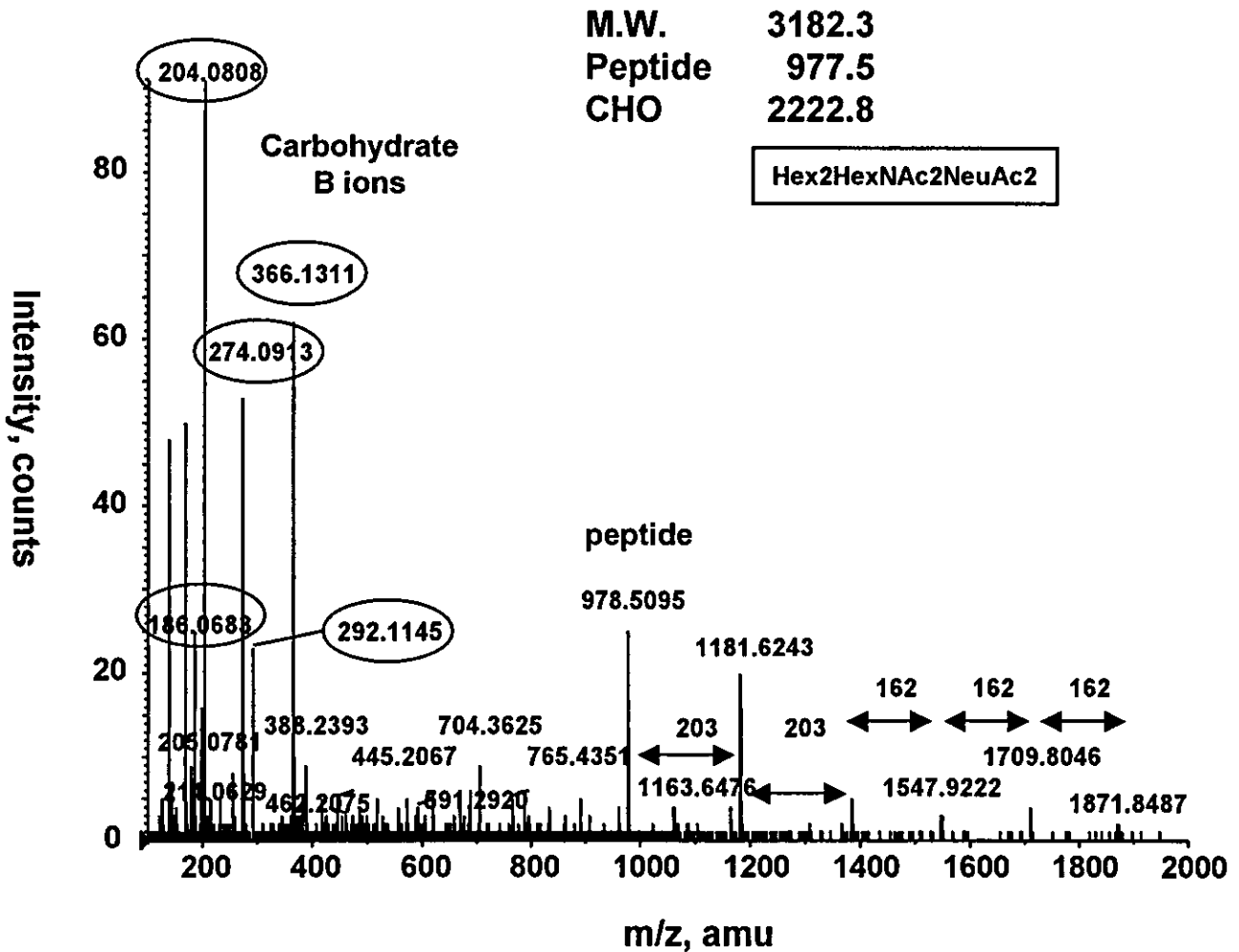
(B) TIC for the product ion scan at m/z 100 - 2000



(C) Product ion scan at m/z 204

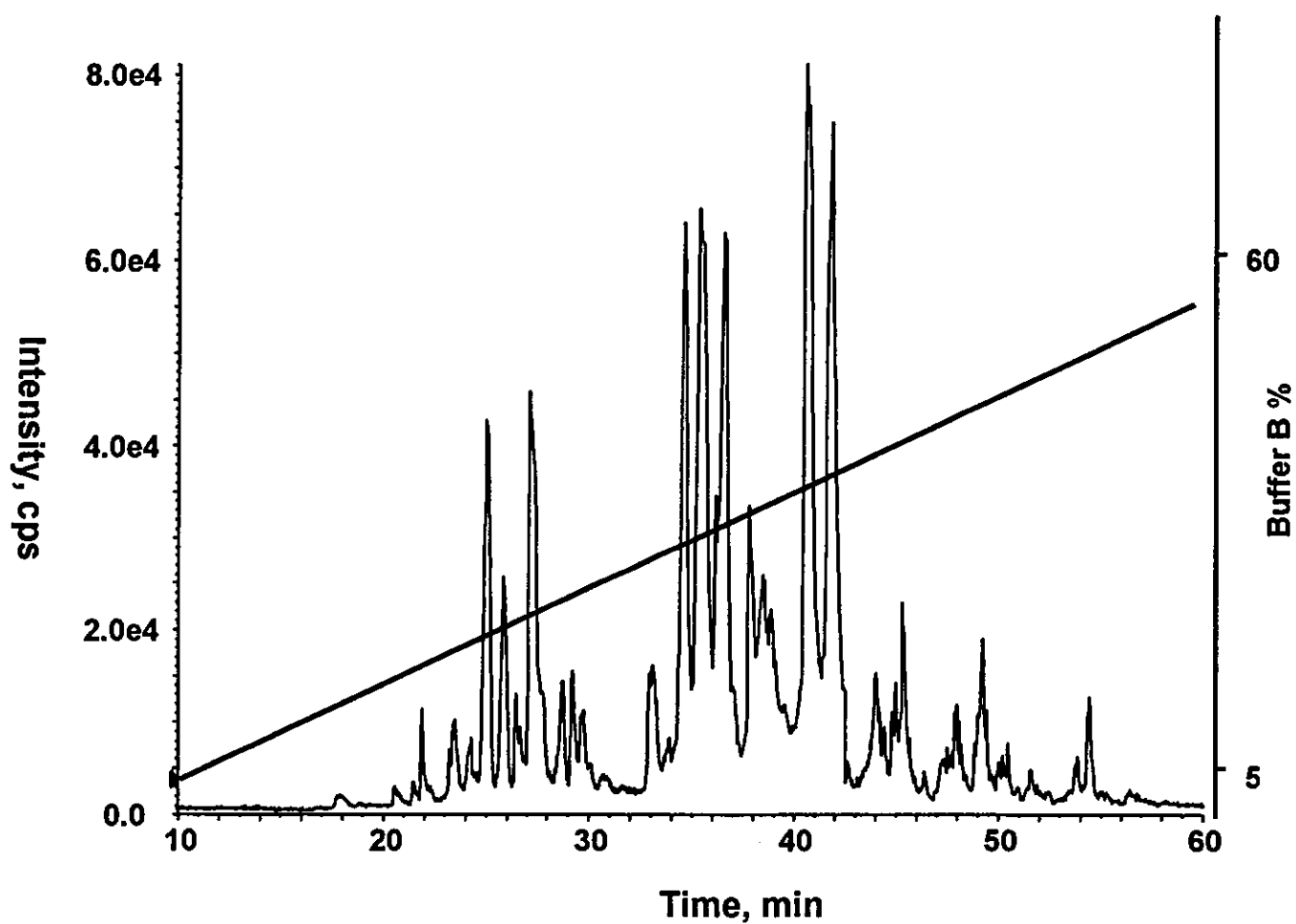


| Residue | Mass | b | b-NH3 | y | y-NH3 |
|---------|--------|--------|--------|--------|--------|
| V | 99.07 | 100.08 | 83.05 | 978.53 | 961.50 |
| N | 114.04 | 214.12 | 197.09 | 879.46 | 862.43 |
| F | 147.07 | 361.19 | 344.16 | 765.41 | 748.39 |
| T | 101.05 | 462.23 | 445.21 | 618.35 | 601.32 |
| 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 |



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

(A) TIC for the full scan m/z 1000-2000



☒35 LC-MS/MS of tryptic digest of ceruloplasmin

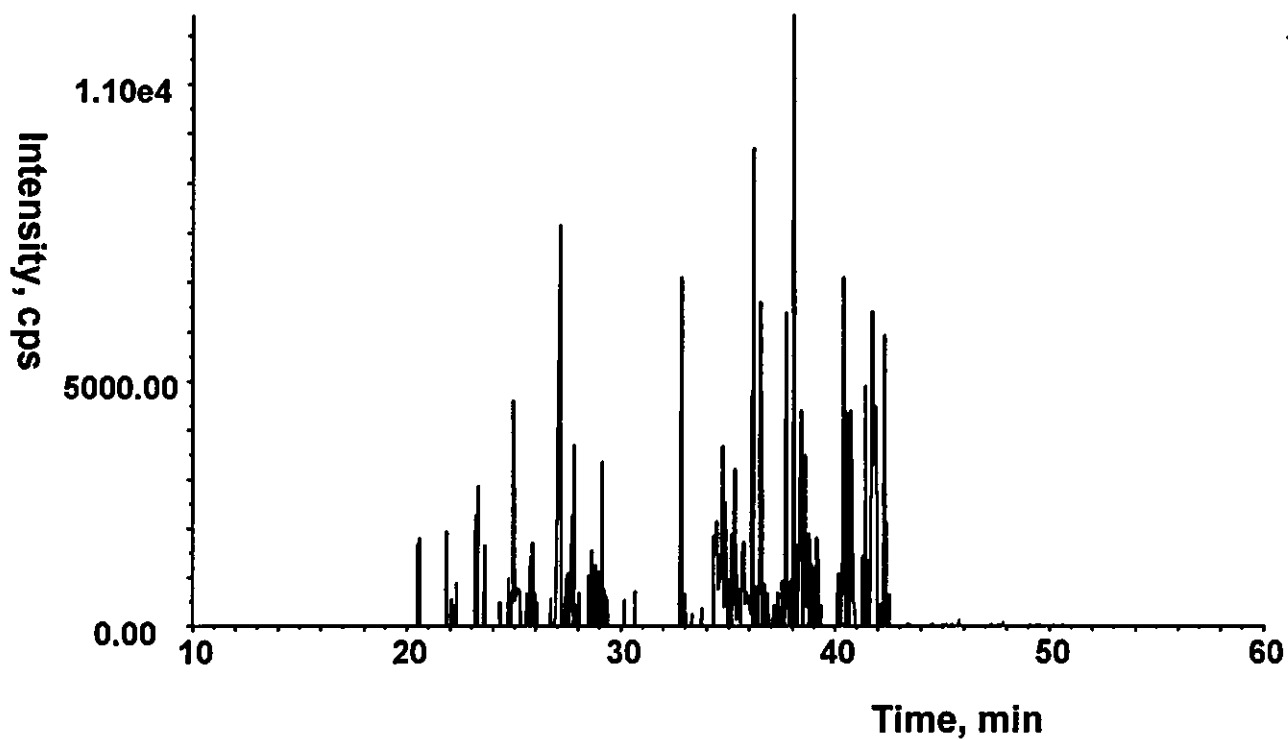
Column Magic C18

3 μ , 0.2*50 mm, 2 μ l/min

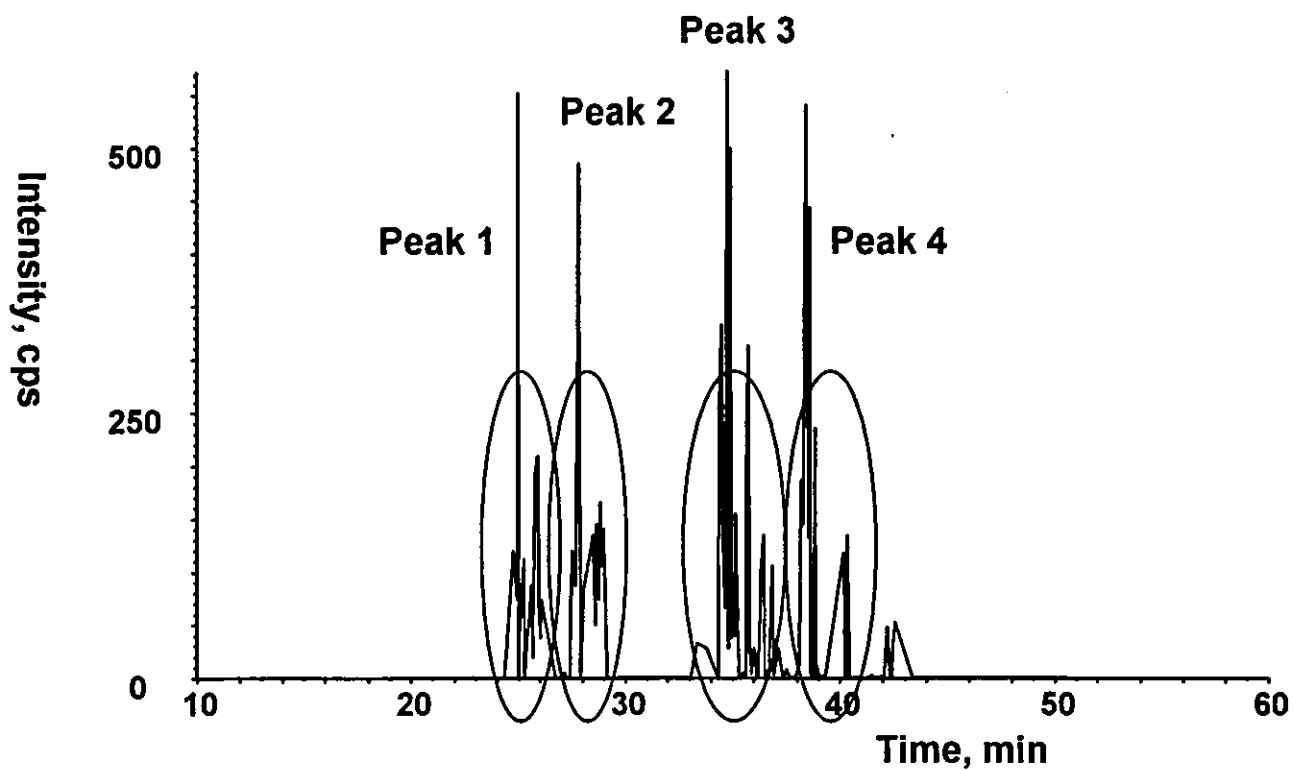
A: 2 % CH_3CN + 0.1 % formic acid

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



M.W. 4096.6
 Peptide 1891.8
 CHO 2222.8

Hex₂HexNAc₂NeuAc₂

| 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 |

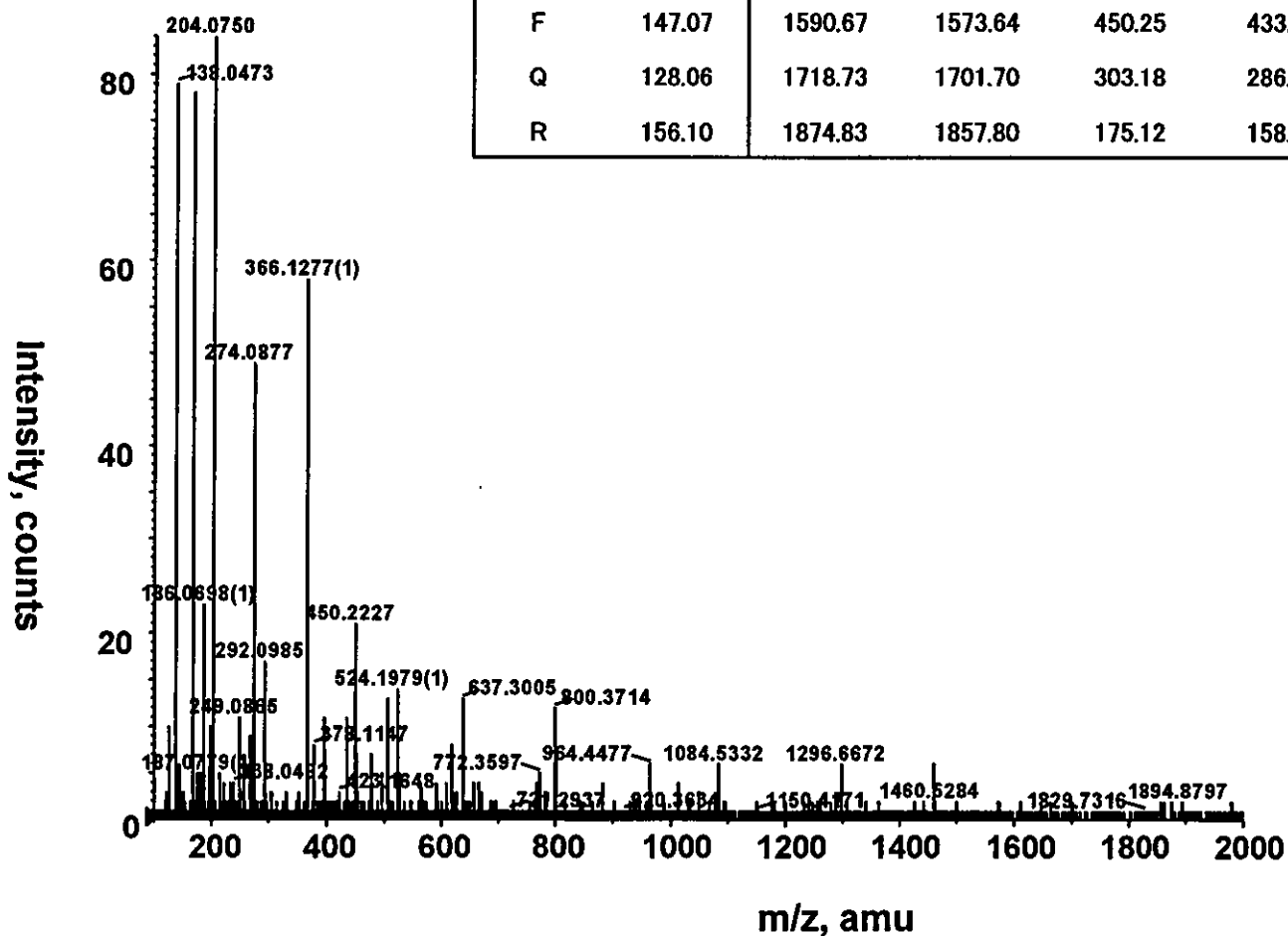
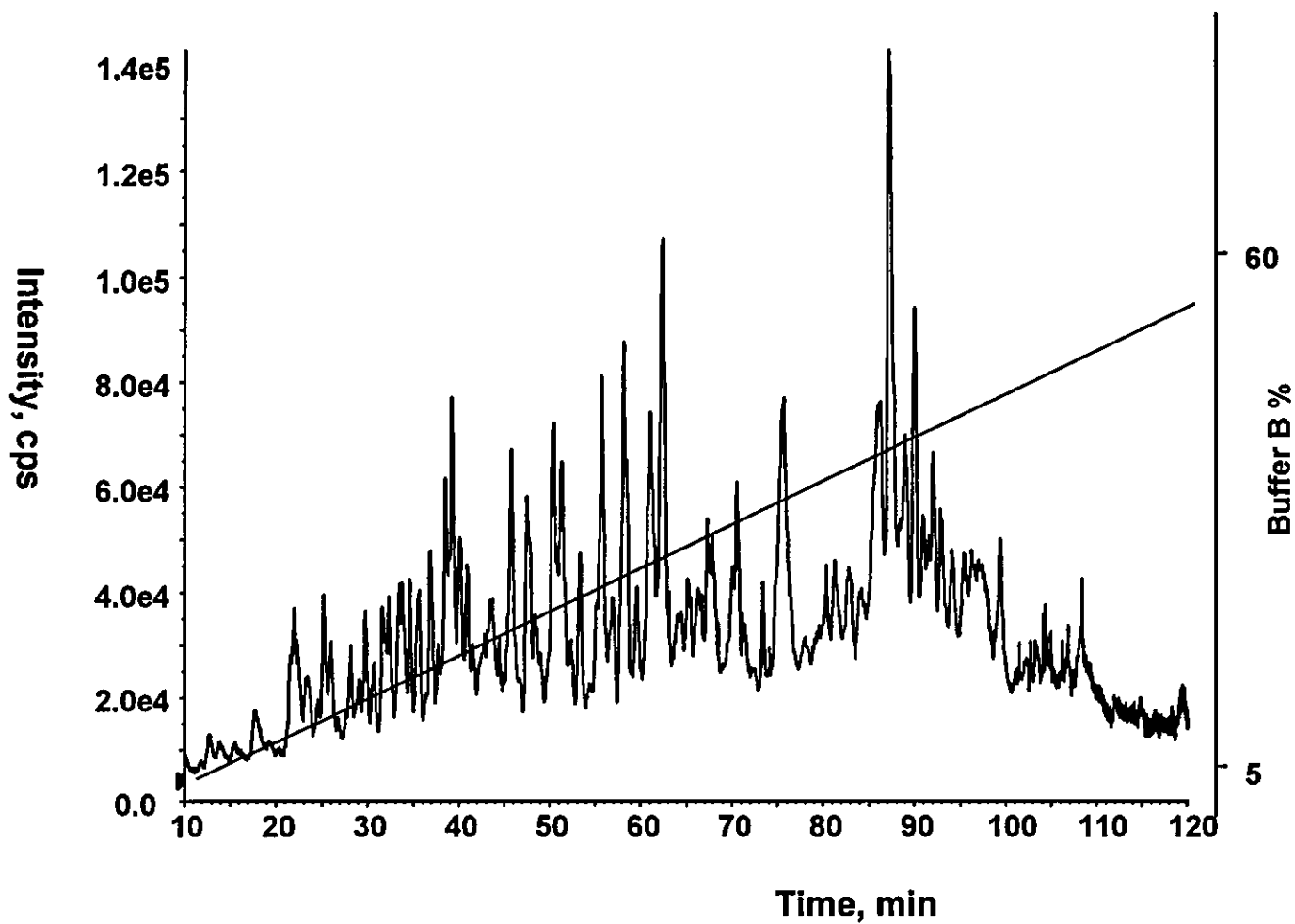


Fig 36 Product ion spectrum of M⁺(m/z1366.6³⁺) at 25 min

(A) TIC for the full scan m/z 400-2000



☒37 LC-MS/MS of tryptic digest of human serum

Column Magic C18

3 μ , 0.2*50 mm, 2 μ l/min

A: 2 % CH_3CN + 0.1 % formic acid

B: 90 % CH_3CN + 0.1 % formic acid