

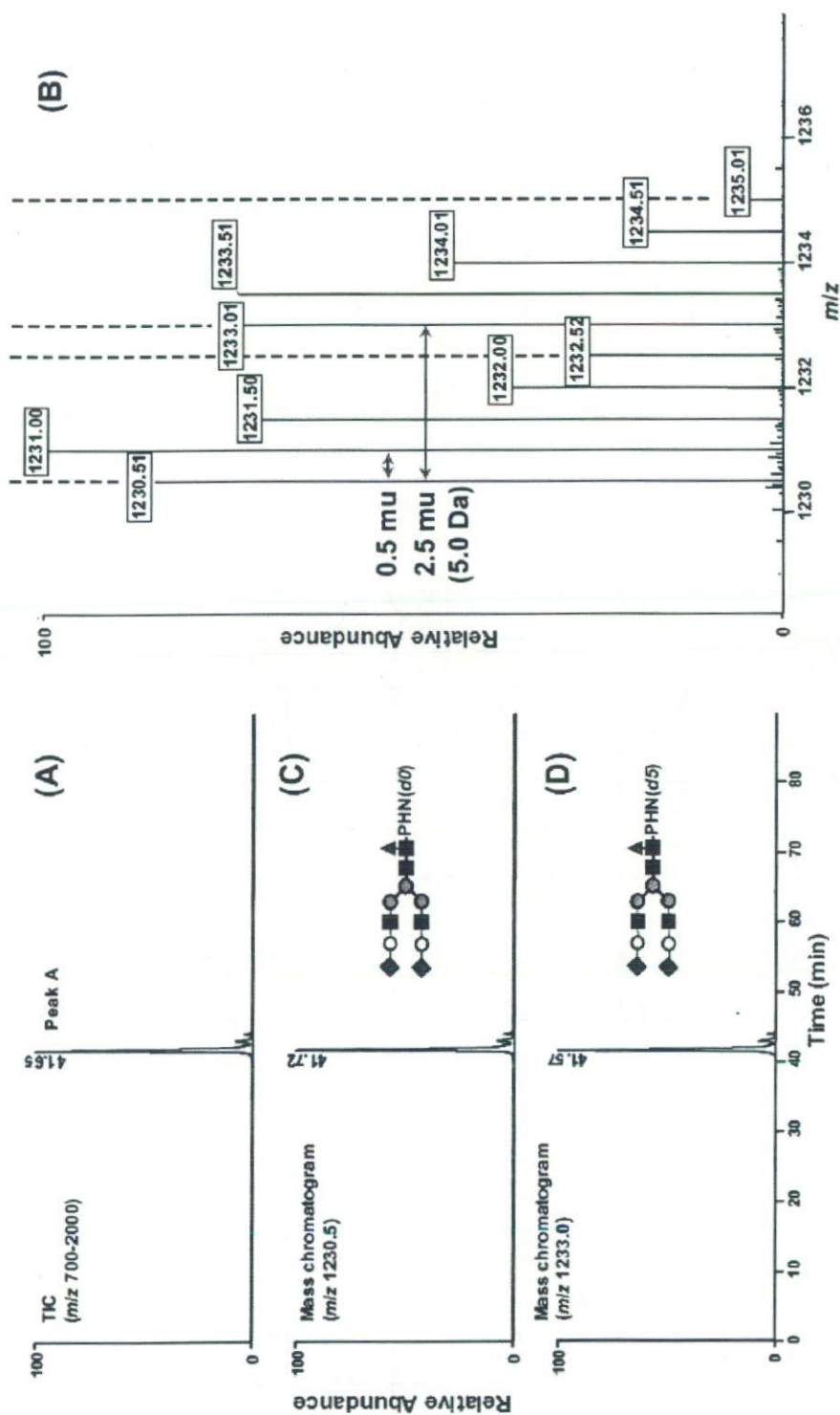


Fig. 12 d_0 -及び d_5 -PHN糖鎖のLC/FTMS. (A), d_0 -及び d_5 -PHN糖鎖の標準ジシアリル2本鎖糖鎖混合物のLC/MS/MSにより得られたTIC; (B), peak A のマススペクトル; (C), d_0 -PHN糖鎖のマスクロマトグラム; (D), d_5 -PHN糖鎖のマスクロマトグラム.

LC: Instrument, NanoFrontier nLC (Hitachi); Column, Hypercarb (0.075 x 150 mm); Trap cartridge, Graphitized carbon cartridge (0.3 x 5 mm), (Flow rate, 200 nL/min; A buffer, 2 % $\text{CH}_3\text{CN}/5$ mM ammonium acetate (pH 9.6); B buffer, 80 % $\text{CH}_3\text{CN}/5$ mM ammonium acetate (pH 9.6); Gradient, 2 – 90 % of B in 60 min; MS: Instrument, LTQ-FT (ThermoFisher Scientific); Ion mode, positive ion.
 ◆, N-アセチルノイラミン酸; ○, ガラク トース; ■, N-アセチルグルコサミン; ●, マンノース; ▲ フコース; PHN, フェニルヒ ドラジン.

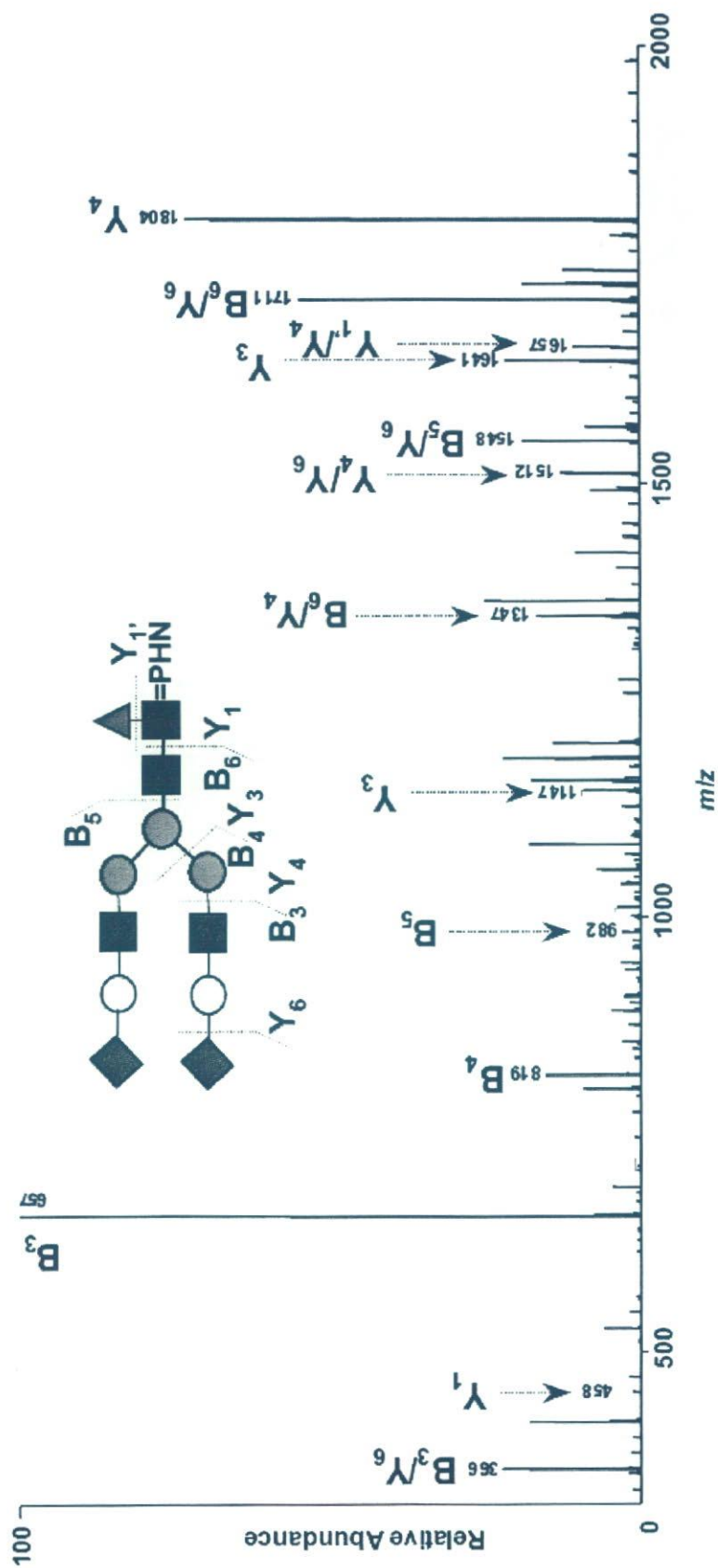


Fig.13 d_0 -PHN糖鎖-のMS/MSスペクトル

◆, N-アセチルノイラミン酸; ○, ガラクトース; ■, N-アセチルグルコサミン; ●, マンノース; ▲, フコース; PHZ, フェニルヒドラジン.

モデル細胞: rapid growth HL-60 細胞

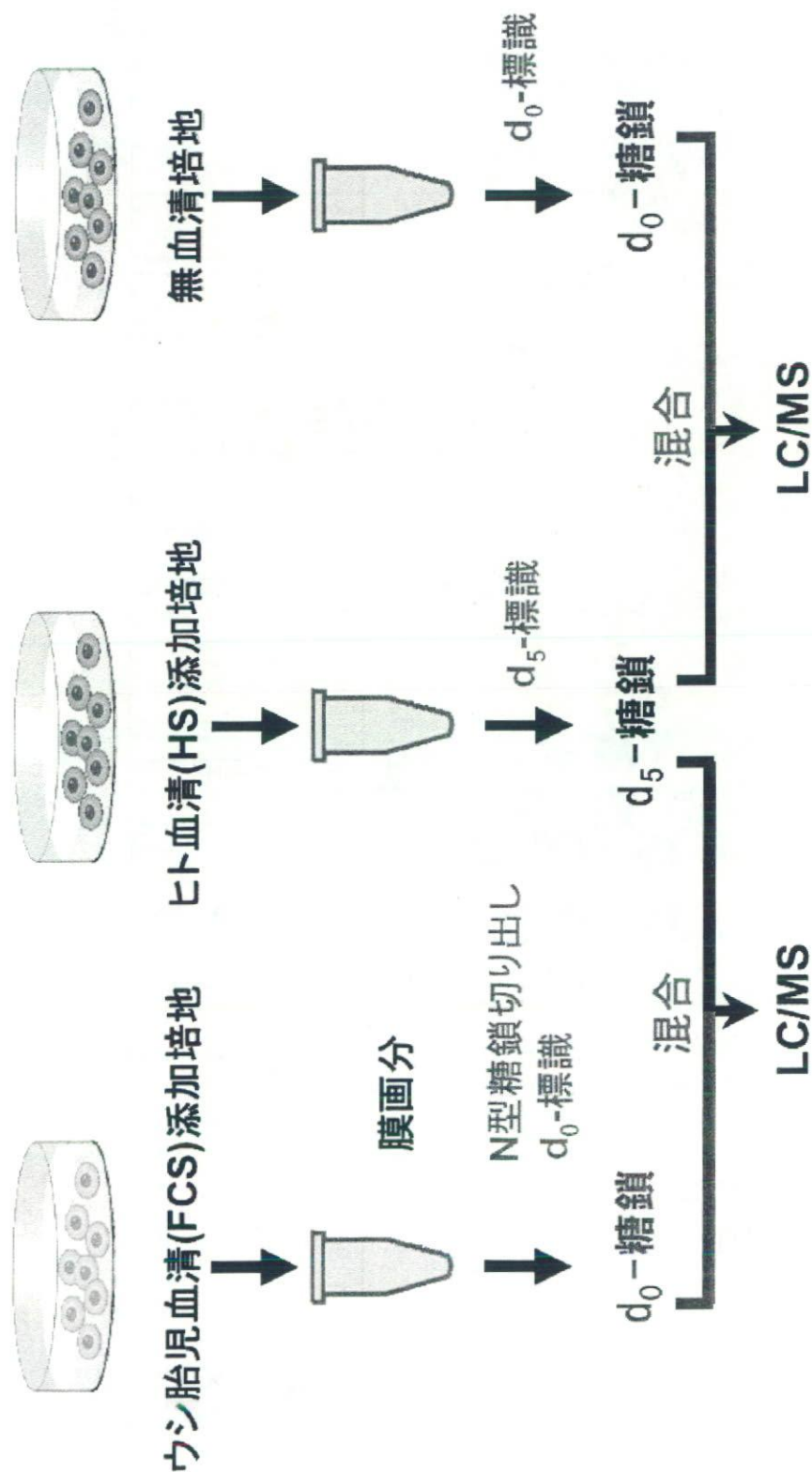


Fig.14 異なる培養条件で培養した細胞の膜画分由来糖鎖の差異解析

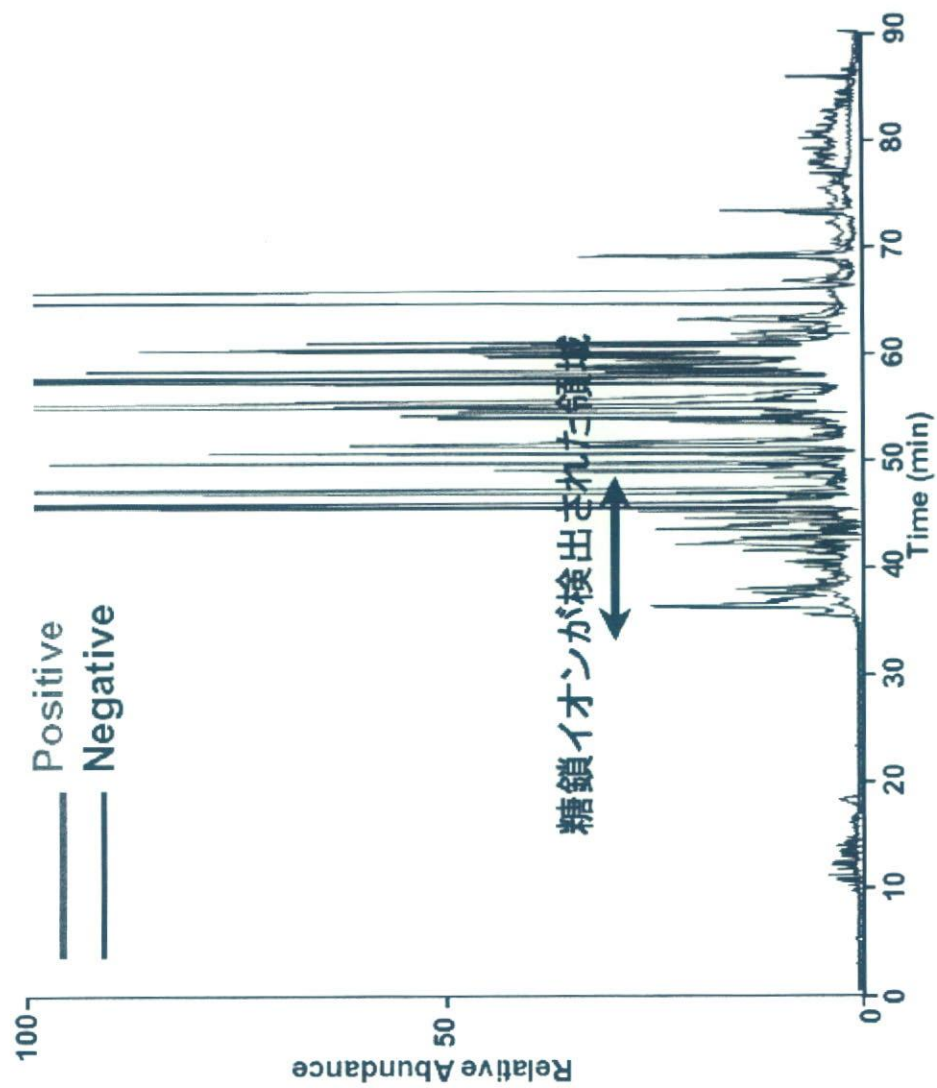


Fig.15 FCS及びHS添加培地で培養した細胞から調製した d_0 -PHN糖鎖 及び d_5 -PHN-HS糖鎖混合試料のLC/MSにより得られたトータルイオンクロマトグラム (TIC)

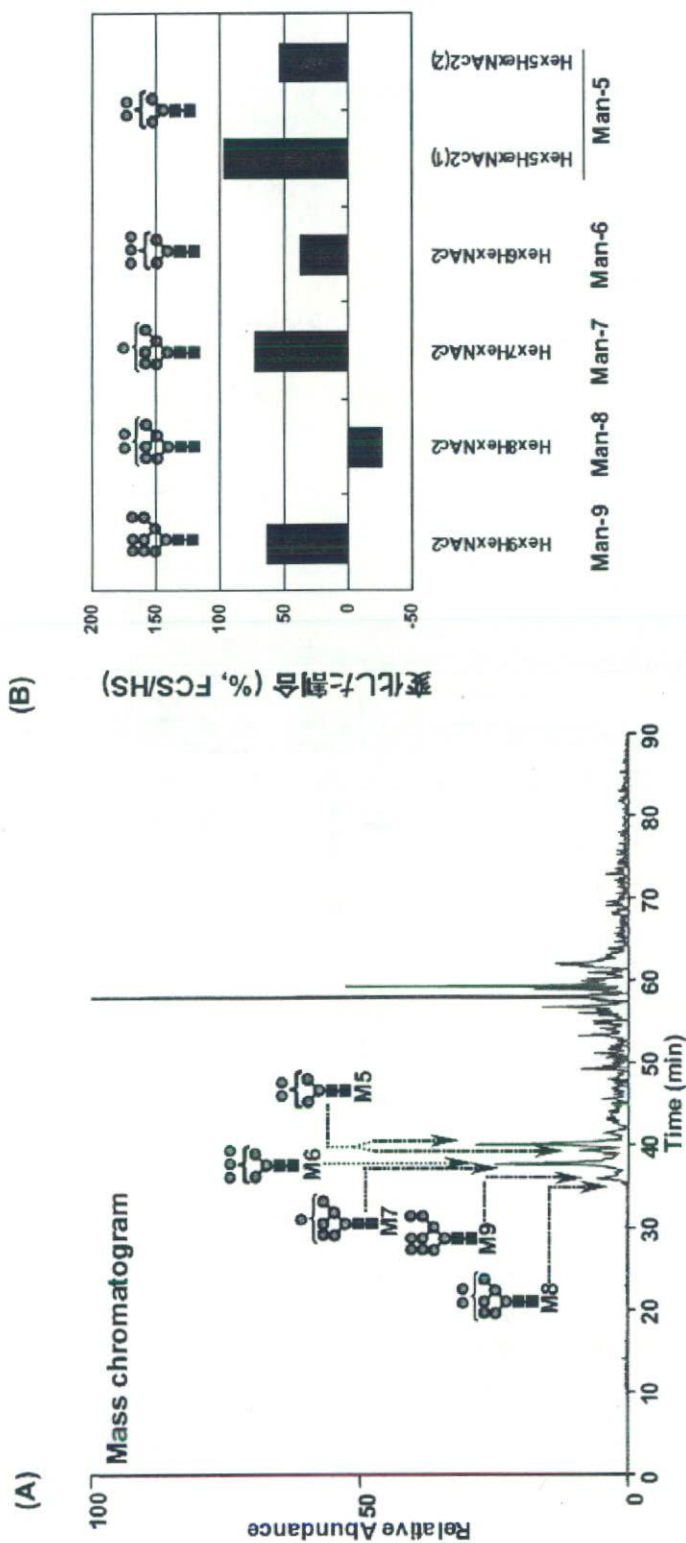
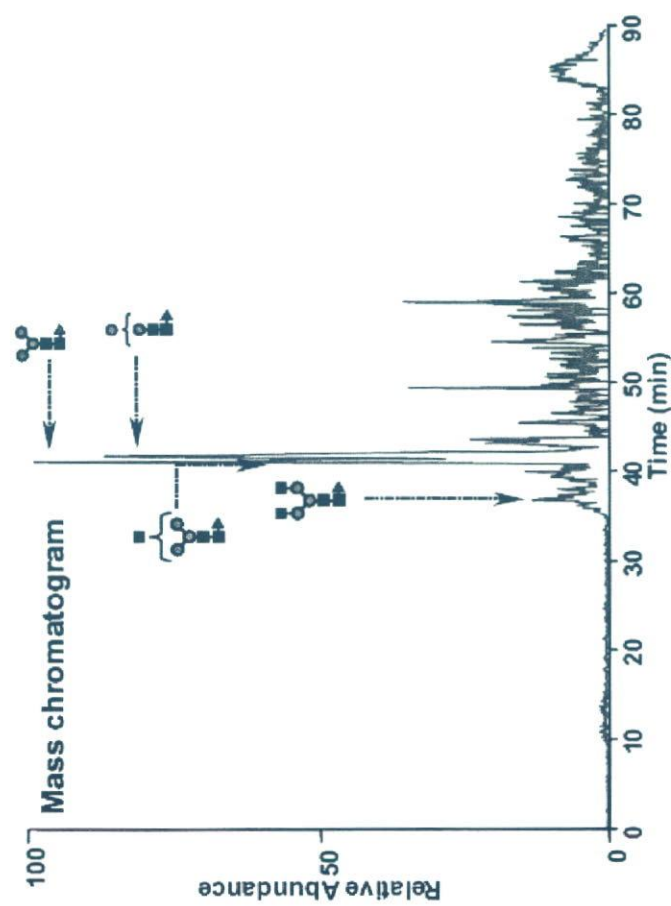


Fig.16 高マンノース型糖鎖の比較定量解析 (FCS vs HS)

(A)



(B)

変化した割合 (% FCS/HS)

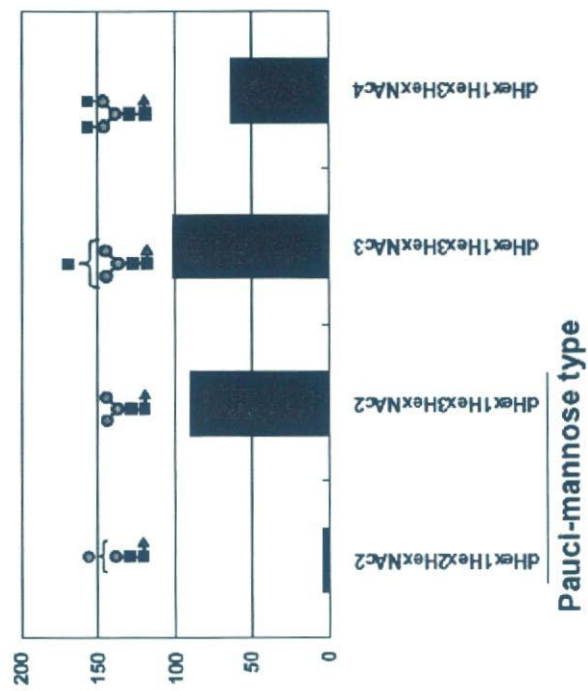


Fig.17 低分子量糖鎖の比較定量解析 (FCS vs HS)

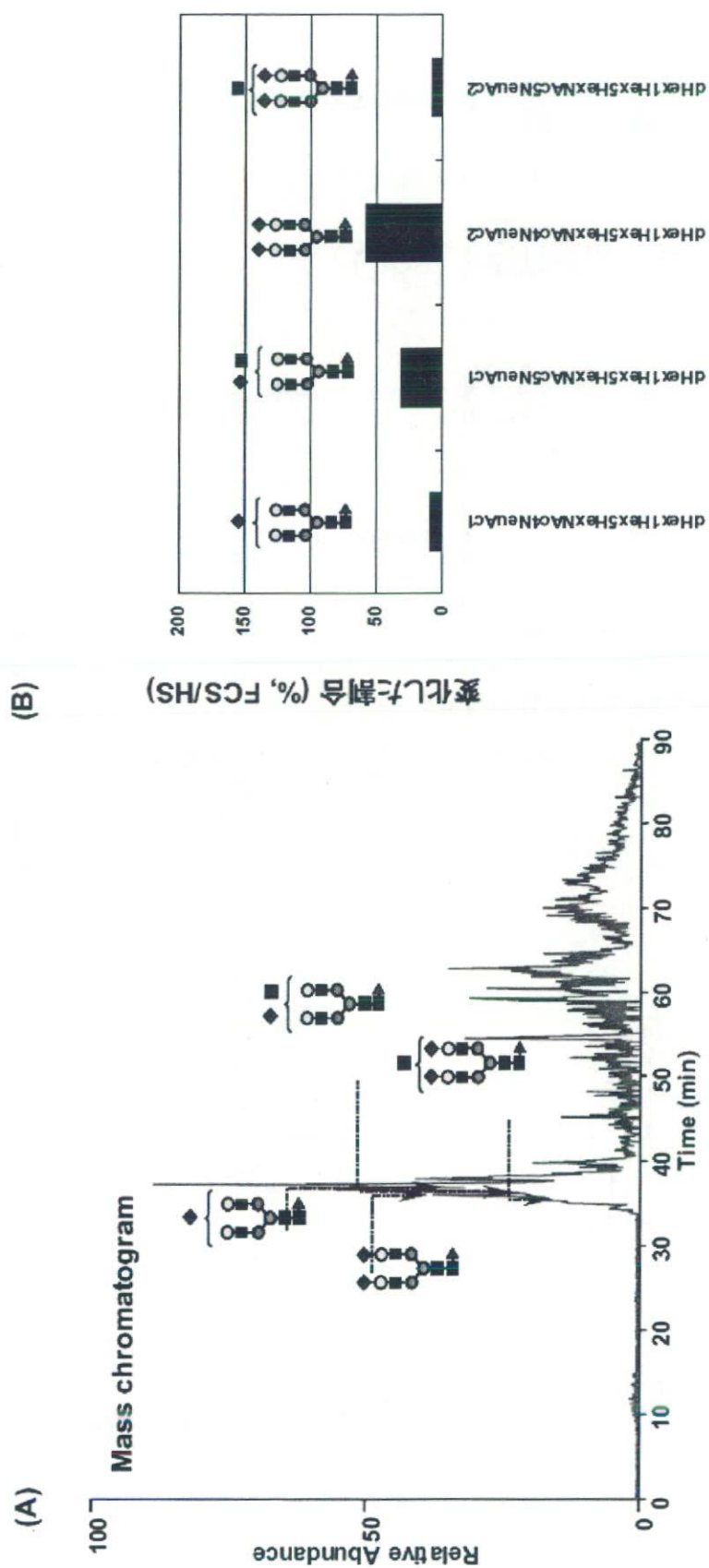


Fig.18 2本鎖糖鎖の比較定量解析 (FCS vs HS)

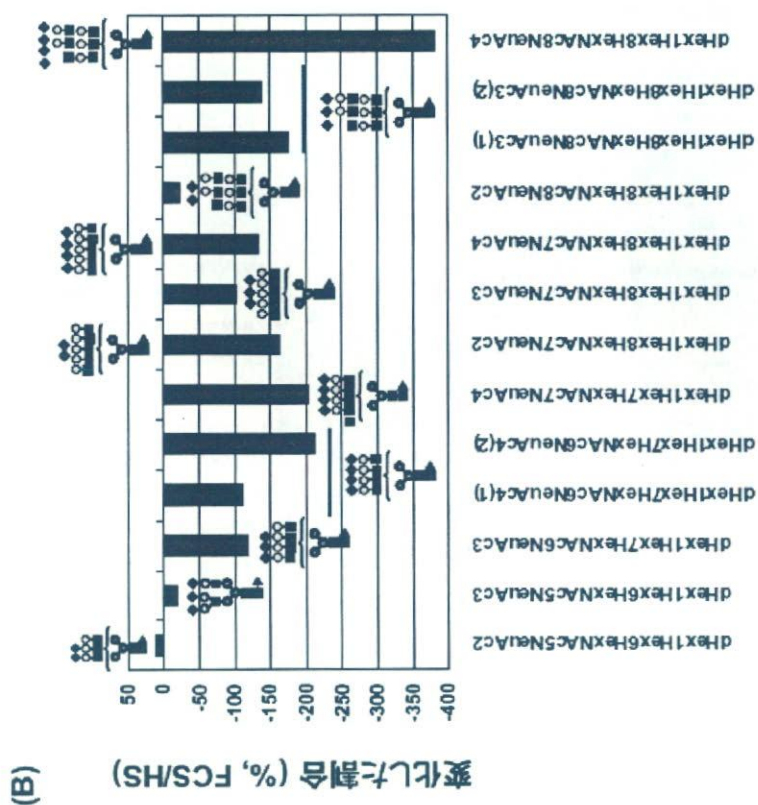
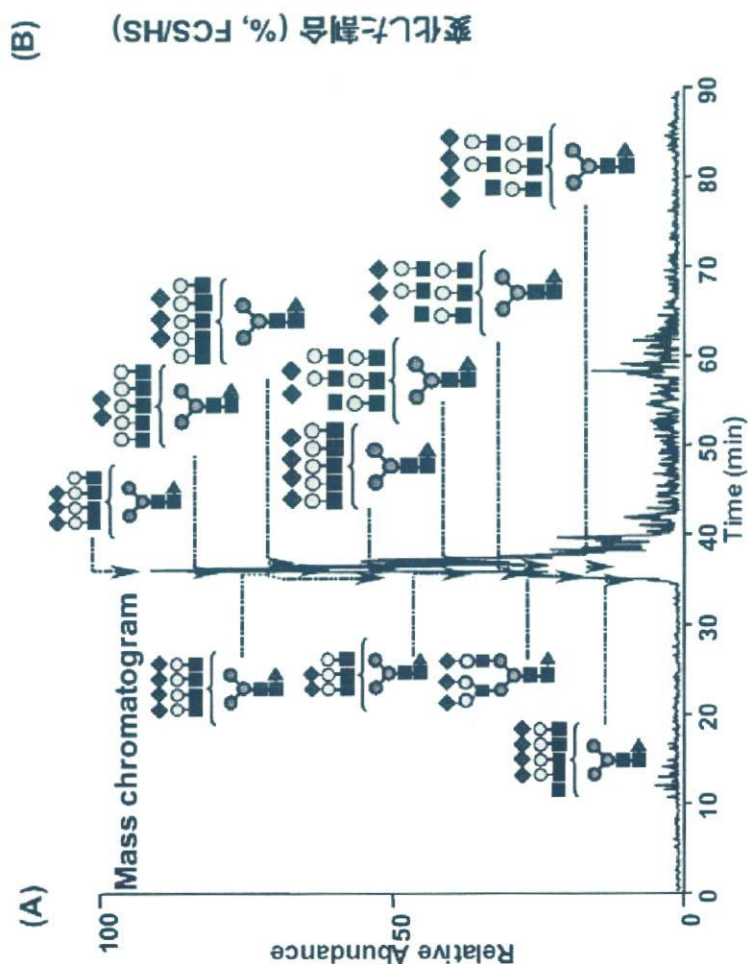


Fig.19 3及び4本鎖糖鎖の比較定量解析 (FCS vs HS)

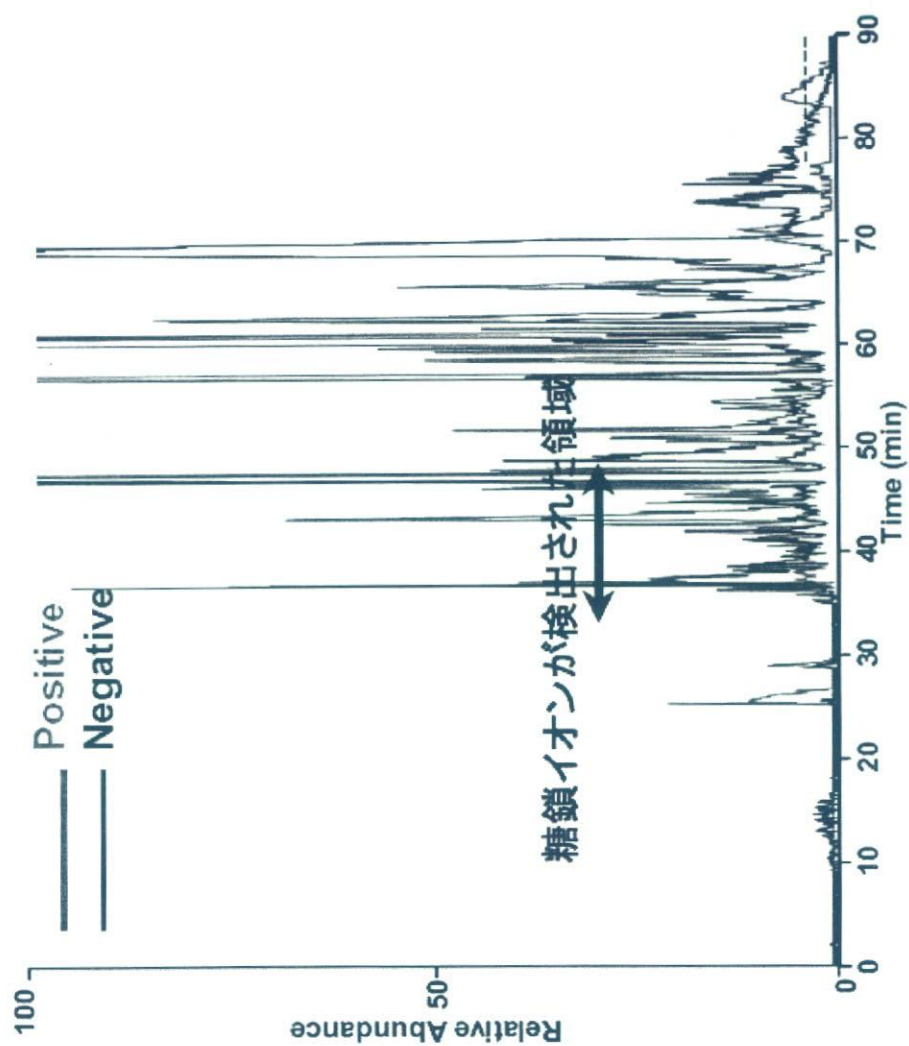
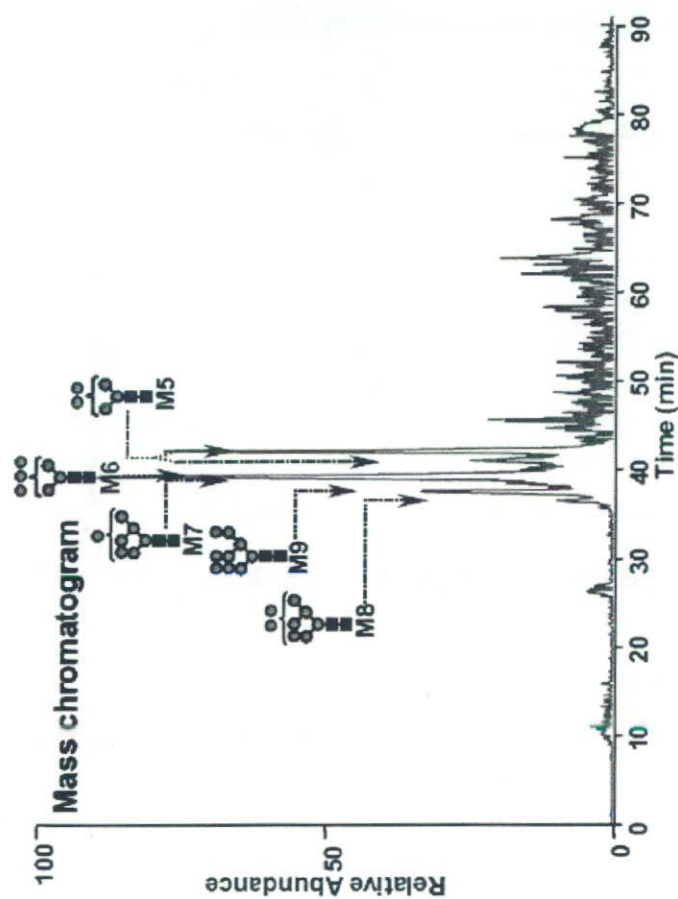


Fig.21 無血清培地及びHS添加培地で培養した細胞から調製した d_0 -PHN糖鎖及び d_5 -PHN-HS糖鎖混合試料のLC/MSにより得られたトータルイオンクロマトグラム (TIC)

(A)



(B)

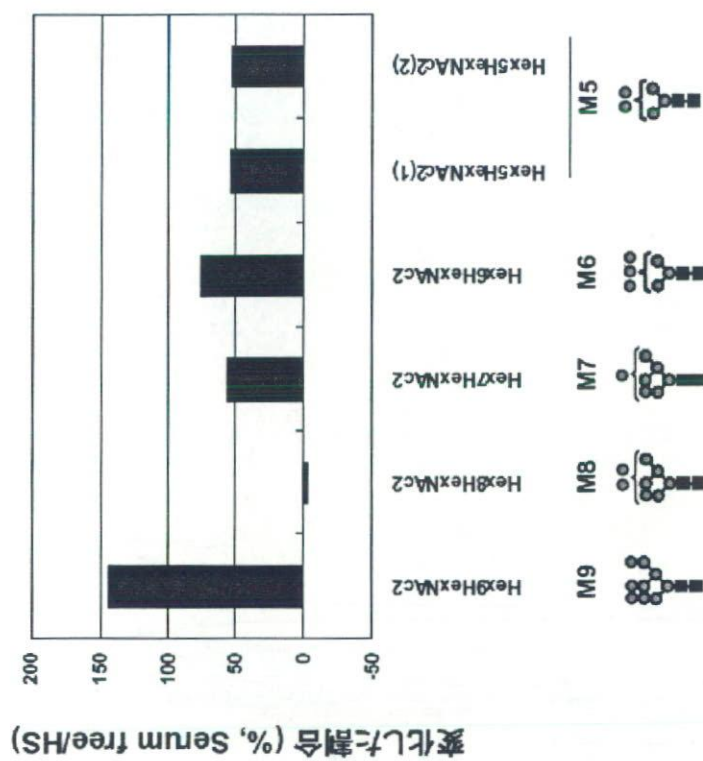
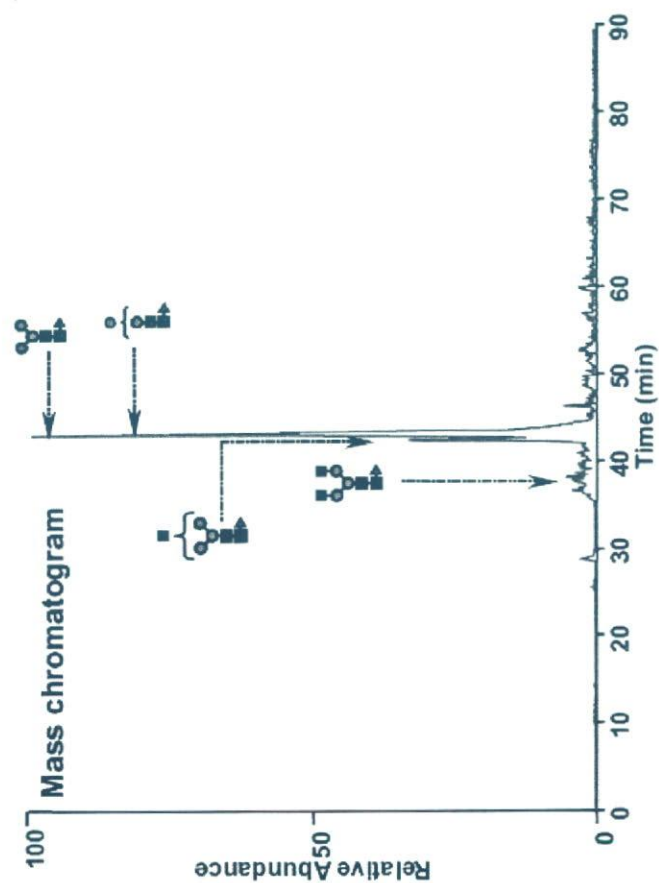


Fig.22 高マンノース型糖鎖の比較定量解析 (serum free vs HS)

(A)



(B)

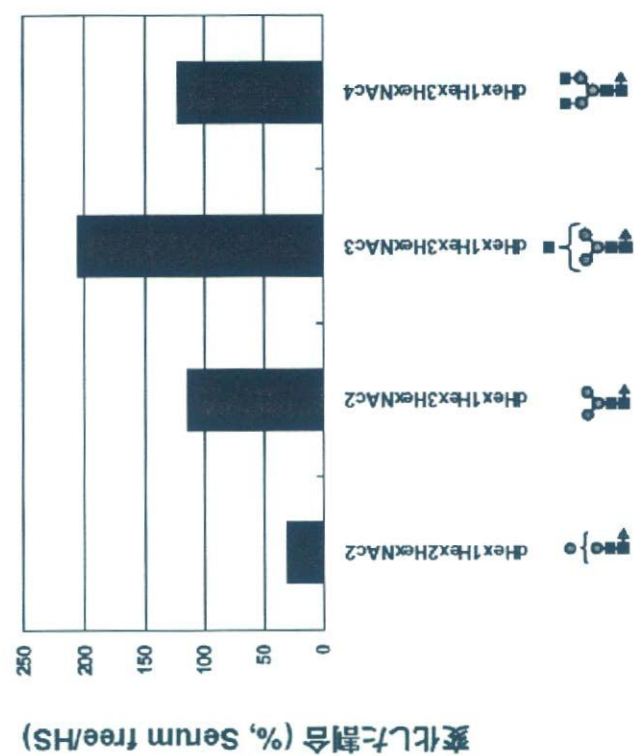
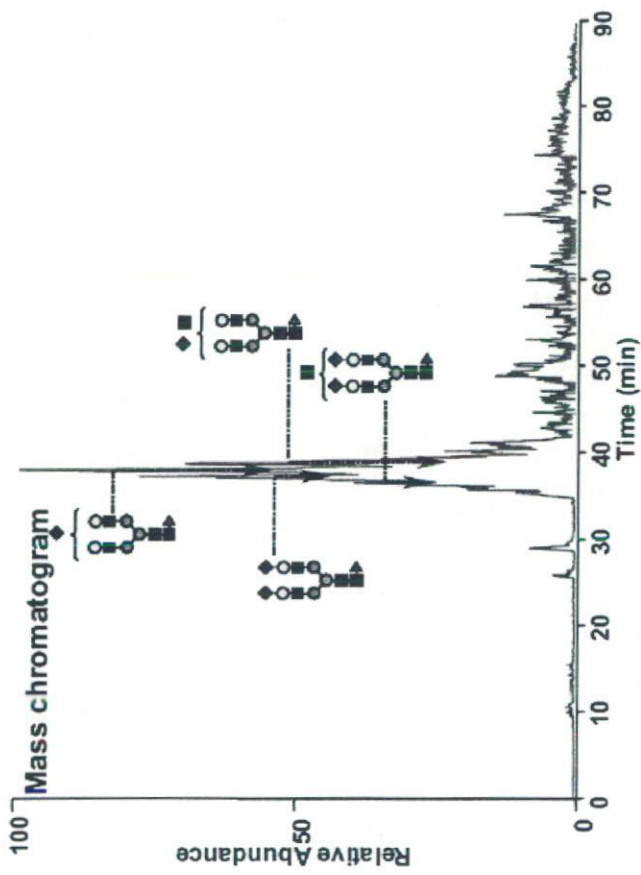


Fig.23 低分子量糖鎖の比較定量解析 (serum free vs HS)

(B)



変化した割合 (% , Serum free/HS)

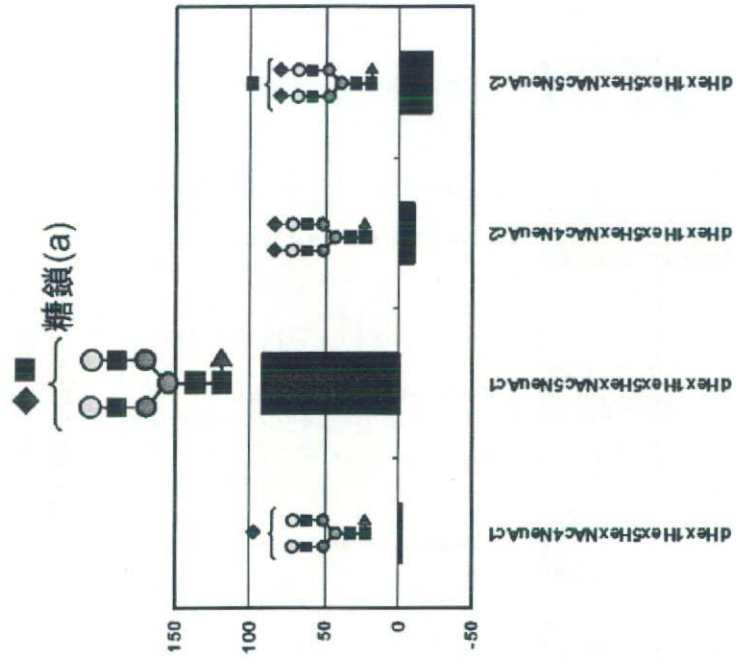


Fig.24 2本鎖糖鎖の比較定量解析 (serum free vs HS)

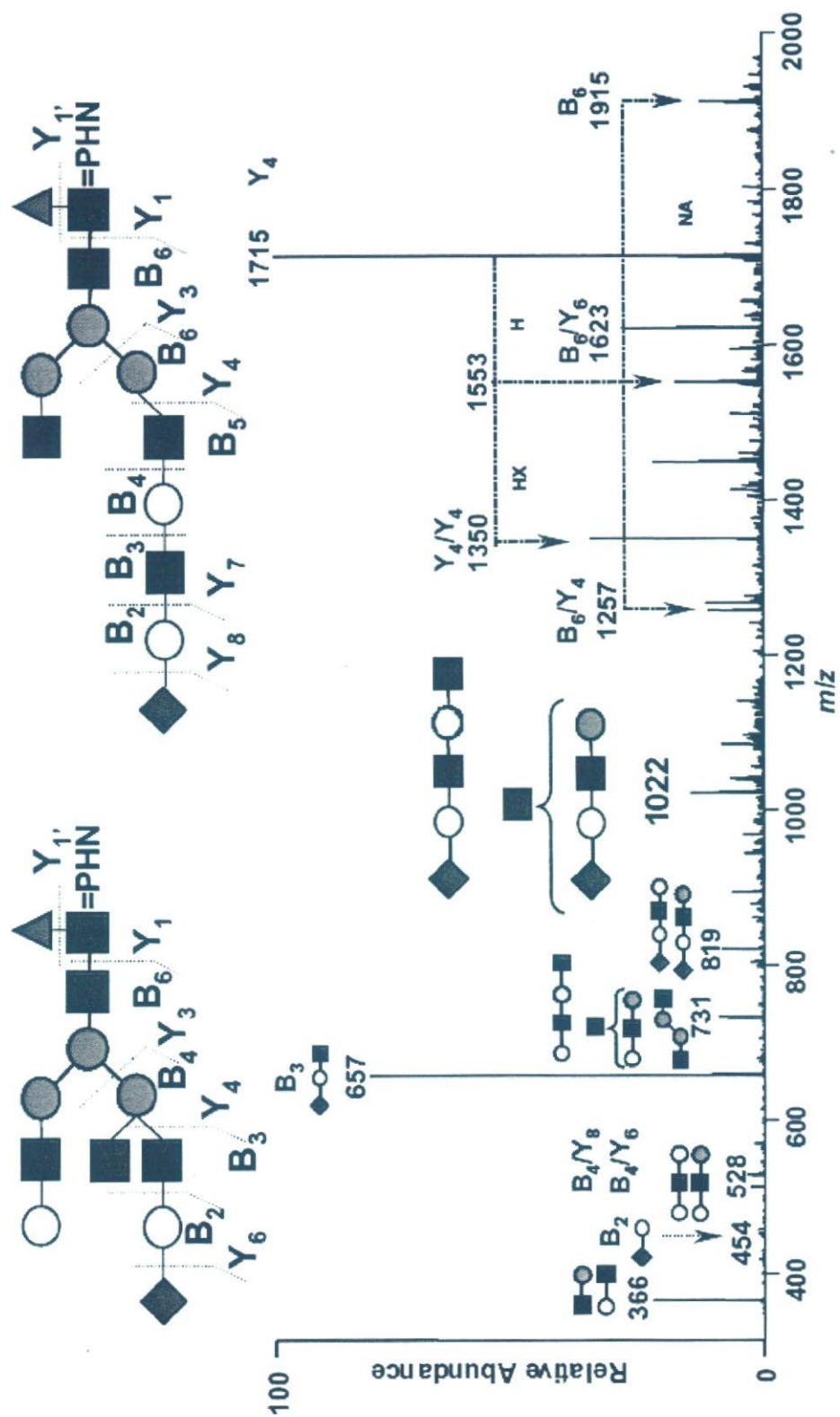


Fig.25 糖鎖(a) の MS/MS スペクトル

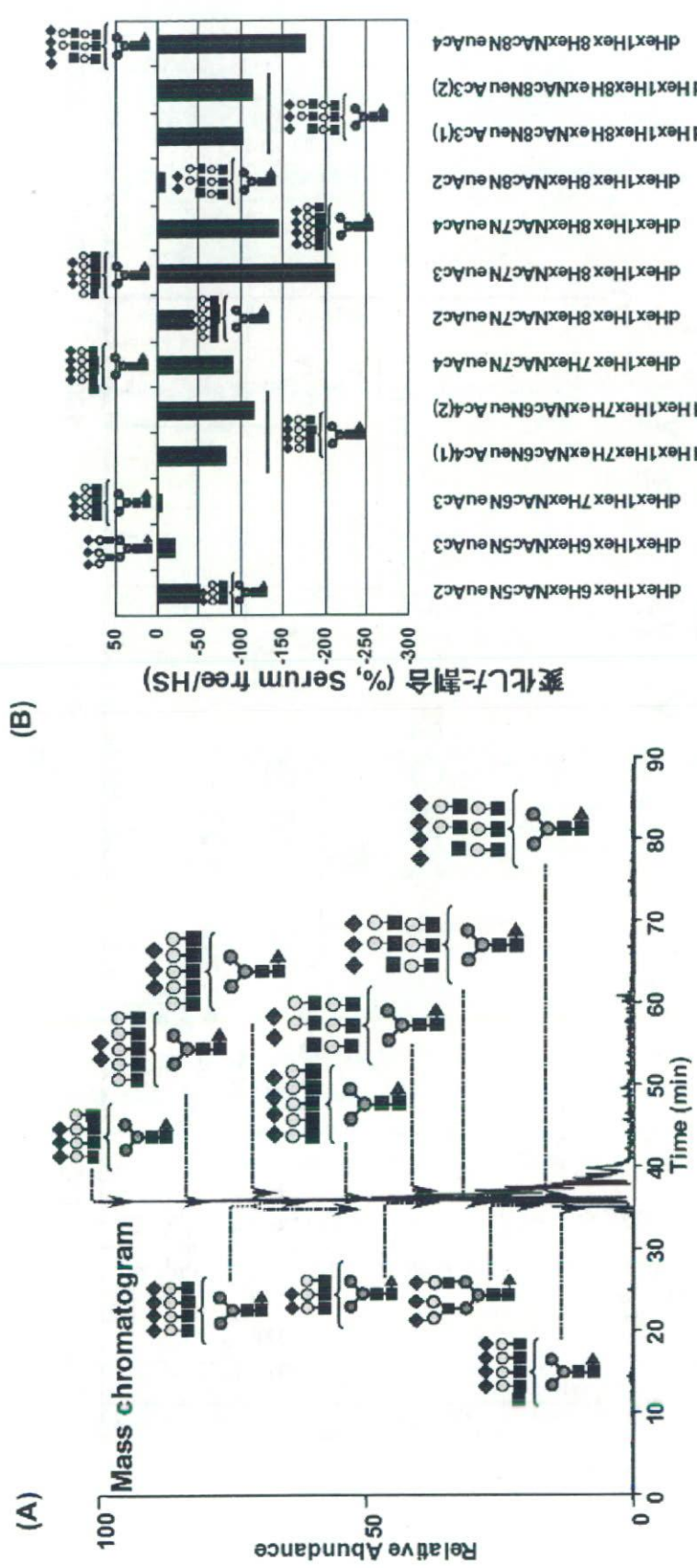


Fig.26 3及び4本鎖糖鎖の比較定量解析 (serum free vs HS)

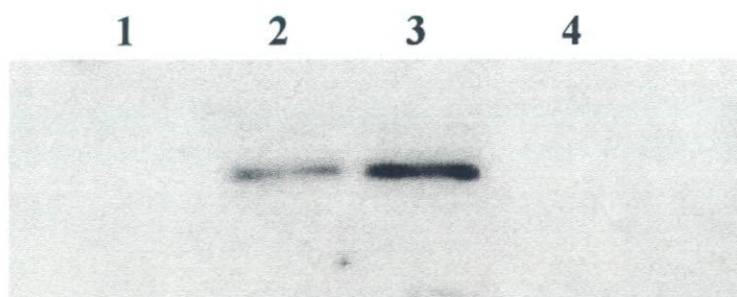


Fig.28 Expression of Bcl-xL and Bcl-FNK following Ad35 vector transduction in human bone-marrow CD34+ cells. Lane 1; mock, lane 2; Ad35-BclxL, lane 3; Ad35-BclFNK, lane 4; Ad35-GFP. The CD34+ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hr. Following a 48 hr-incubation, the cells were collected and expression of Bcl-xL and Bcl-FNK was assessed by Western blotting analysis. One representative experiment of three is shown.

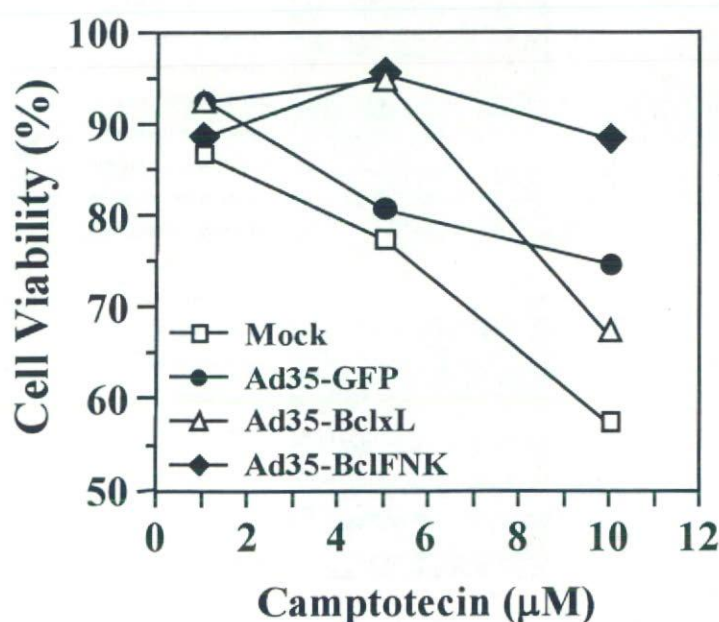


Fig.29 Survival of human bone-marrow CD34+ cells in the presence of Camptotecin. The CD34+ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hr. Following a 6 day-incubation, Camptotecin was added to the cells at the indicated concentrations, and incubated for 48 hrs. Viability of the cells was assessed by trypan blue exclusion assays. The results shown are mean of two experiments.

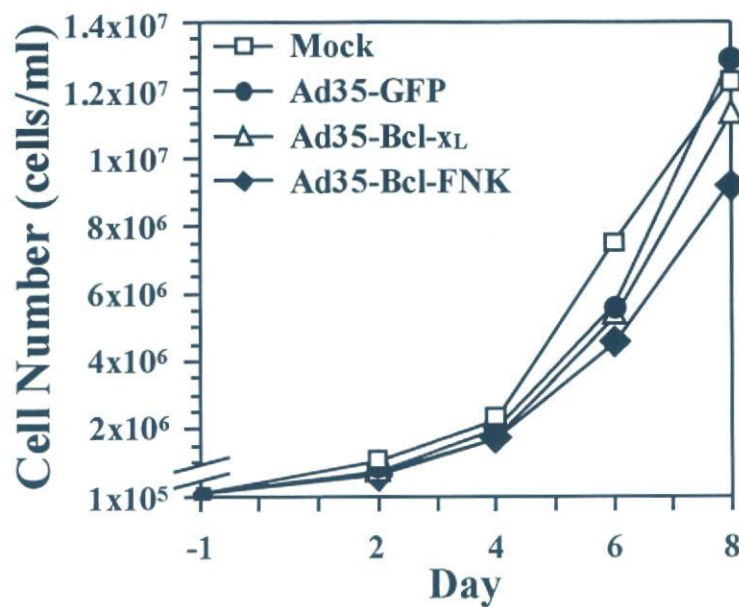


Fig.30 Growth ratio of human bone-marrow CD34⁺ cells following Ad35 vector Transduction. The CD34⁺ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hr. Cell numbers were measured at the indicated time points. The results shown are mean of 2 experiments.

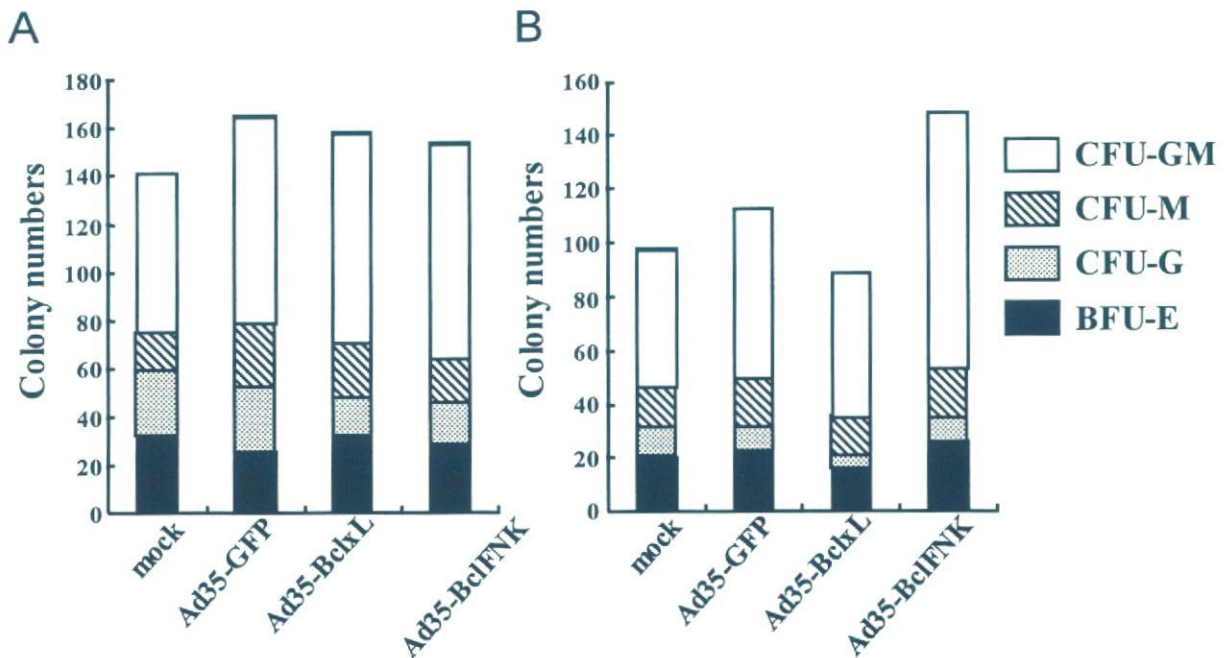


Fig.31 Colony forming assay of human bone-marrow CD34⁺ cells following Ad35 vector Transduction. The CD34⁺ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hr. The cells were collected and subjected to colony forming assay at (A) 2 days or 8 days after transduction. The results shown are the mean of 4 experiments .

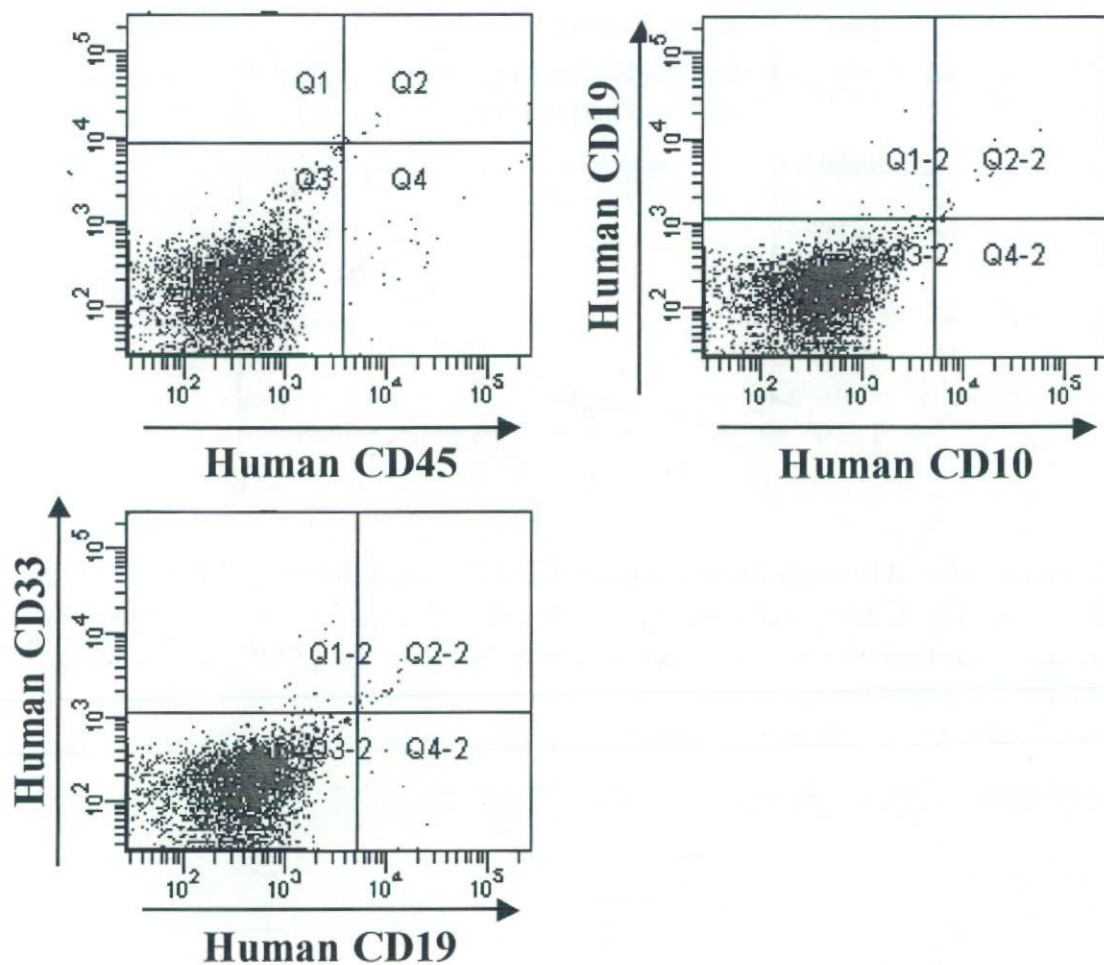


Fig.32 Analysis of human hematopoietic cells in NOG recipients. The CD34⁺ cells were transduced with Bcl-FNK-expressing Ad35 vectors at 6000 VP/cell for 6 hr. Following a 48 hr-incubation, 1×10^5 cells were transplanted into irradiated NOG mice. Bone marrow cells were recovered and analyzed using flowcytometry 20 weeks after transplantation.

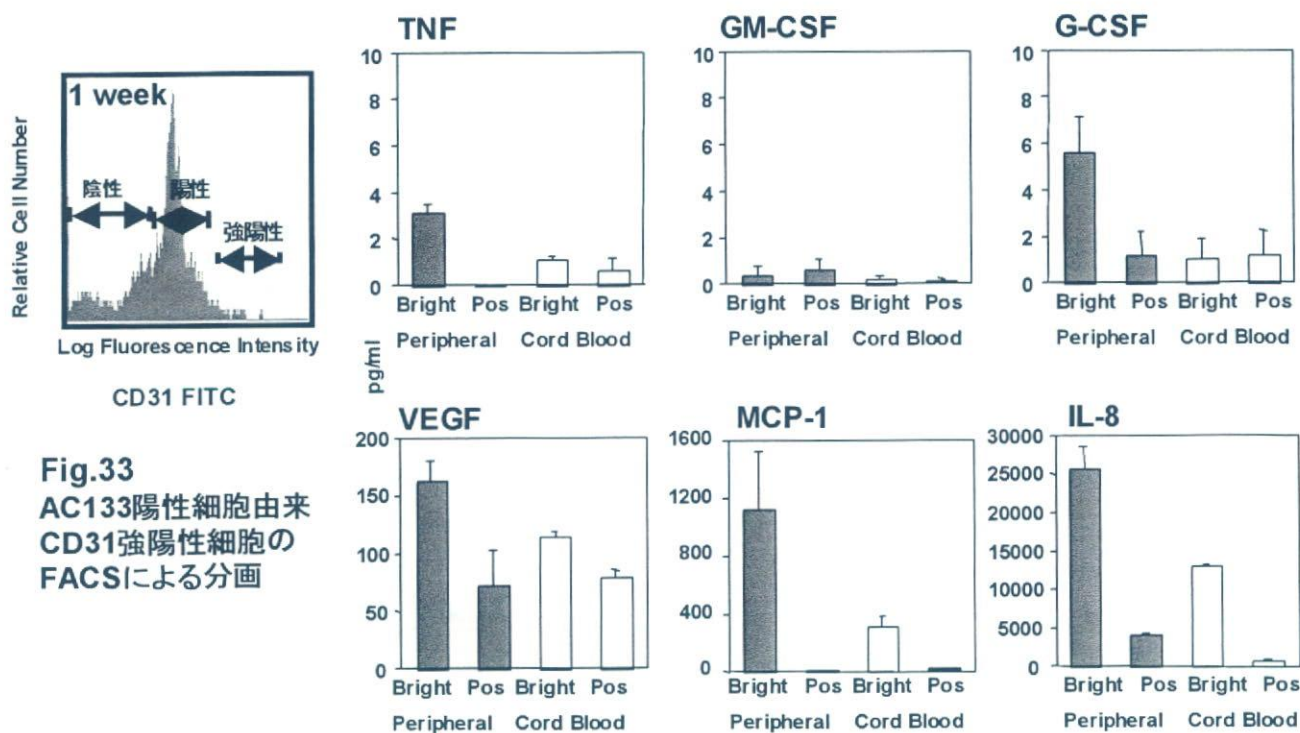


Fig.34 EPCが放出するサイトカインの解析

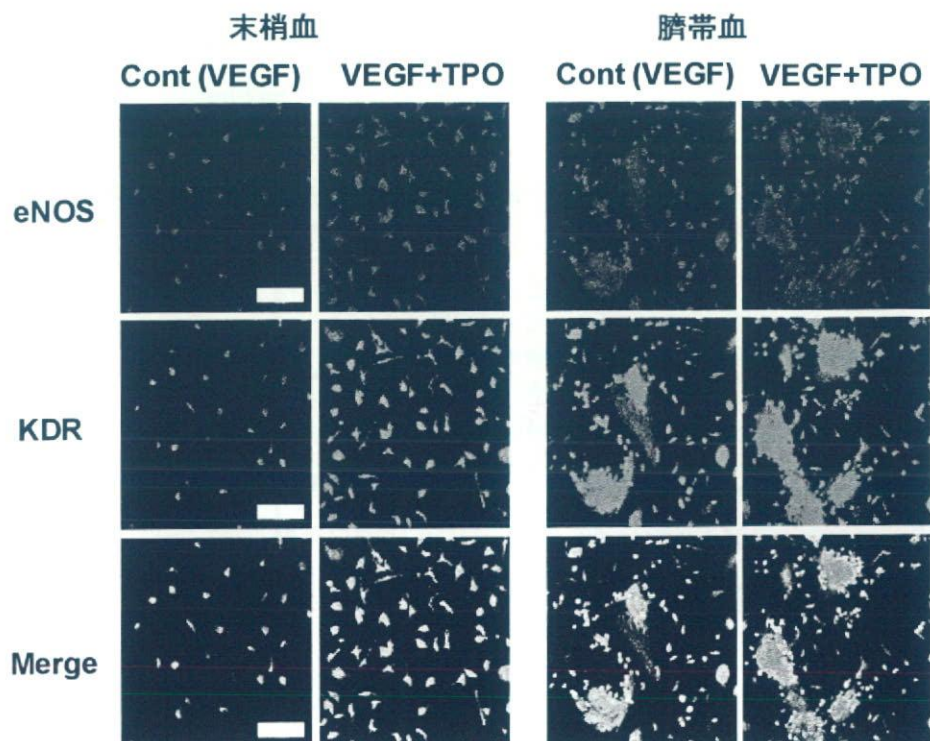


Fig.35 TPOのEPCの増幅作用