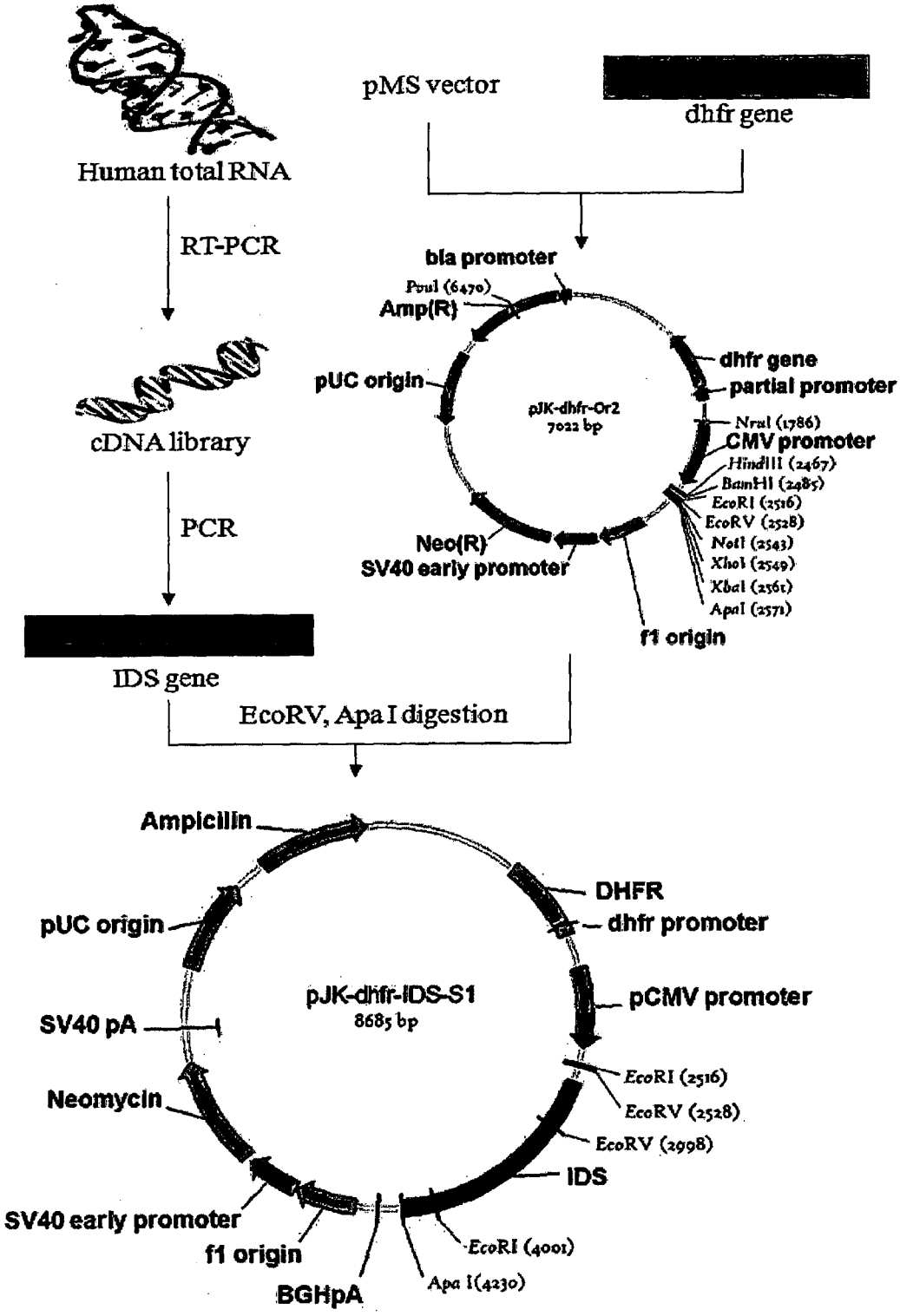
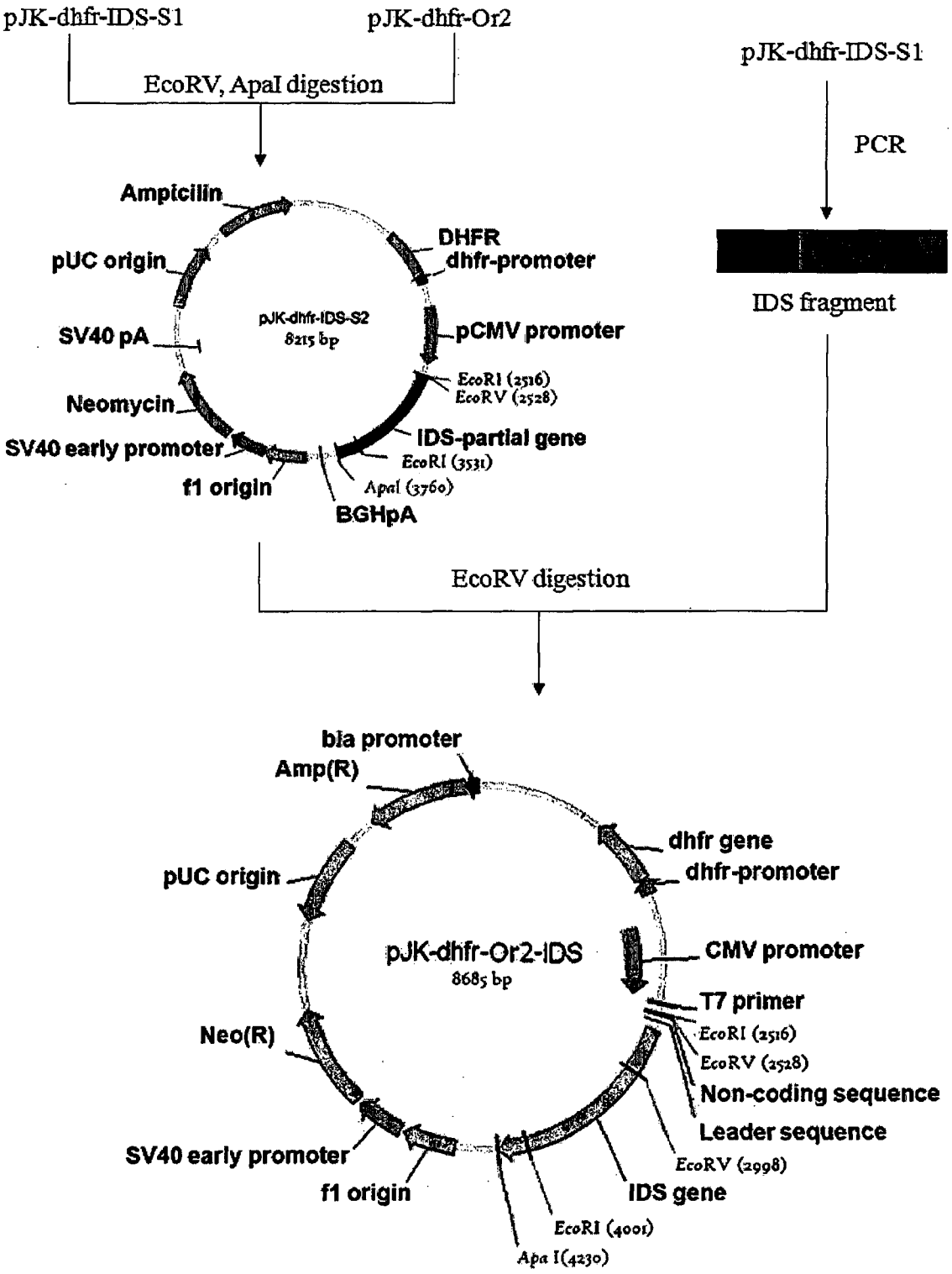
【DRAWINGS】

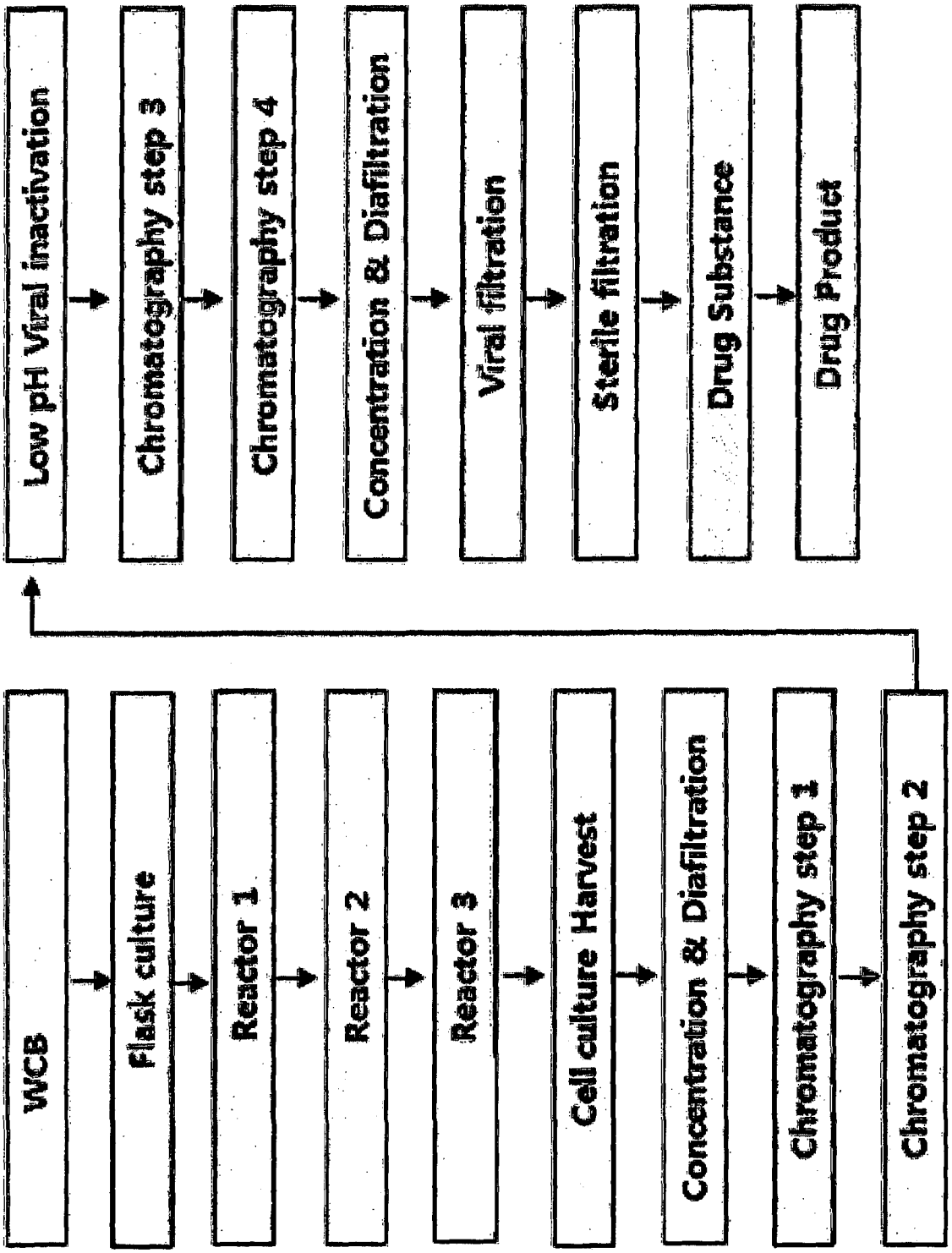
【Fig. 1】



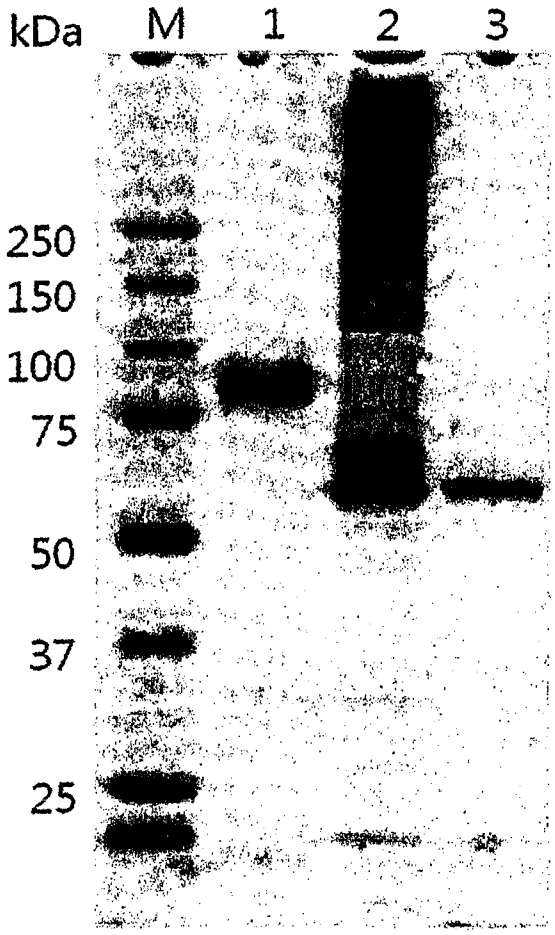
【Fig. 2】


pJK-dhfr-IDS-S1
pJK-dhfr-Or2
EcoRV, ApaI digestion
pJK-dhfr-IDS-S1
PCR
IDS fragment
Ampicilin
pUC origin
SV40 pA
Neomycin
SV40 early promoter
f1 origin
BGHpA
DHFR
dhfr-promoter
pCMV promoter
EcoRI (2516)
EcoRV (2528)
IDS-partial gene
EcoRI (3531)
ApaI (3760)
pJK-dhfr-IDS-S2
8215 bp
EcoRV digestion
bla promoter
Amp(R)
pUC origin
Neo(R)
SV40 early promoter
f1 origin
dhfr gene
dhfr-promoter
CMV promoter
T7 primer
EcoRI (2516)
EcoRV (2528)
Non-coding sequence
Leader sequence
EcoRV (2998)
IDS gene
EcoRI (4001)
Apa I(4230)
pJK-dhfr-Or2-IDS
8685 bp

[Fig. 3]


WCB
Flask culture
Reactor 1
Reactor 2
Reactor 3
Cell culture Harvest
Concentration & Diafiltration
Chromatography step 1
Chromatography step 2
Low pH Viral inactivation
Chromatography step 3
Chromatography step 4
Concentration & Diafiltration
Viral filtration
Sterile filtration
Drug Substance
Drug Product

[Fig. 4]



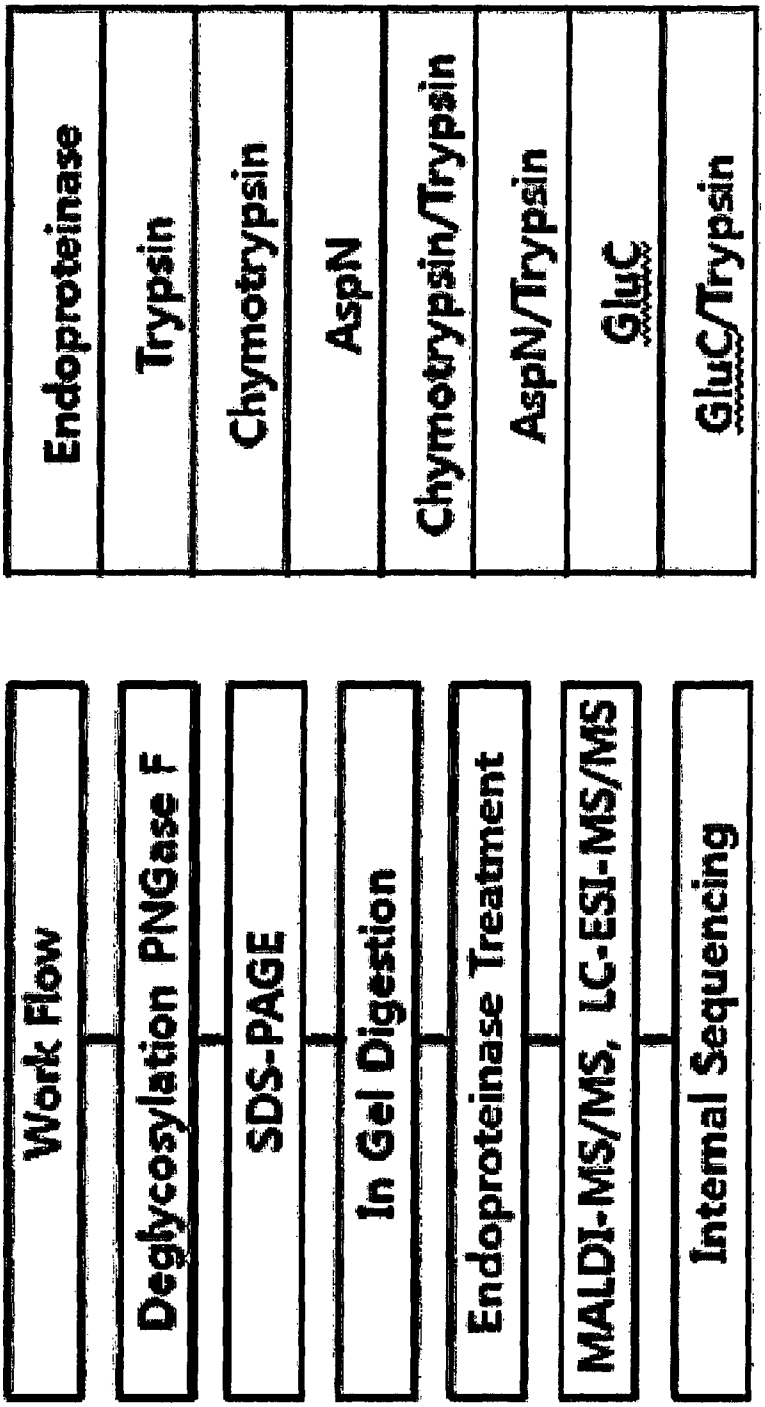
Lane M: Protein size marker

Lane 1: glycosylated IDS

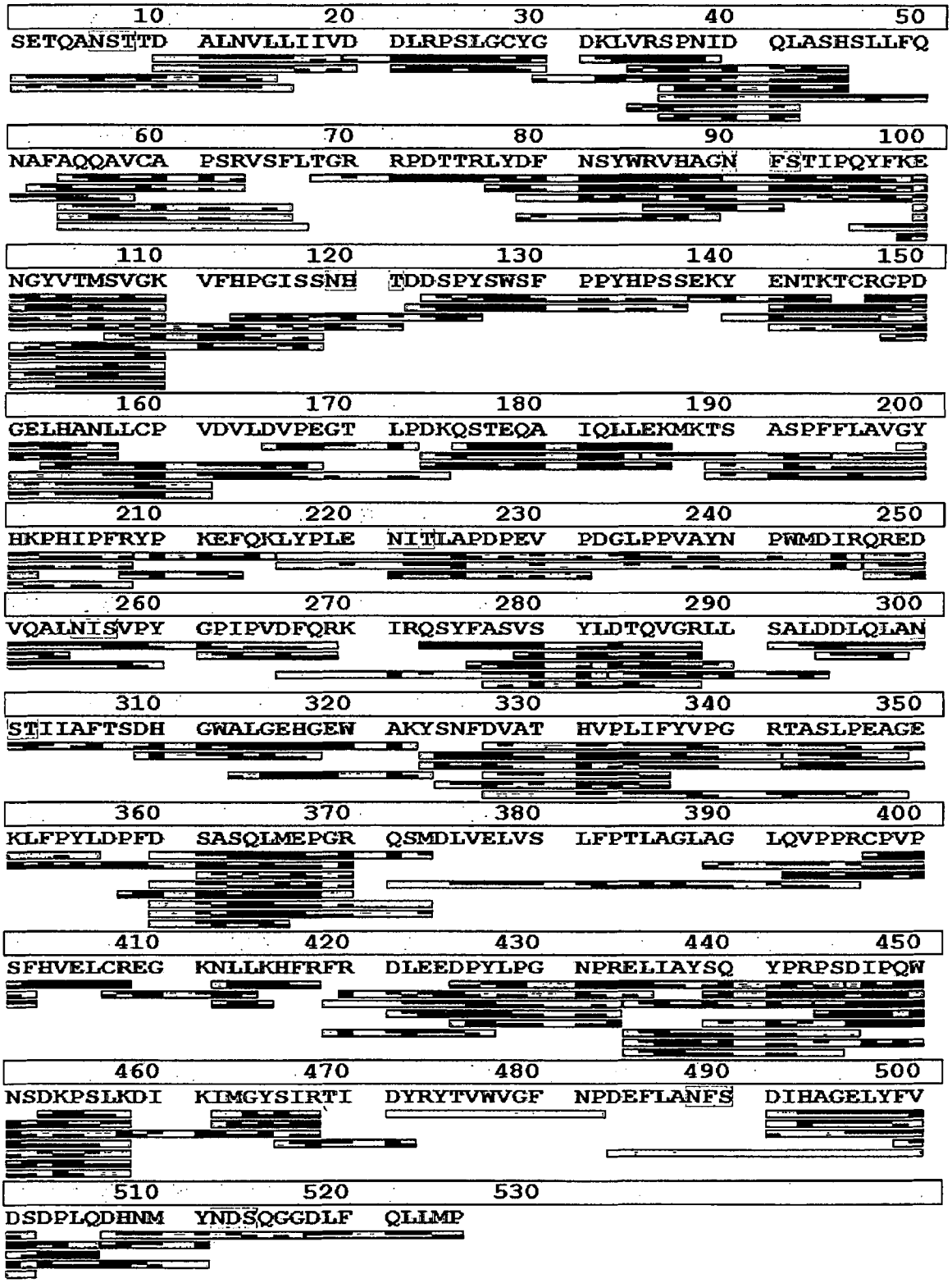
Lane 2: IDS treated with PNGase F

Lane 3: deglycosylated IDS

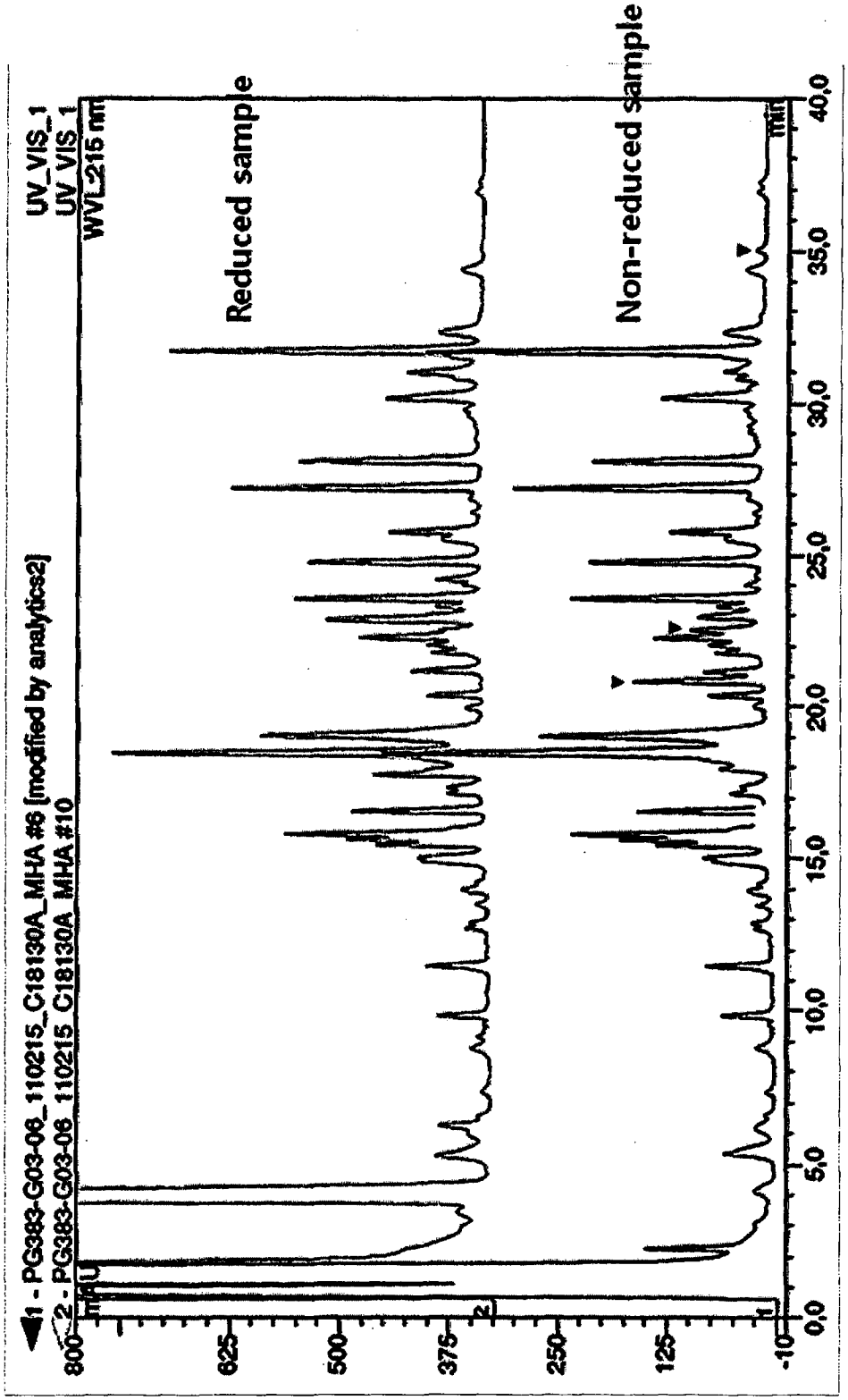
【Fig. 5】


Work Flow
Deglycosylation PNGase F
SDS-PAGE
In Gel Digestion
Endoproteinase Treatment
MALDI-MS/MS, LC-ESI-MS/MS
Intemal Sequencing
Endoproteinase
Trypsin
Chymotrypsin
AspN
Chymotrypsin/Trypsin
AspN/Trypsin
GluC
GluC/Trypsin

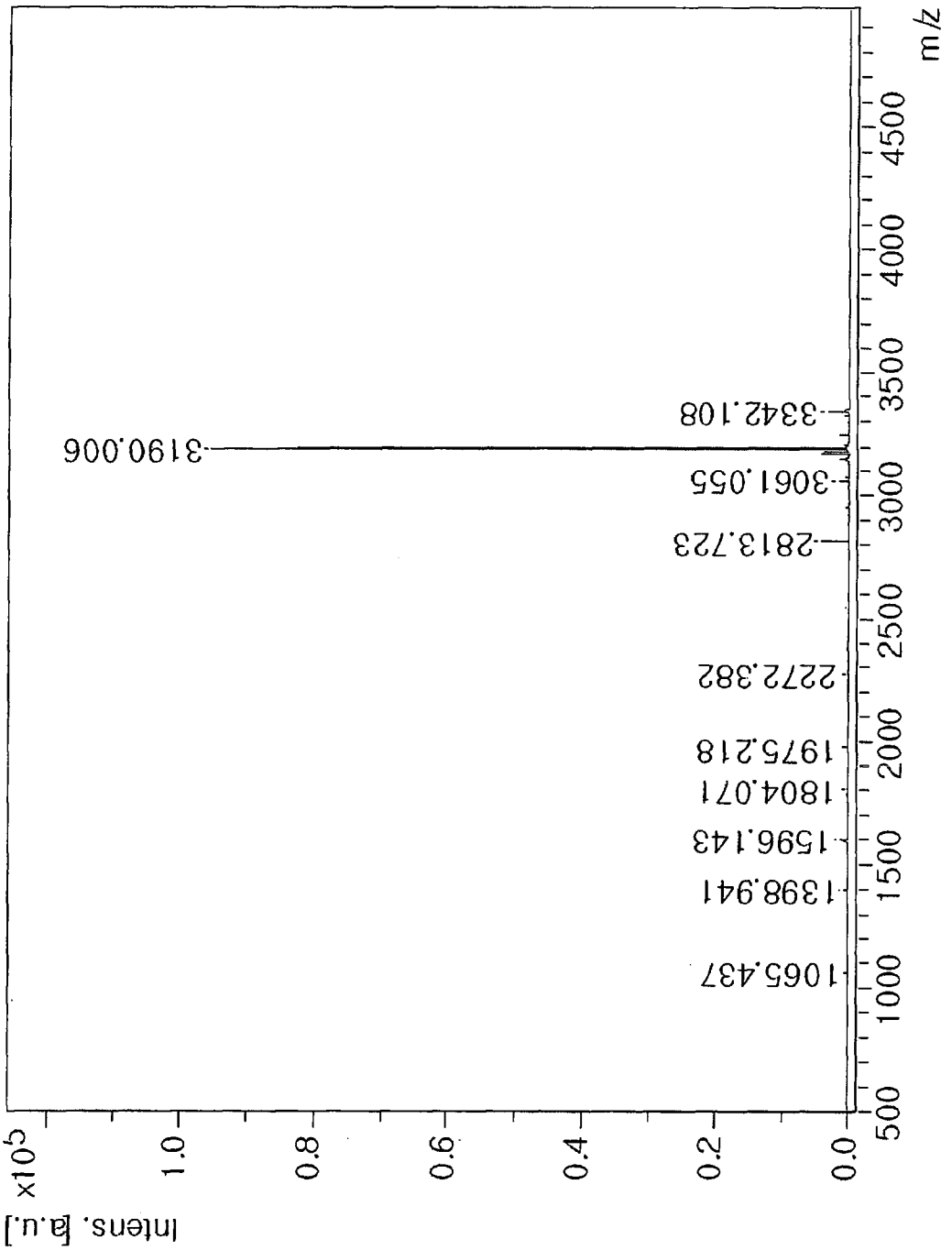
【Fig. 6】


10 20 30 40 50
SETQANSTTD ALNVLLIIVD DLRPSLGCYG DKLVRSPNID QLASHSLLFQ
60 70 80 90 100
NAFAQQAVCA PSRVSFLTGR RPDTTRLYDF NSYWRVHAGN FSTIPQYFKE
110 120 130 140 150
NGYVTMSVGK VFHPGISSNH TDDSPYSWSF PPYHPSSEKY ENTKTCRGPD
160 170 180 190 200
GELHANLLCP VDVLDVPEGT LPDKQSTEQA IQLLEKMKTS ASPFFLAVGY
210 220 230 240 250
HKPHIPFRYP KEFQKLYPLE NITLAPDPEV PDGLPPVAYN PWMDIRQRED
260 270 280 290 300
VQALNISVPY GPIPVDFQRK IRQSYFASVS YLDTQVGRLL SALDDLQLAN
310 320 330 340 350
STIIAFTSDH GWALGEHGEW AKYSNFDVAT HVPLIFYVPG RTASLPEAGE
360 370 380 390 400
KLFPYLDPFD SASQLMEPGR QSMDLVELVS LFPTLAGLAG LQVPPRCPVP
410 420 430 440 450
SFHVELCREG KNLLKHFRFR DLEEDPYLPG NPRELIAYSQ YPRPSDIPQW
460 470 480 490 500
NSDKPSLKDI KIMGYSIRTI DYRYTVWVGF NPDEFLANFS DIHAGELYFV
510 520 530
DSDPLQDHNM YNDSQGGDLF QLLMP

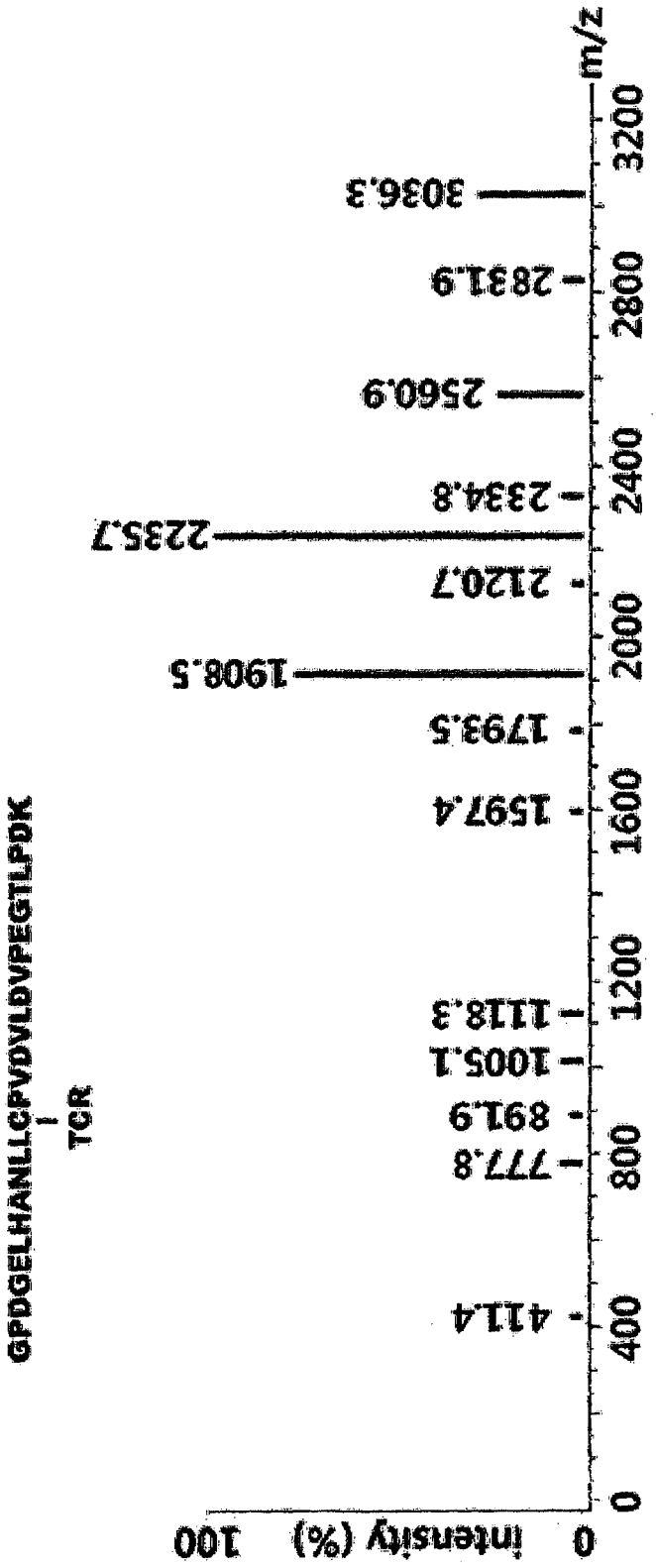
【Fig. 7】


Reduced sample
Non-reduced sample
1 - PG383-G03-06_110215_C18130A_MHA #6 [modified by analytics2]
2 - PG383-G03-06_110215 C18130A MHA #10
UV_VIS_1
UV_VIS_1
WVL:215 nm
mAU
min
800
625
500
375
250
125
-10
0,0
5,0
10,0
15,0
20,0
25,0
30,0
35,0
40,0

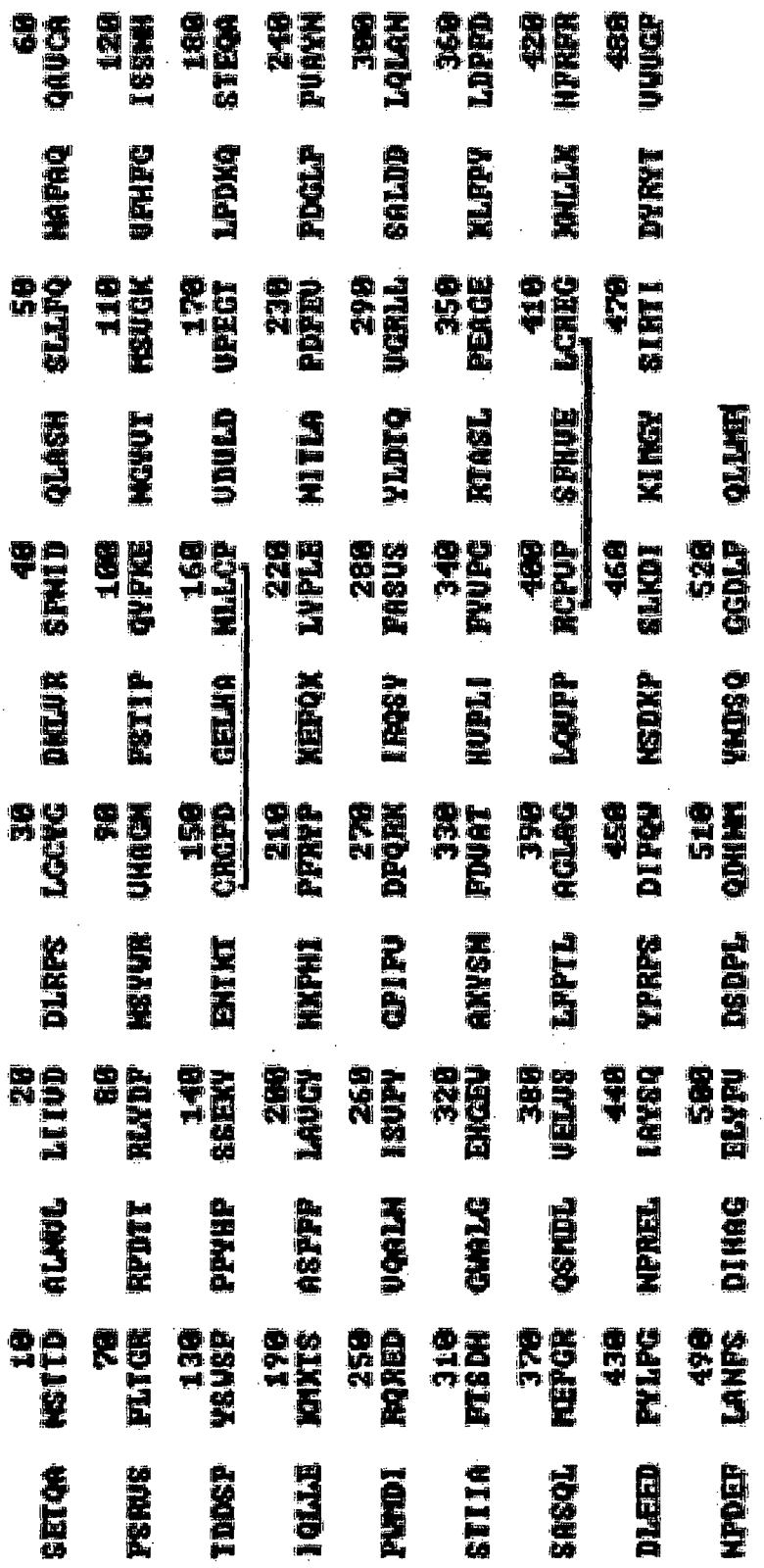
【Fig. 8】


Intens. [a.u.]
x10^5
1.0
0.8
0.6
0.4
0.2
0.0
500
1000
1500
2000
2500
3000
3500
4000
4500
m/z
1065.437
1398.941
1596.143
1804.071
1975.218
2272.382
2813.723
3061.055
3190.006
3342.108

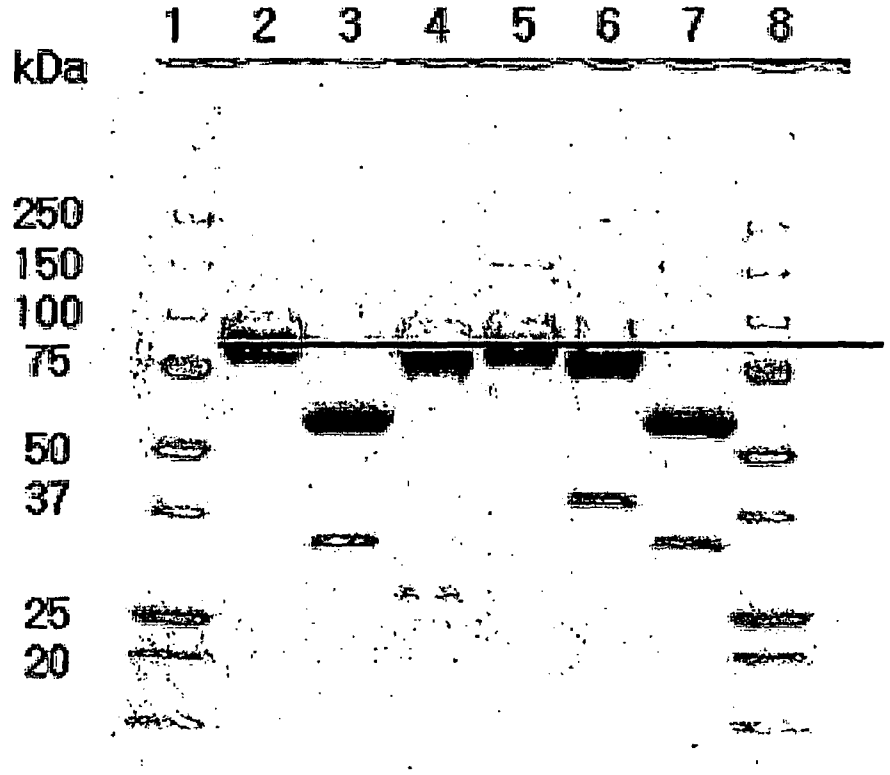
[Fig. 9]


m/z
3200
2800
2400
2000
1600
1200
800
400
0
3036.3
2831.9
2560.9
2334.8
2235.7
2120.7
1908.5
1793.5
1597.4
1118.3
1005.1
891.9
777.8
411.4
intensity (%)
100
0
GPDGELHANLLCPVDVLDVPEGTLPDK
TCR

【Fig. 10】

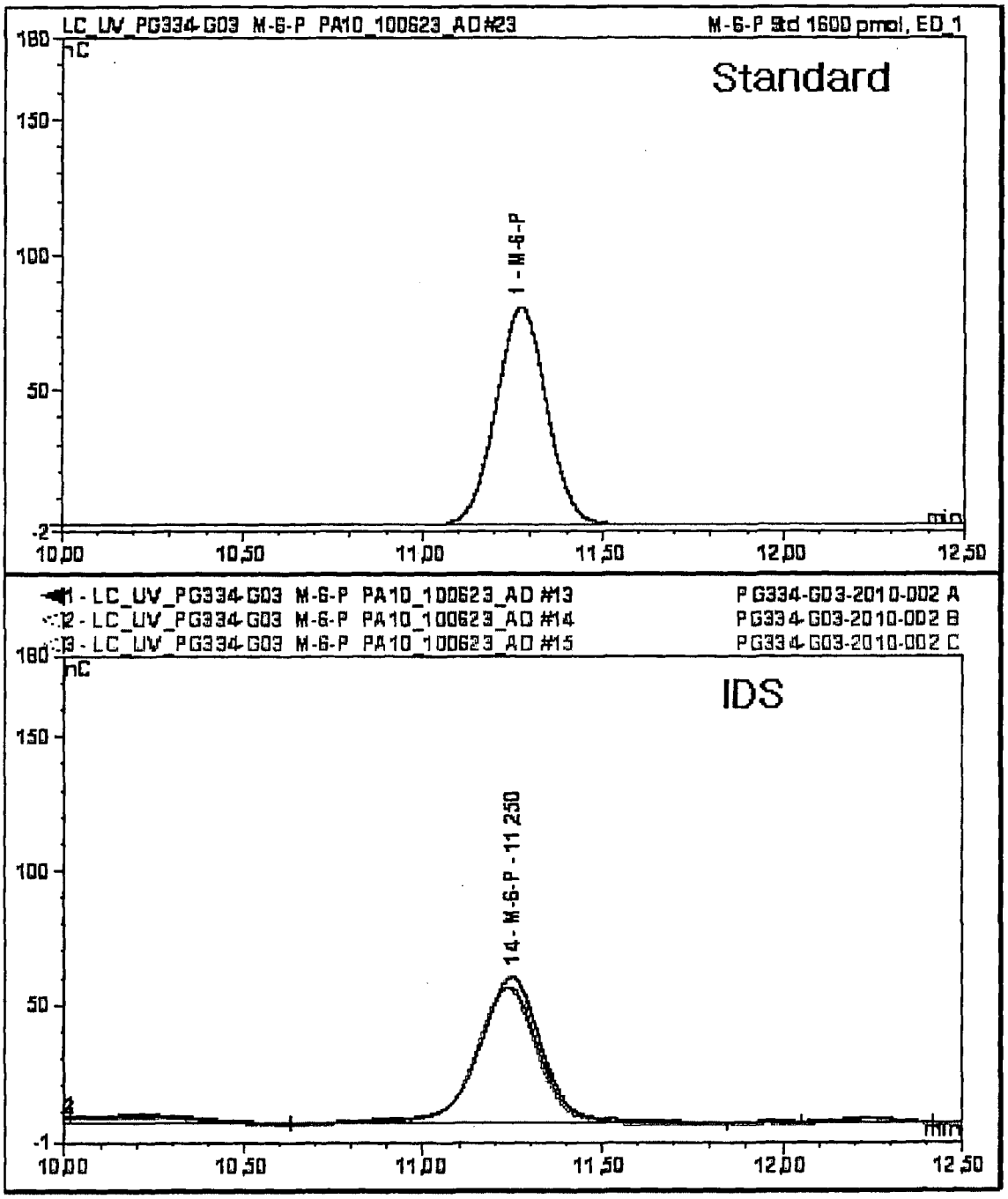

10 NSTTD AIAWVL 20 LIIVD DLRRFS 30 LCCHG DNEJVR 40 SFWID QLASH 50 SLLTQ 60 HAFRQ QAUCA
70 FLTGR RPDTTI 80 RLVDP HSYWR 90 UHRGM FSTIP 100 QVPKE HGHVT 110 HSUIGK UPHPG 120 ISSMH
130 YSUSP PPVHP 140 SSEKV ENTMT 150 CRGPD GELHA 160 HLLCP KLASH 170 UPEGT LPDKQ 180 STEQA
190 KDNTS ASFPP 200 LAUGV HMPHI 210 PPRKP KEPQK 220 LVPLE UDUUD 230 PDFEU FDGLP 240 PVAYN
250 RQRED VQRLH 260 ISUPV CPIPU 270 DPQRK IRQSY 280 FASUS NITLA 290 YLDTQ 300 VGRLL 310 LQLAH
310 FTSDH GWALG 320 EKGEU ANYSH 330 PDVAT HVPLI 340 PYVPG RTASL 350 PERGE 360 LDPFD
370 KEPGR QSNDL 380 VEEJS LFPTL 390 AGLAG LQUPP 400 RCPUP SFNVE 410 LGREG 420 HPREPR
430 PYLPC NPREL 440 LAYSQ YPRPS 450 DIPQW NSDXP 460 SLKDI KLNGY 470 SIHTI 480 DYRYT
490 LAWPS DIHAG 500 ELYPU DSDPL 510 QDHIM YHDSQ 520 GGDLP QLLMH VHUGP

【Fig. 11】

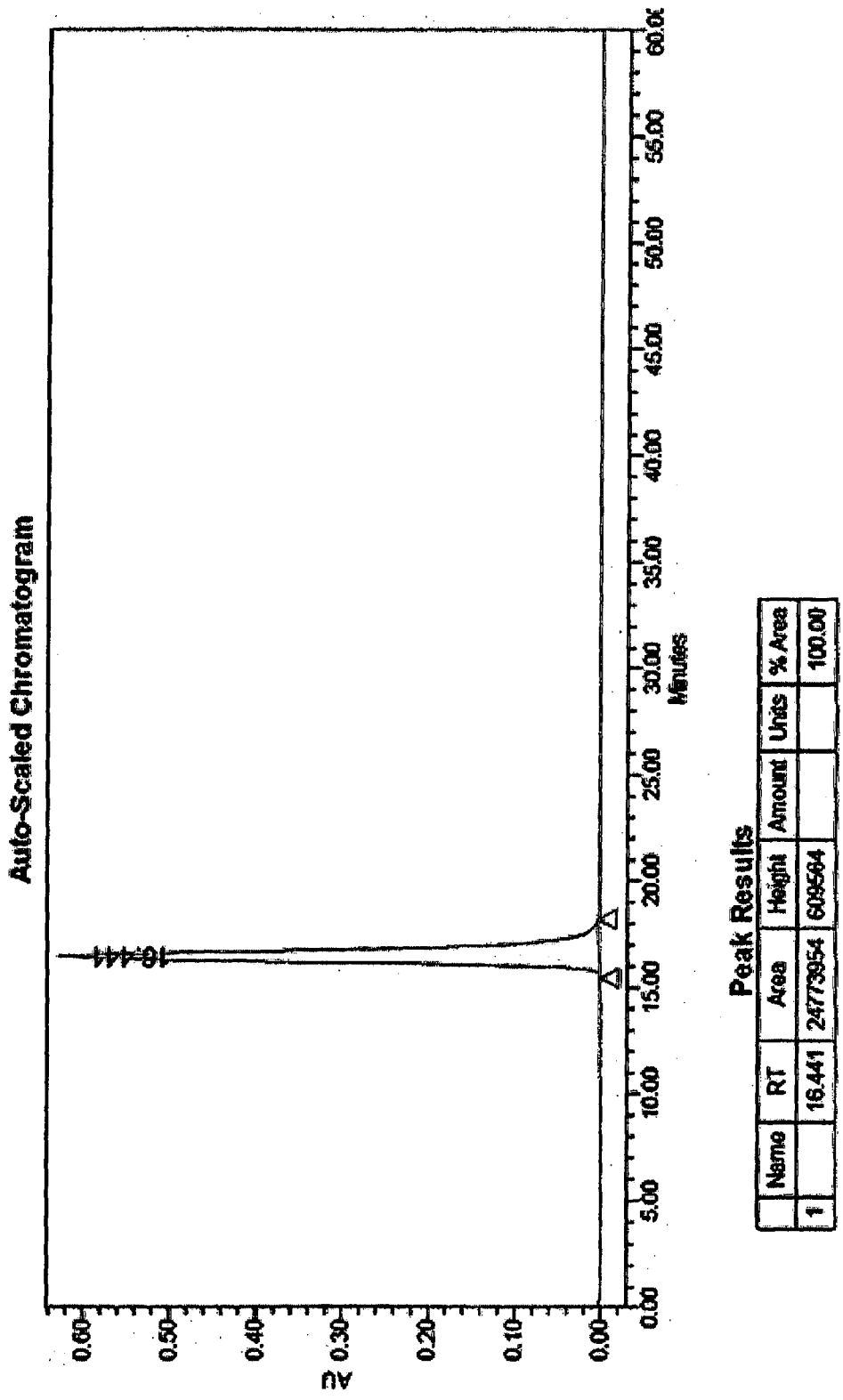


Lane 1, 8: Molecular size marker
Lane 2: IDS
Lane 3: IDS treated with PNGase F
Lane 4: IDS treated with Endo H
Lane 5: IDS treated with O-glycosidase
Lane 6: IDS treated with Sialidase
Lane 7: IDS treated with PNGase F and O-glycosidase

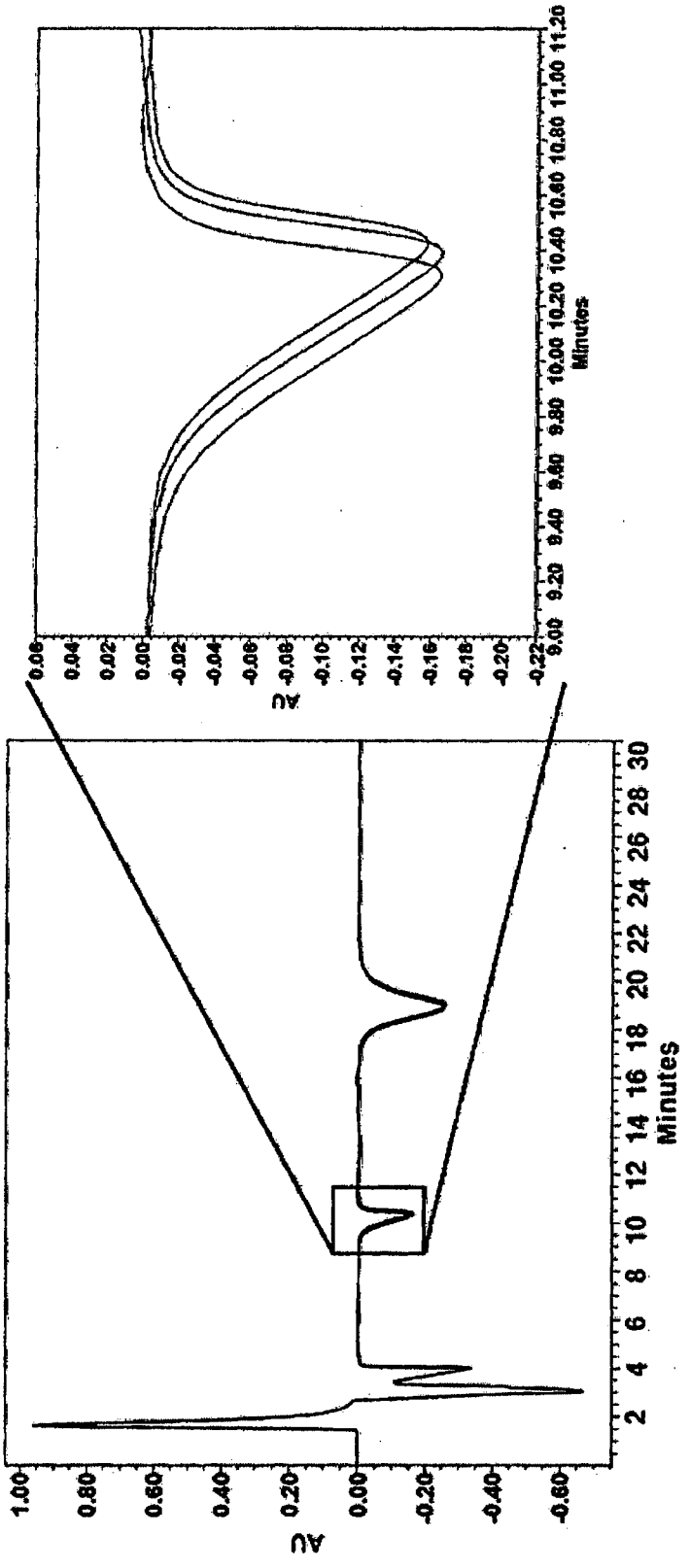
【Fig. 12】


LC_UV_PG334-G03 M-6-P PA10_100623_AD#23
M-6-P Std 1600 pmol, ED_1
Standard
1 - M-6-P
1 - LC_UV_PG334-G03 M-6-P PA10_100623_AD #13
2 - LC_UV_PG334-G03 M-6-P PA10_100623_AD #14
3 - LC_UV_PG334-G03 M-6-P PA10_100623_AD #15
PG334-G03-2010-002 A
PG334-G03-2010-002 B
PG334-G03-2010-002 C
IDS
14 - M-6-P - 11.250
nC
min

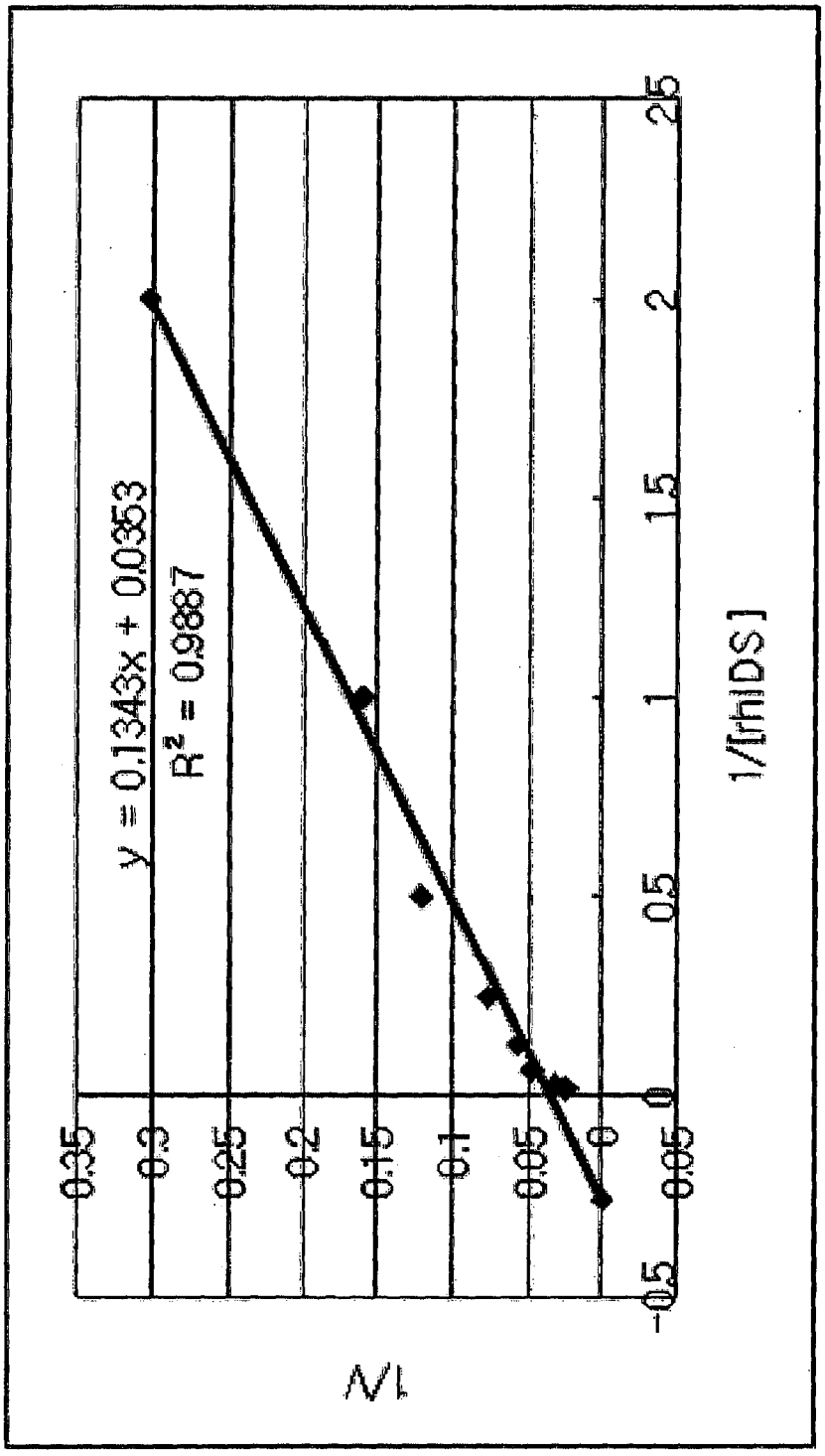
[Fig. 13]


Auto-Scaled Chromatogram
16.441
AU
Minutes
0.00
0.10
0.20
0.30
0.40
0.50
0.60
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Peak Results
Name
RT
Area
Height
Amount
Units
% Area
1
16.441
24773954
609564
100.00

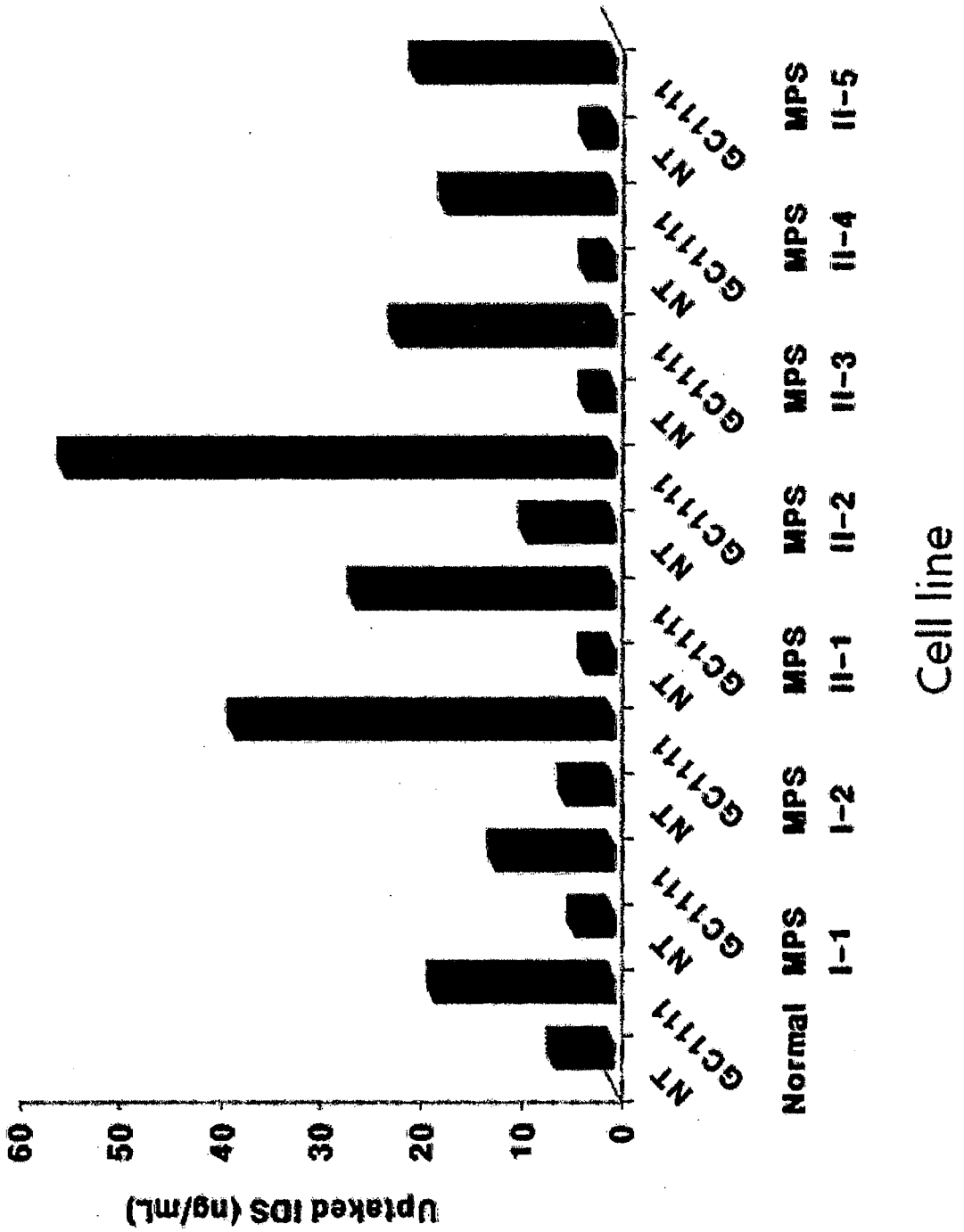
【Fig. 14】


AU
Minutes
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
AU
Minutes
0.06 0.04 0.02 0.00 -0.02 -0.04 -0.06 -0.08 -0.10 -0.12 -0.14 -0.16 -0.18 -0.20 -0.22
9.00 9.20 9.40 9.60 9.80 10.00 10.20 10.40 10.60 10.80 11.00 11.20

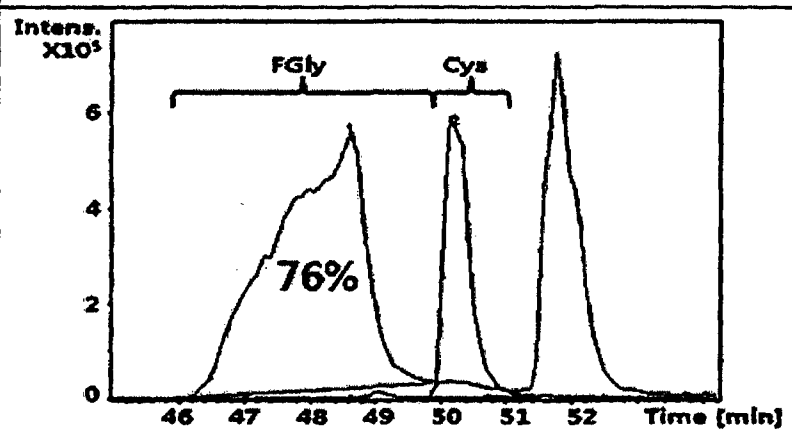
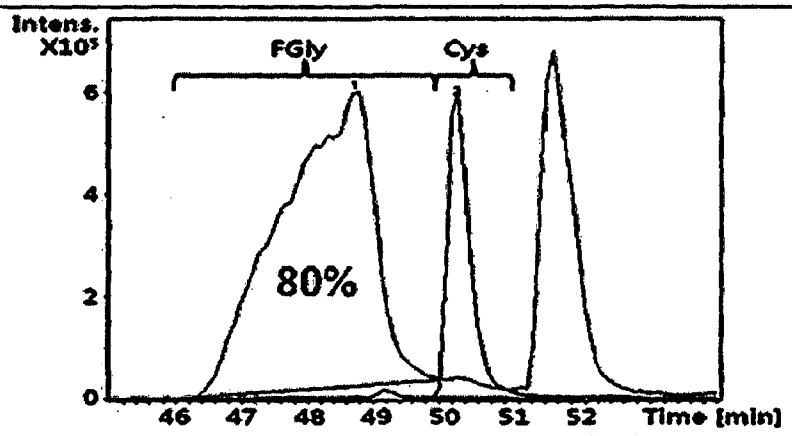
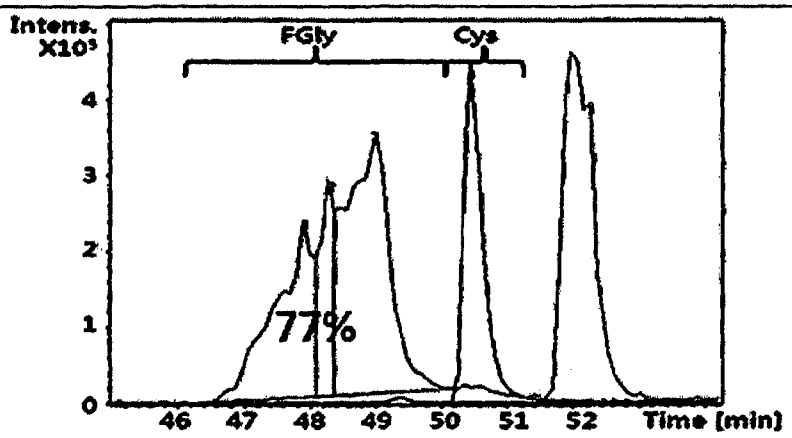
【Fig. 15】


y = 0.1343x + 0.0353
R² = 0.9887
1/[rhiDS]
VI
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.5
0.05
0.5
1
1.5
2
25

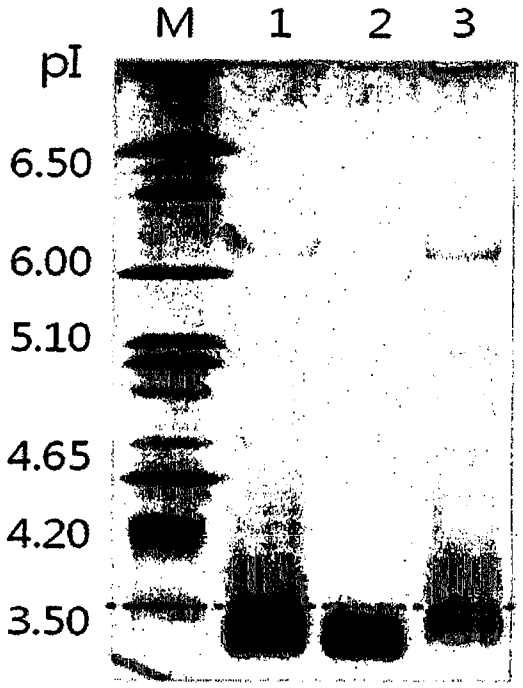
【Fig. 16】


Uptaked IDS (ng/mL)
60
50
40
30
20
10
0
Normal
MPS I-1
MPS I-2
MPS II-1
MPS II-2
MPS II-3
MPS II-4
MPS II-5
NT
GC1111
Cell line

【Fig. 17】

No. of Analysis	GC1111
1	
2	
3	
FGly content mean	78 ± 2%

【Fig. 18】



Lane M: size marker

Lane 1: a loaded sample for cation exchange chromatography

Lane 2: an eluate of cation exchange chromatography

Lane 3: a regeneration solution after cation exchange chromatography