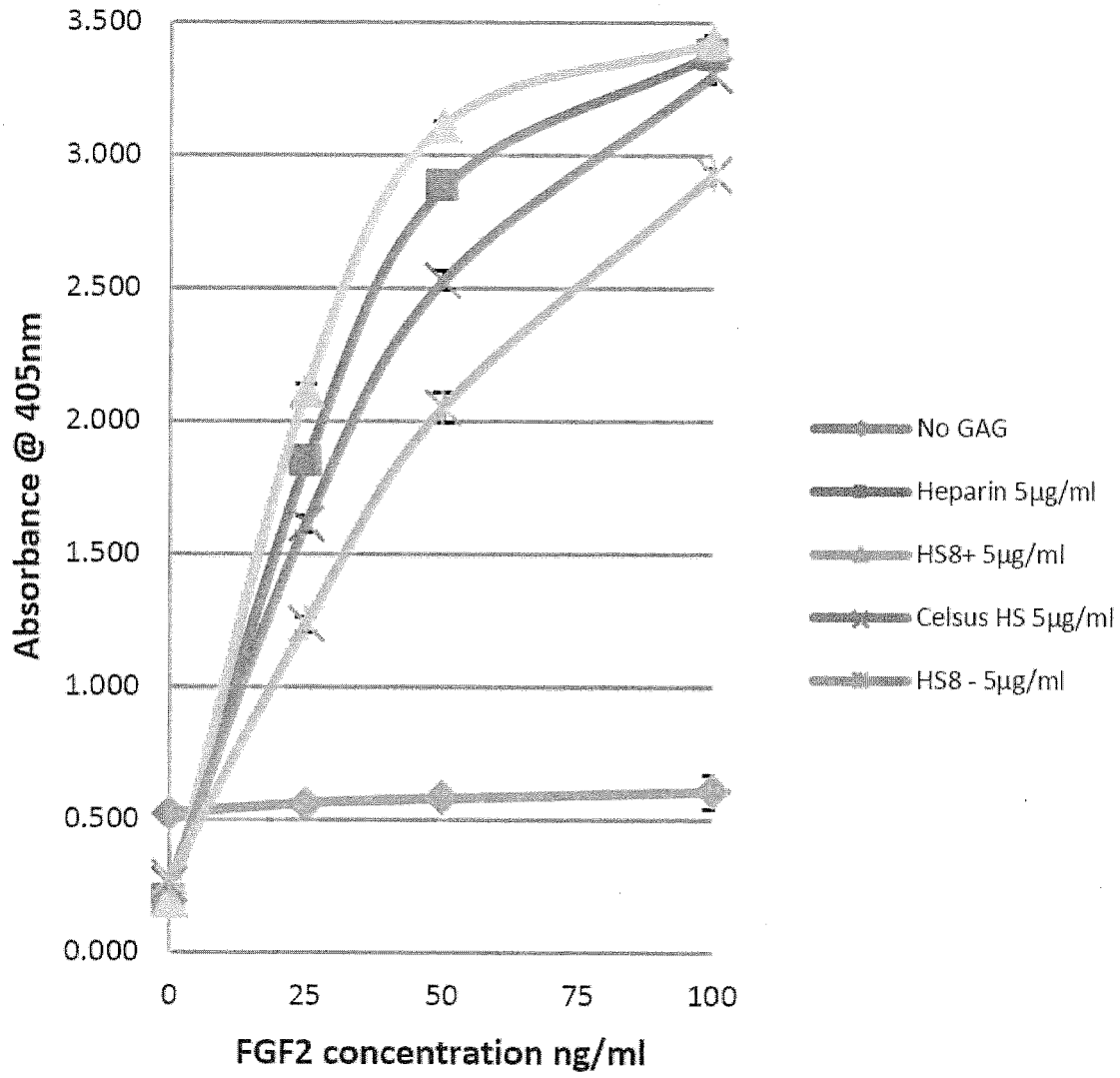

3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
No GAG
Heparin 5µg/ml
HS8+ 5µg/ml
Celsus HS 5µg/ml
HS8 - 5µg/ml

Figure 1

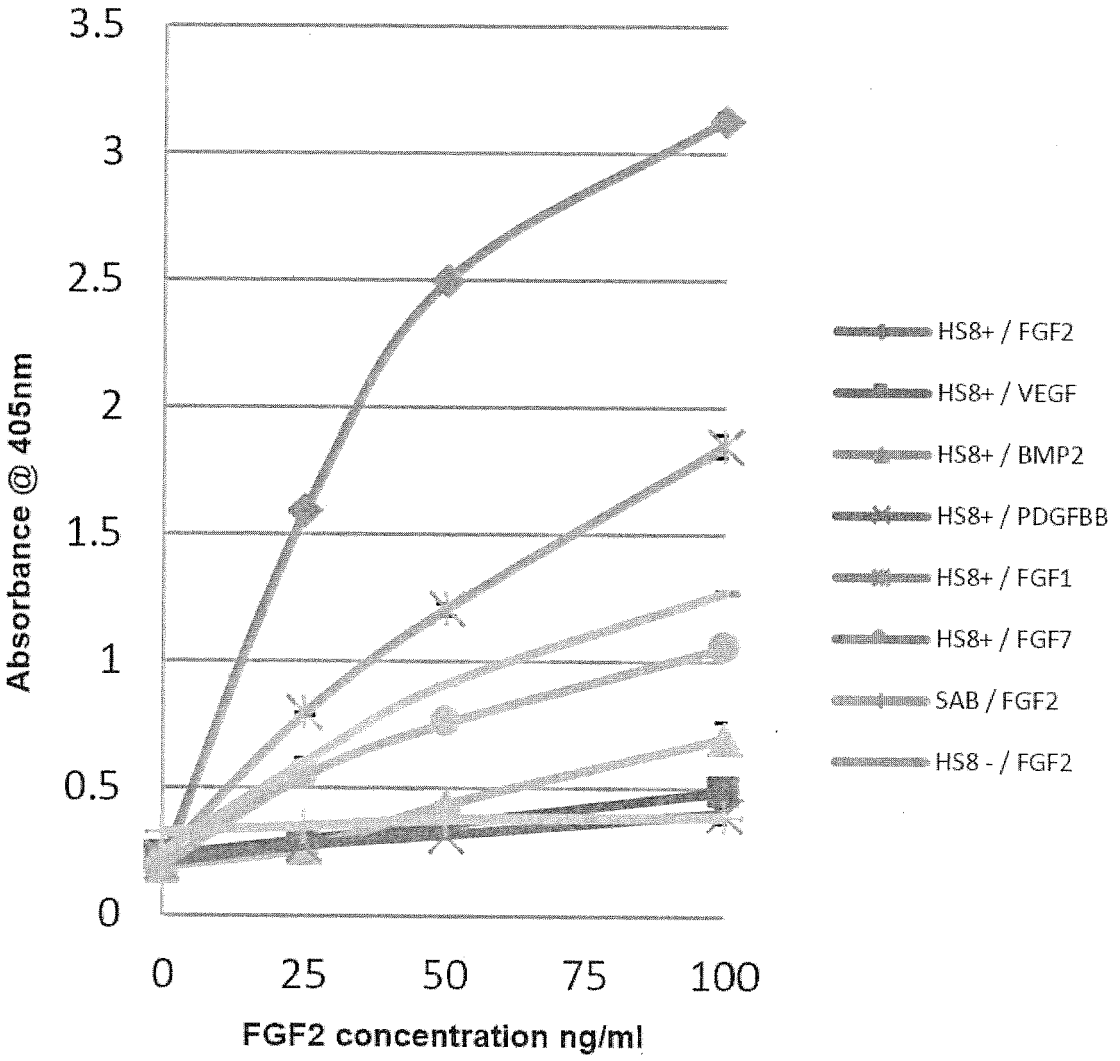

3.5
3
2.5
2
1.5
1
0.5
0
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
HS8+ / FGF2
HS8+ / VEGF
HS8+ / BMP2
HS8+ / PDGFBB
HS8+ / FGF1
HS8+ / FGF7
SAB / FGF2
HS8 - / FGF2

Figure 2

| | Residue/ Size | Sequence | Reference |
|---|---|---|---|
| 1 | **248-254/ 7aa** | $^{248}$YKRSRYT$^{254}$ -paper<br>Have to be $^{248}$**YRSRKYT**$^{254}$ | Lee *et al*, 2007 |
| 2 | **261-277/17aa** | $^{261}$KRTGQYKLGSKTGPGQK$^{277}$ | |
| 3 | **260-277aa/18aa** | $^{260}$LKRTGQYKLGSKTGPGQK$^{277}$ | Hileman *et al*, 1998 |
| 5 | **235-262/ 28aa** | $^{235}$FFFERLESNNYNTYRSRKYTSWYVALKR$^{262}$ | Baird *et al*, 1998 |
| 6 | **157-172/ 16aa** | $^{157}$GHFKDPKRLYCKNGGF$^{172}$ | Gandhi *et al*, 2008 |
| 8 | **185-194/ 10aa** | $^{185}$VREKSDPHIK$^{194}$ | Kinsella,*et al*, 1998 |
| 9 | **250-259/ 10aa** | $^{250}$SRKYTSWYVA$^{259}$ | |
| 10 | **260-273/14aa** | $^{260}$LKRTGQYKLGSKTG$^{273}$ | |
| 11 | **260-280/21aa** | $^{260}$LKRTGQYKLGSKTGPGQKAIL$^{280}$ | Ashikari-Hada *et al*,2004 |
| 13 | **168-169,223,261-277-20aa** | $^{168}$KN$^{169}$ $^{223}$R $^{261}$KRTGQYKLGSKTGPGQK$^{277}$ | Ori *et al*, 2009, Faham *et al*, 1996, Thomson *et al*, 1985 and Baird *et al*, 1998 |
| 14 | **235-262/28aa** | $^{235}$FFFERLESNNYNTYRSRKYTSWYVALKR$^{262}$ | |
| 15 | **216-225/10aa** | $^{216}$LAMKEDGRLL$^{225}$ | |

Figure 3

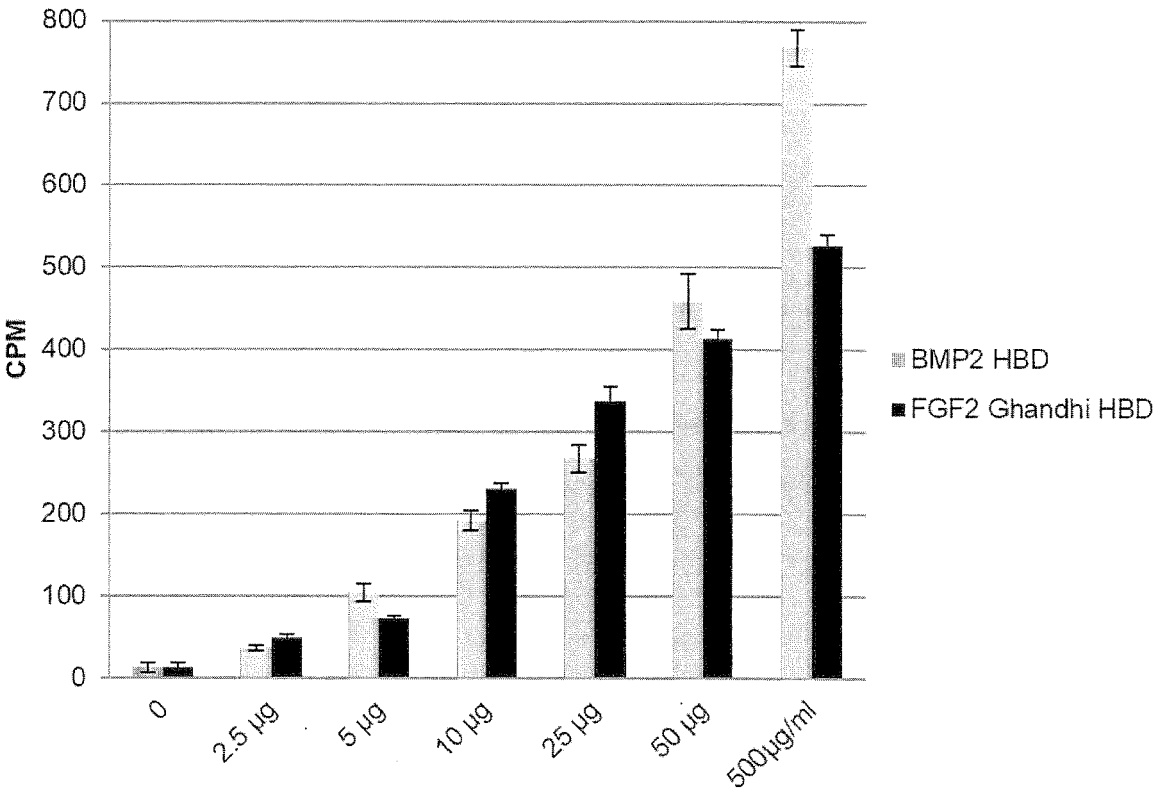

800
700
600
500
400
300
200
100
0
CPM
0
2.5 µg
5 µg
10 µg
25 µg
50 µg
500µg/ml
BMP2 HBD
FGF2 Ghandhi HBD

Figure 4A

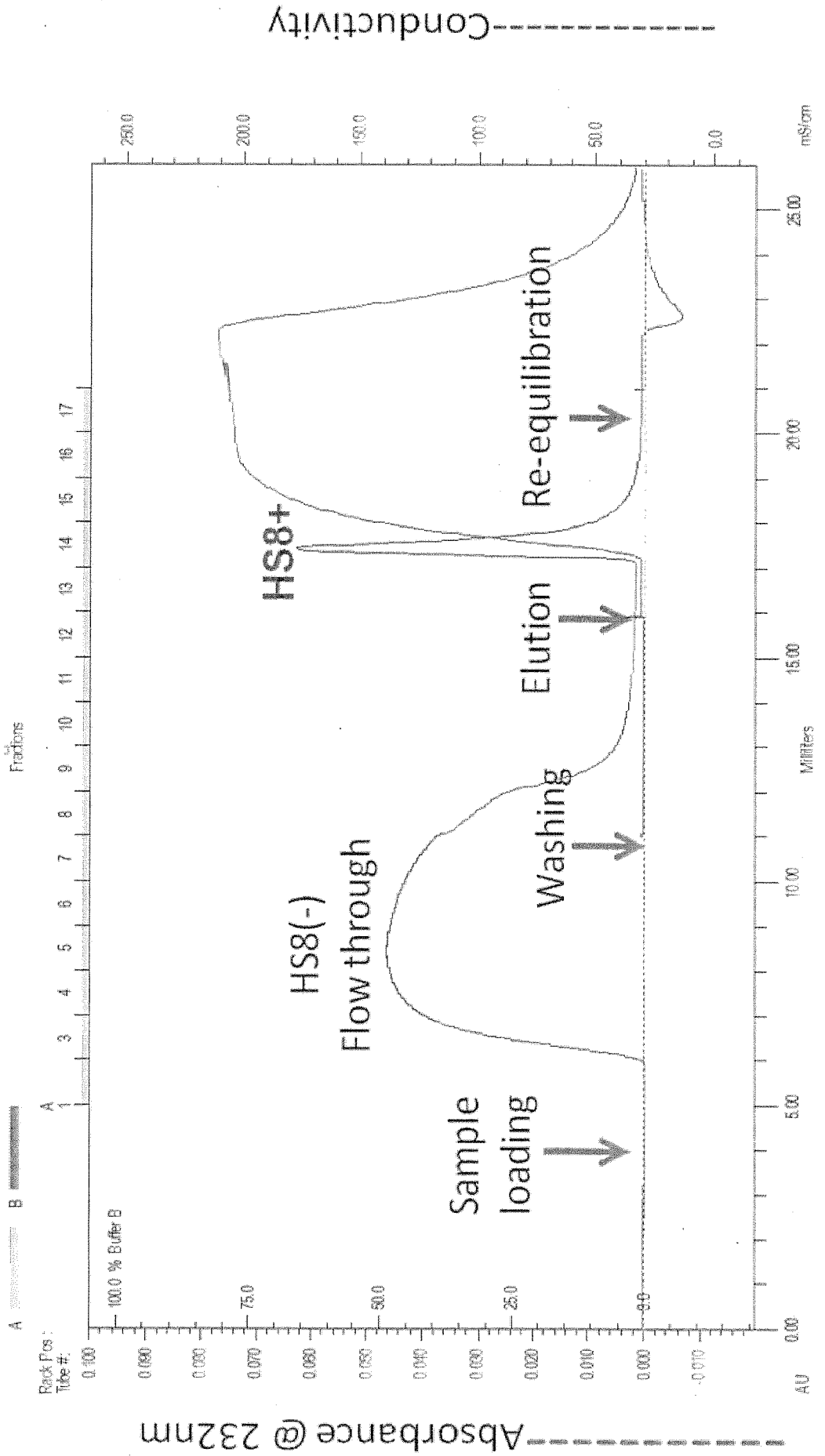

Absorbance @ 232nm
Conductivity
Fraction number
HS8(-)
Flow through
Sample loading
Washing
Elution
HS8+
Re-equilibration
Fractions
Rack Pos :
Tube #:
100.0 % Buffer B
A
B
AU
mS/cm
Milliliters

Figure 4B

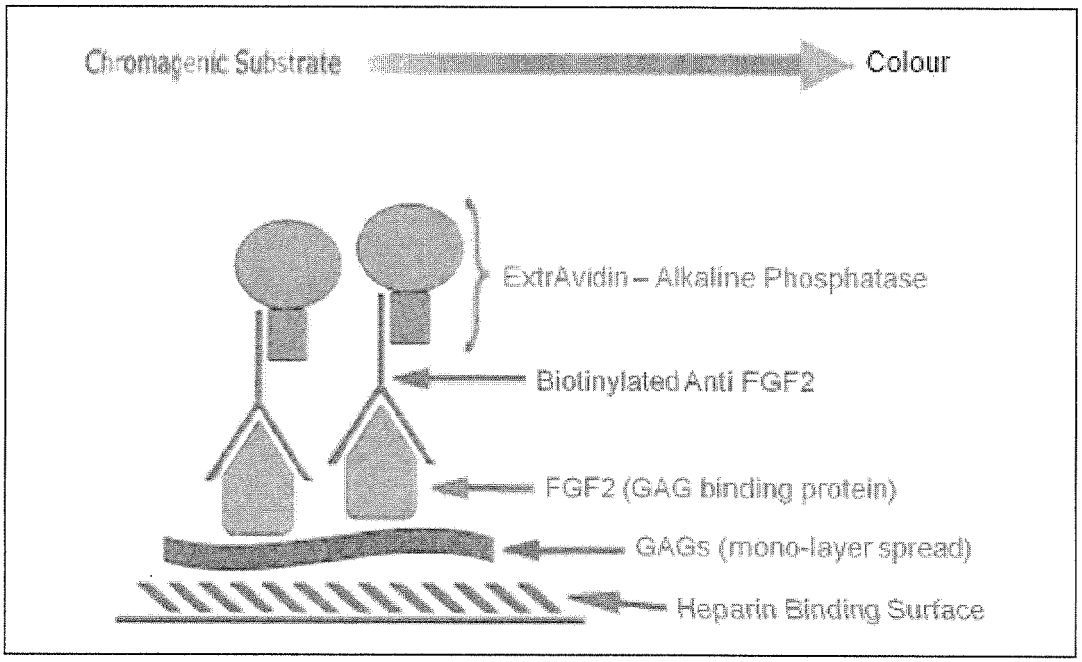

Chromagenic Substrate
Colour
ExtrAvidin – Alkaline Phosphatase
Biotinylated Anti FGF2
FGF2 (GAG binding protein)
GAGs (mono-layer spread)
Heparin Binding Surface

Figure 5

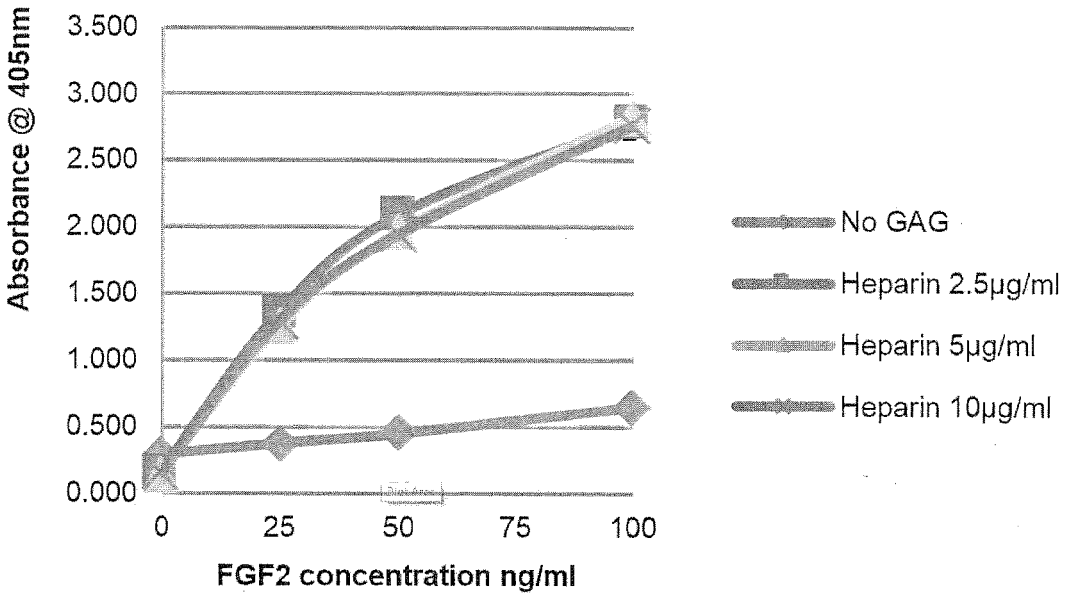



Figure 6A

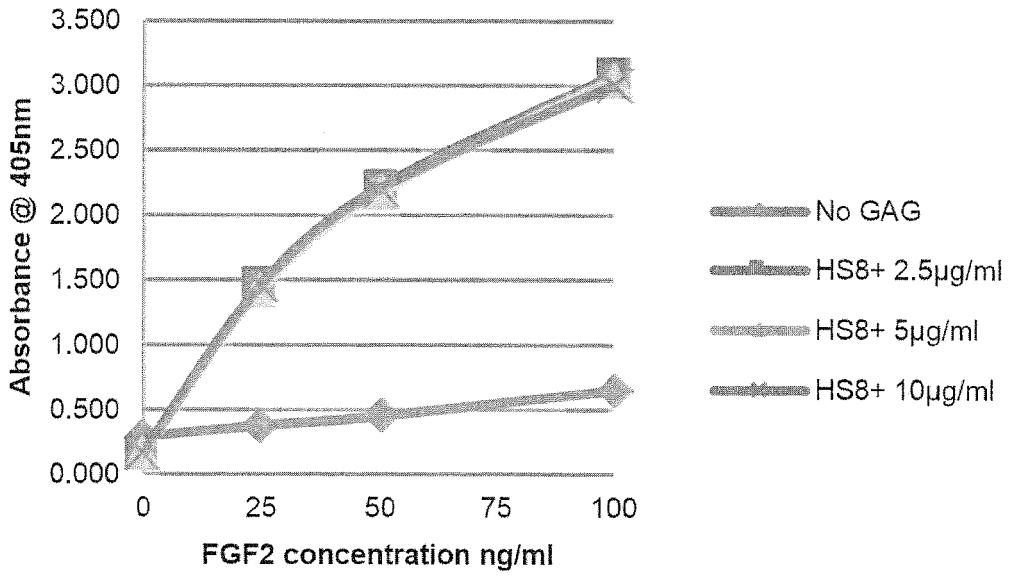



Figure 6B

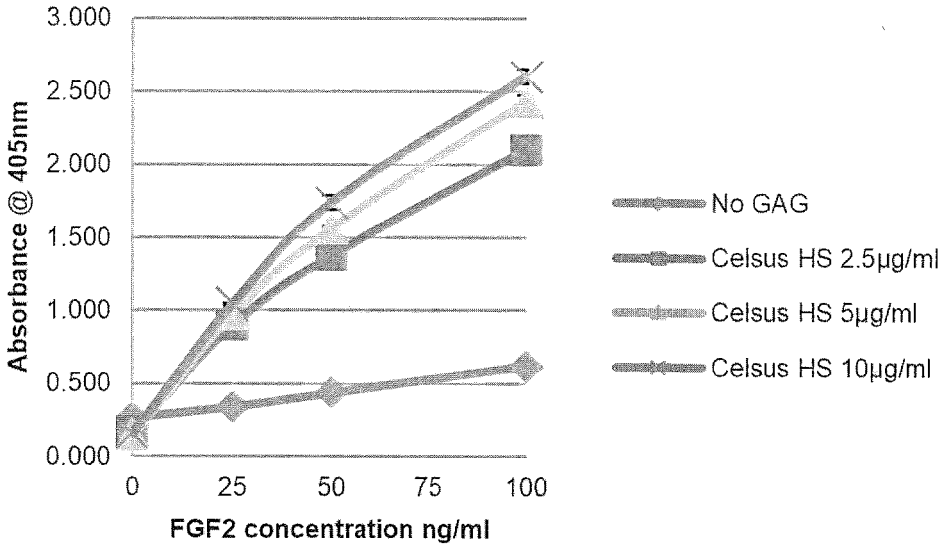



Figure 6C

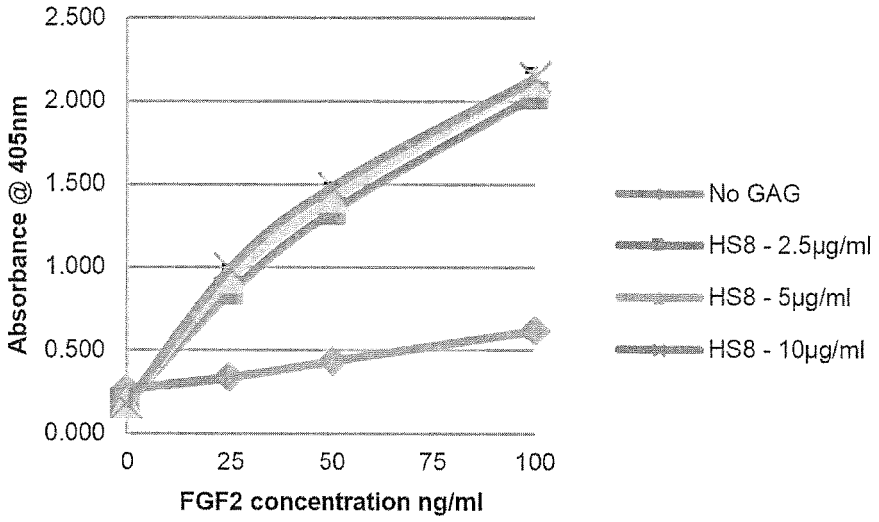



Figure 6D

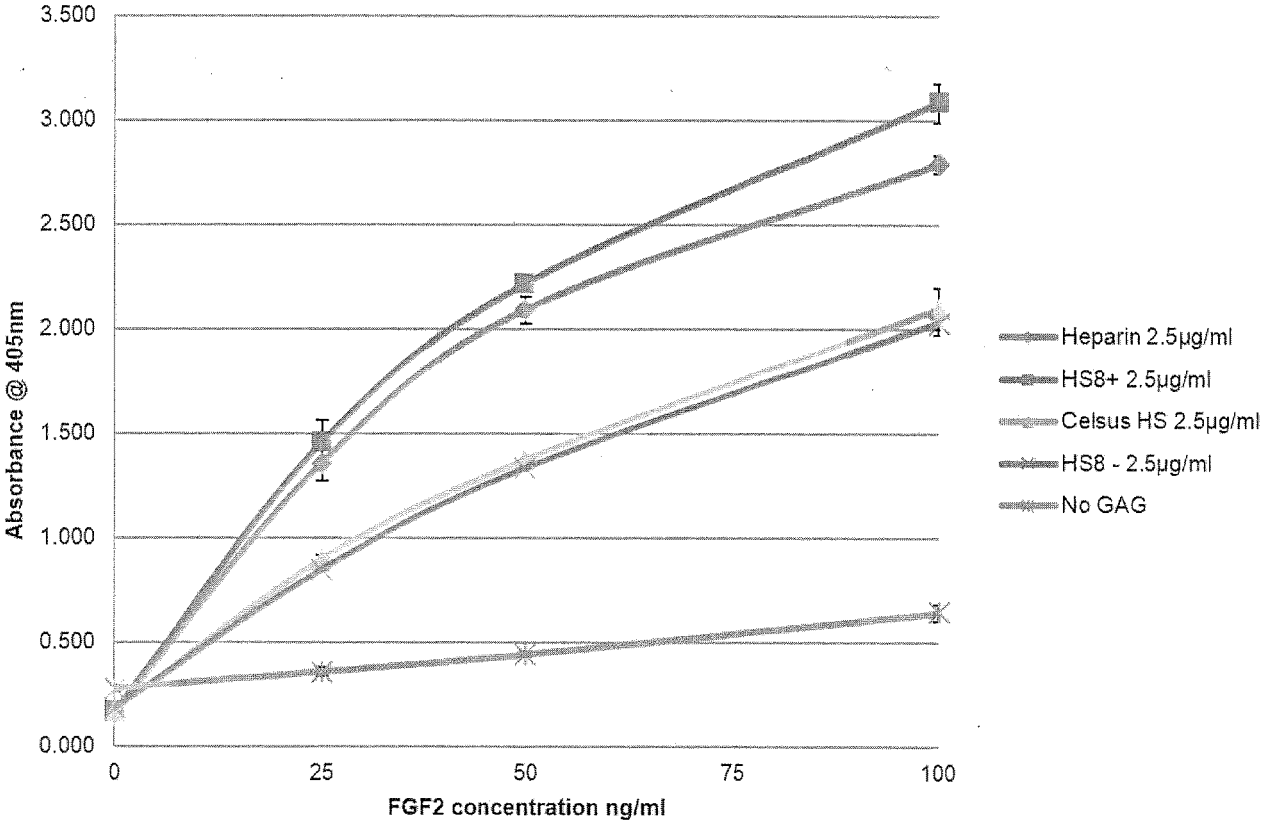

3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
Heparin 2.5µg/ml
HS8+ 2.5µg/ml
Celsus HS 2.5µg/ml
HS8 - 2.5µg/ml
No GAG

Figure 7

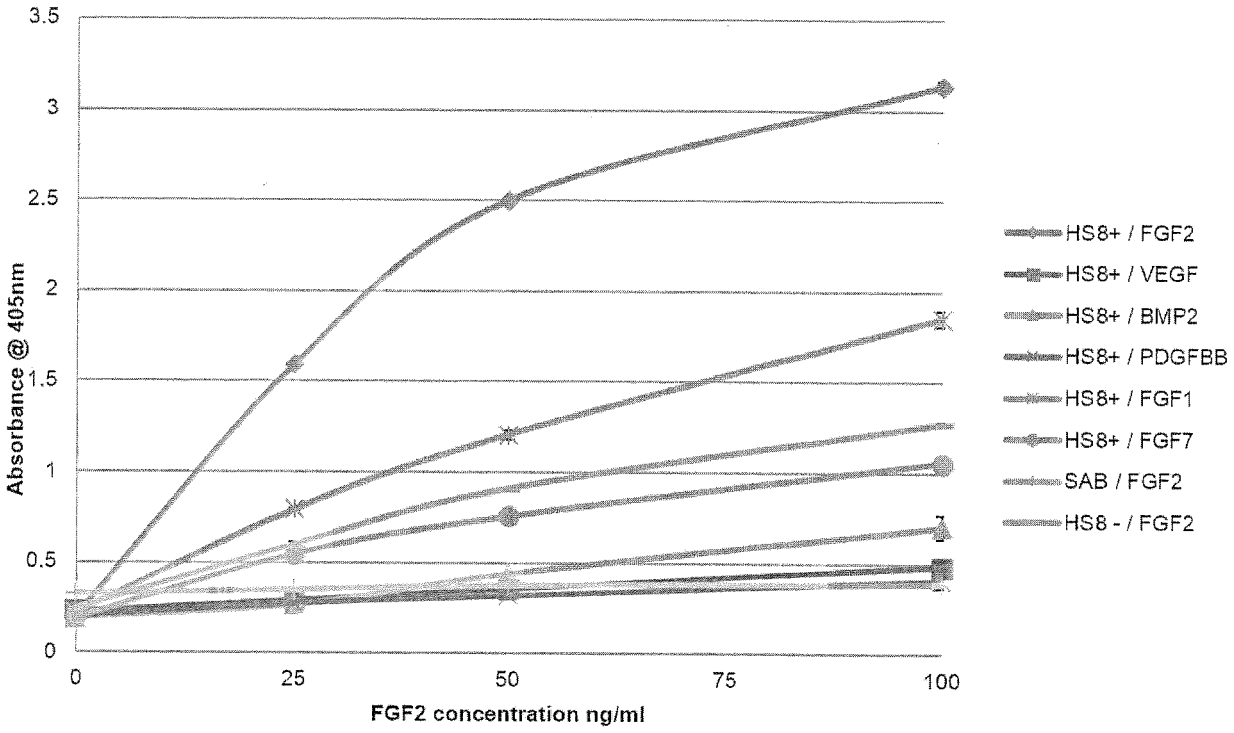



Figure 8A

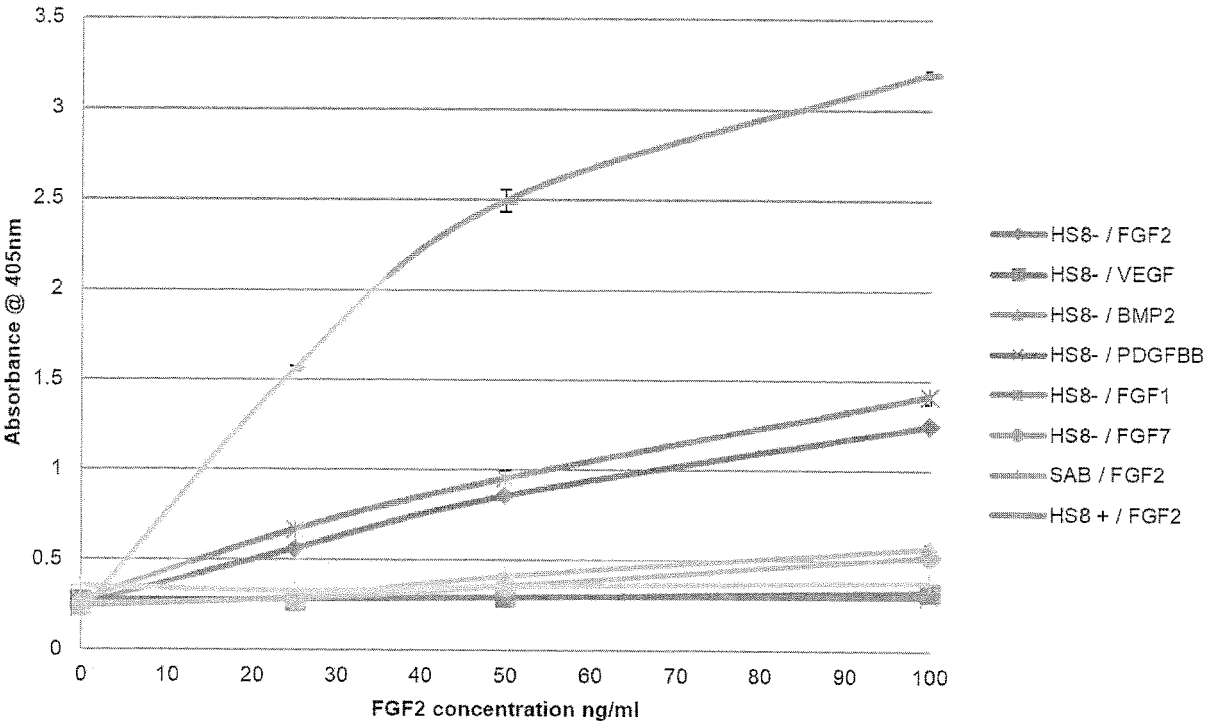



Figure 8B

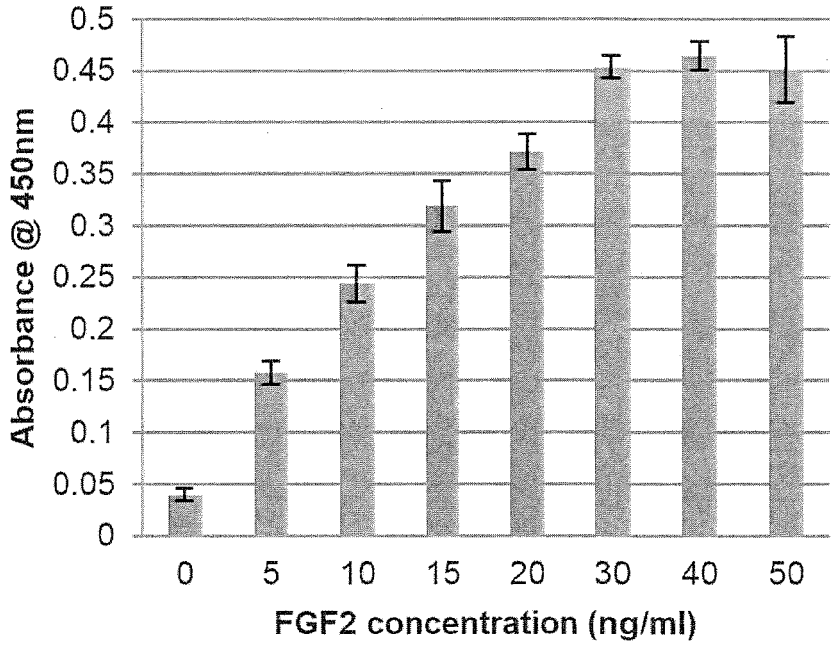



Figure 9A

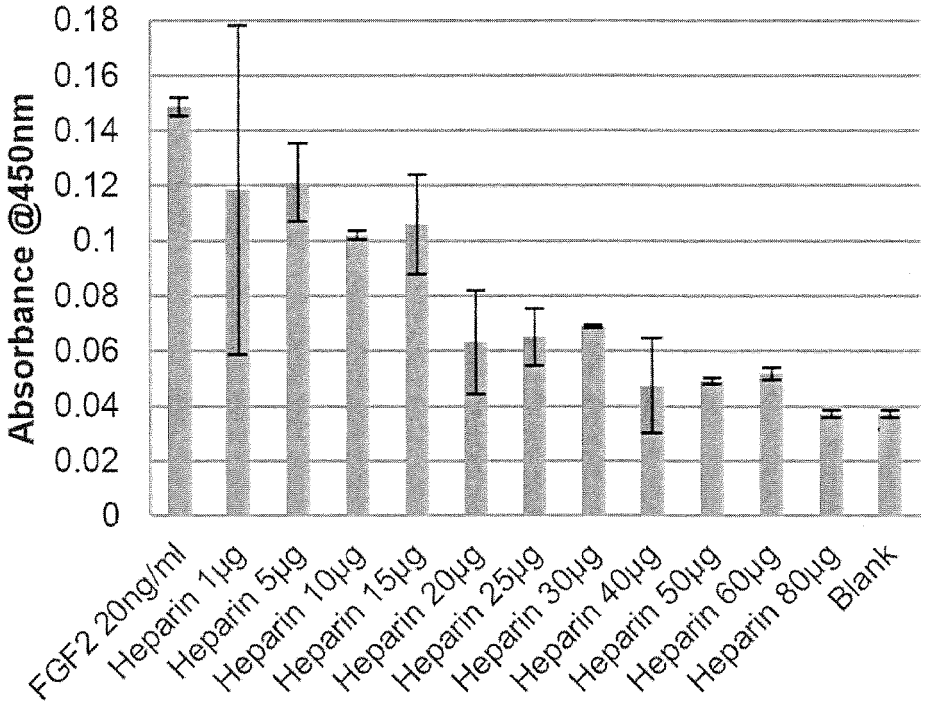



Figure 9B

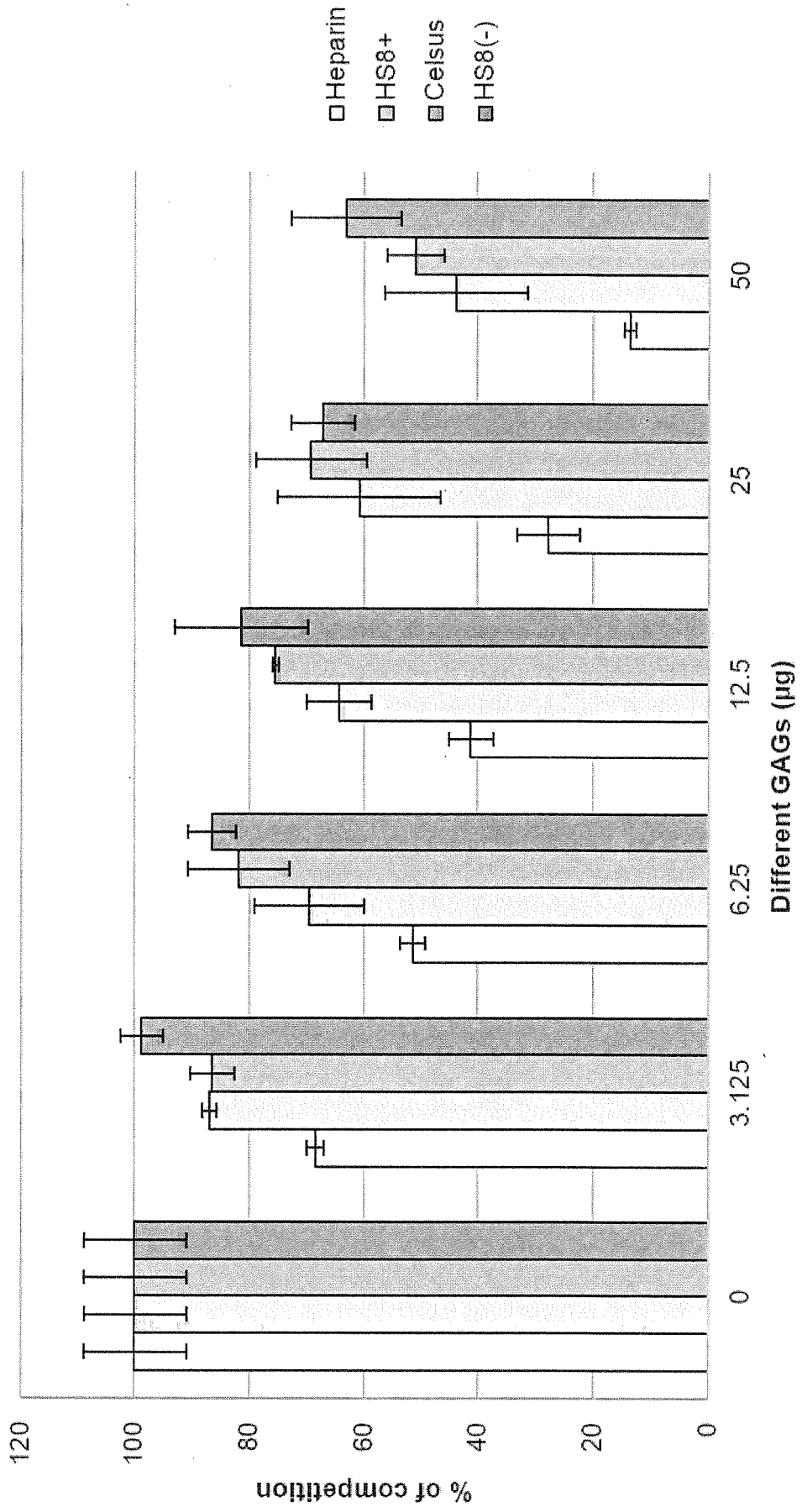

Heparin
HS8+
Celsus
HS8(-)
% of competition
120
100
80
60
40
20
0
0
3.125
6.25
12.5
25
50
Different GAGs (µg)

Figure 9C

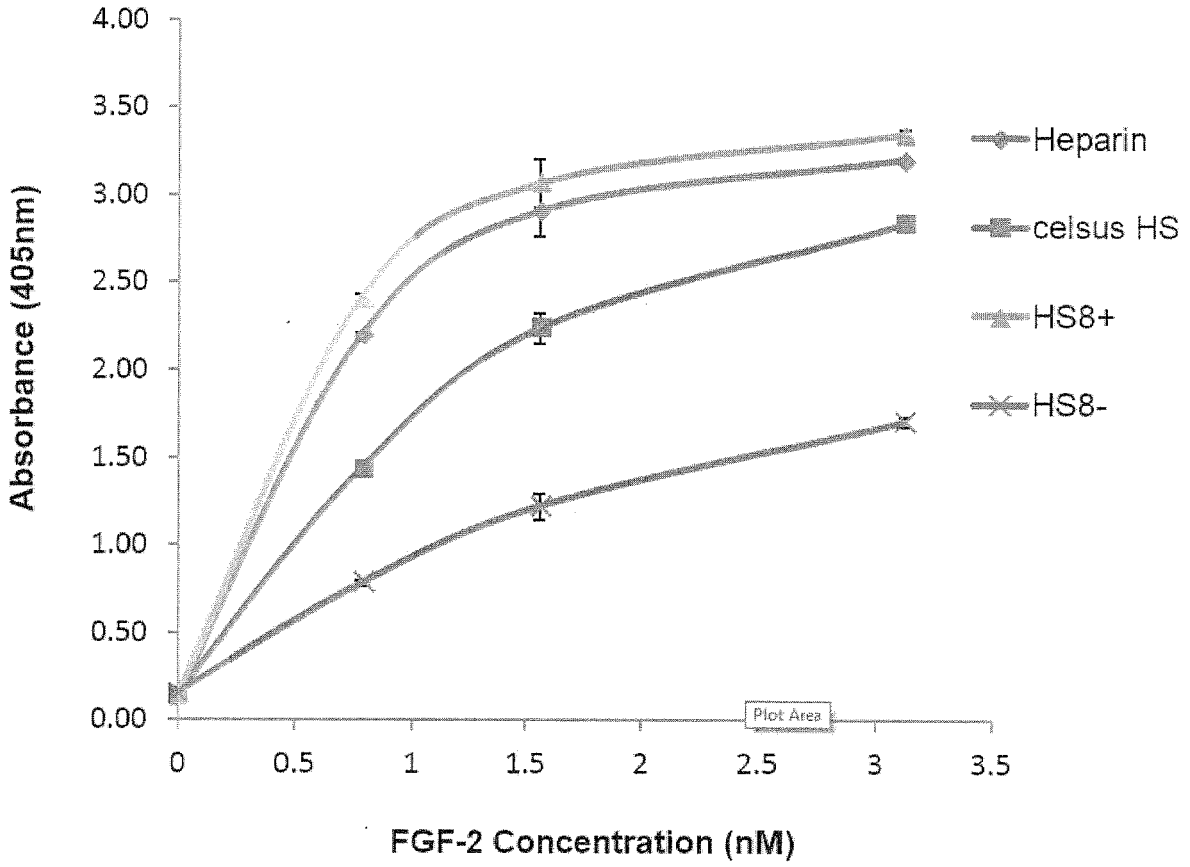

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Absorbance (405nm)
0
0.5
1
1.5
2
2.5
3
3.5
FGF-2 Concentration (nM)
Plot Area
Heparin
celsus HS
HS8+
HS8-

Figure 10

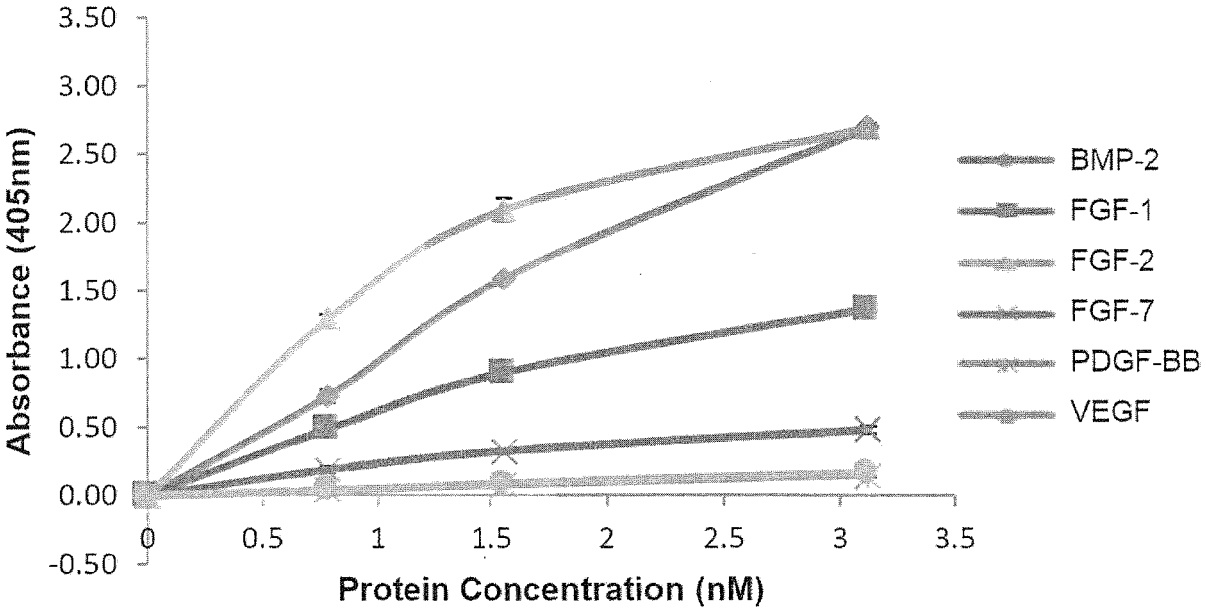



Figure 11A

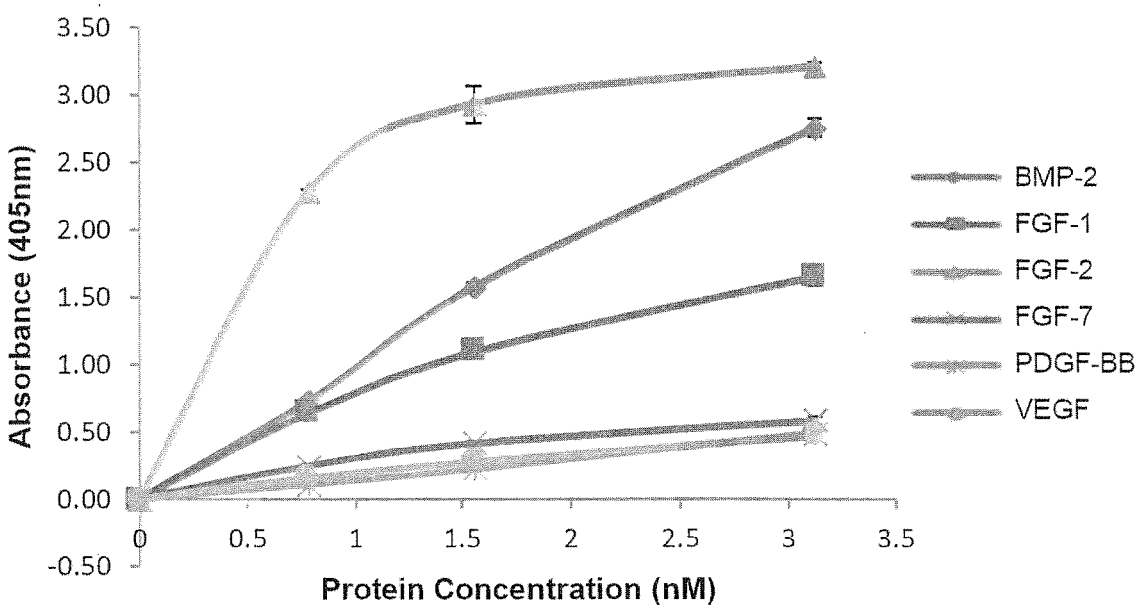



Figure 11B

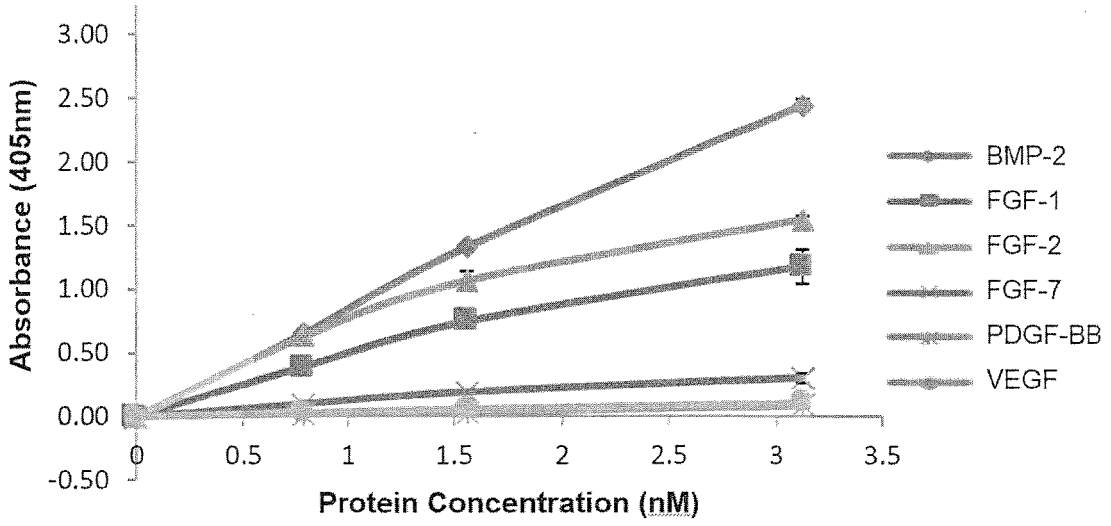



Figure 11C

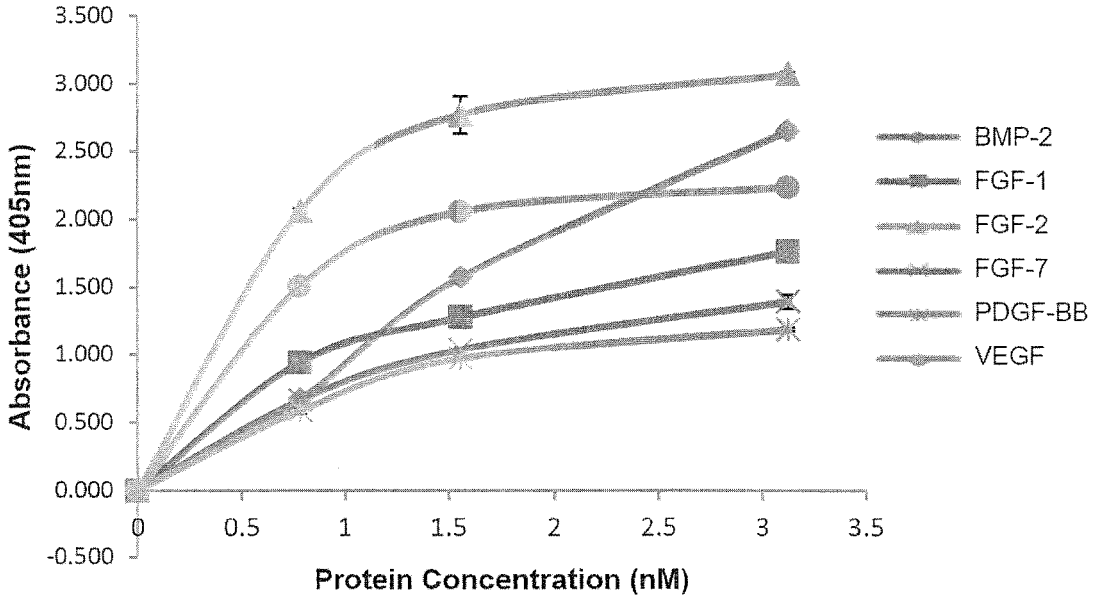



Figure 11D

MVGVGGGDVE DVTPRPGGCQ ISGRGARGCN GIPGAAAWEA ALPRRRPRRH PSVNPRSRAA GSPRTRGRRT EERPSGSRLG DRGRGRALPG GRLGGRGRGR APERVGGRGR GRGTAAPRAA PAARGSRPGP AGTMAAGSIT TLPALPEDGG SGAFPPGHFK DPKRLYCKNG GFFLRIHPDG RVDGVREKSD PHIKLQLQAE ERGVVSIKGV CANRYLAMKE DGRLLASKCV TDECFFFERL ESNNYNTYRS RKYTSWYVAL KRTGQYKLGS KTGPGQKAIL FLPMSAKS

Figure 12

| | %E10 GAG |
|---|---|
| **DISACCHARIDE** | |
| **IdoA/GlcA-AMann$_R$** | 12.9 |
| **IdoA(2S)-AMann$_R$** | 53.4 |
| **GlcA-AMann$_R$(6S)** | 10.25 |
| **IdoA-AMann$_R$(6S)** | 3.4 |
| **IdoA(2S)-AMann$_R$(6S)** | 18.7 |
| **GlcA(2S)-AMann$_R$** | 1.0 |
| **GlcA-AMann$_R$(3S)** | 0.30 |
| **GlcA- AMann$_R$(3,6S)** | 0.15 |
| **UNKNOWN** | 0.0 |

Figure 13

| PEAK NUMBER | DISACCHARIDE | % in E10 GAG |
|---|---|---|
| 1 | ΔHexUA-GlcNAc | 44.8 |
| 3 | ΔHexUA-$GlcNSO_3$ | 21.5 |
| 2 | ΔHexUA-GlcNAc(6S) | 8.0 |
| 7 | ΔHexUA(2S)-GlcNAc | 2.4 |
| 4 | ΔHexUA-$GlcNSO_3$(6S) | 4.0 |
| 5 | ΔHexUA(2S)-$GlcNSO_3$ | 12.4 |
| 8 | ΔHexUA(2S)-GlcNAc(6S) | 0.2 |
| 6 | ΔHexUA(2S)-$GlcNSO_3$(6S) | 4.1 |
| 9 | unknown | 2.4 |

Figure 14

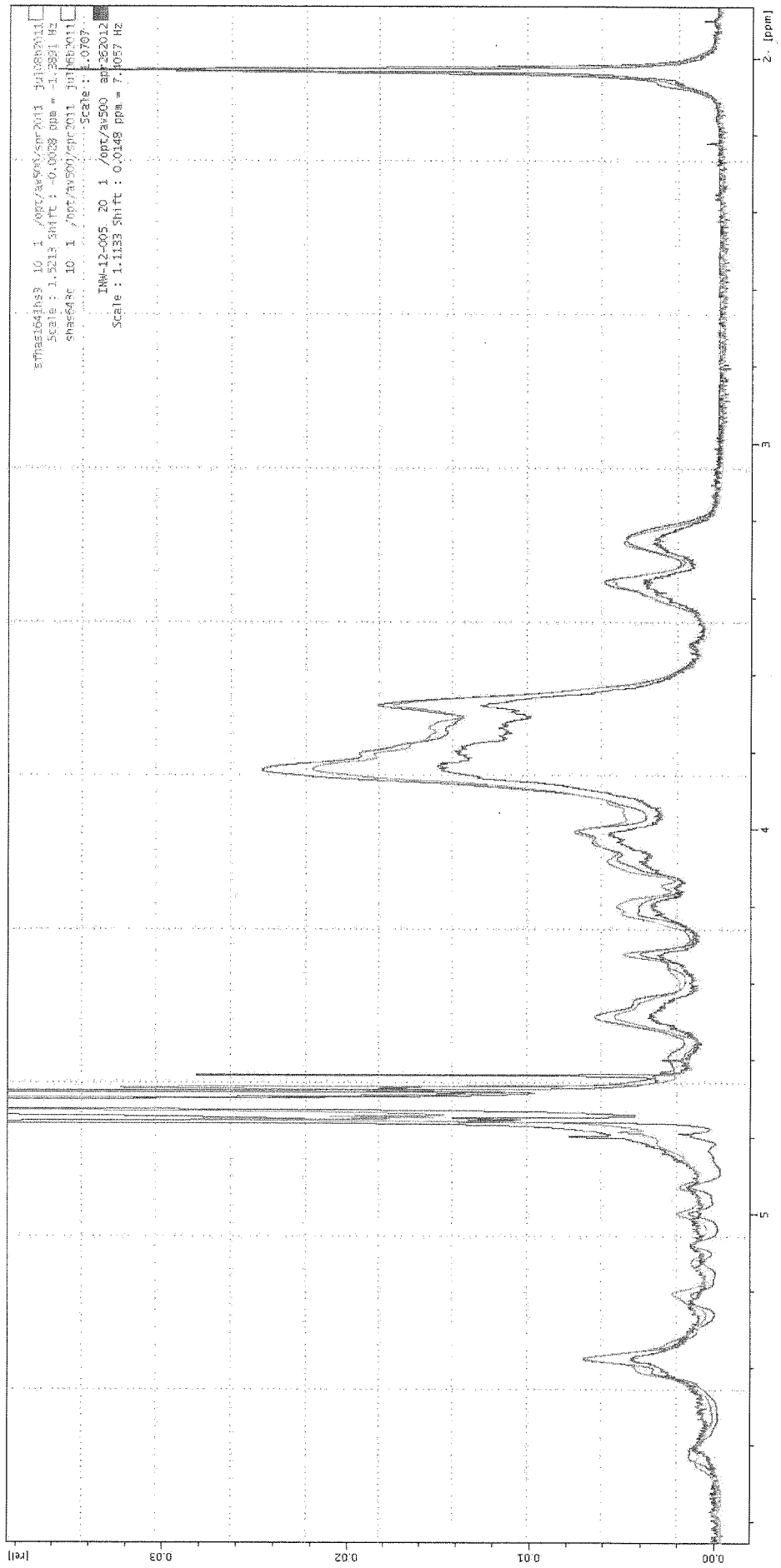



Figure 15

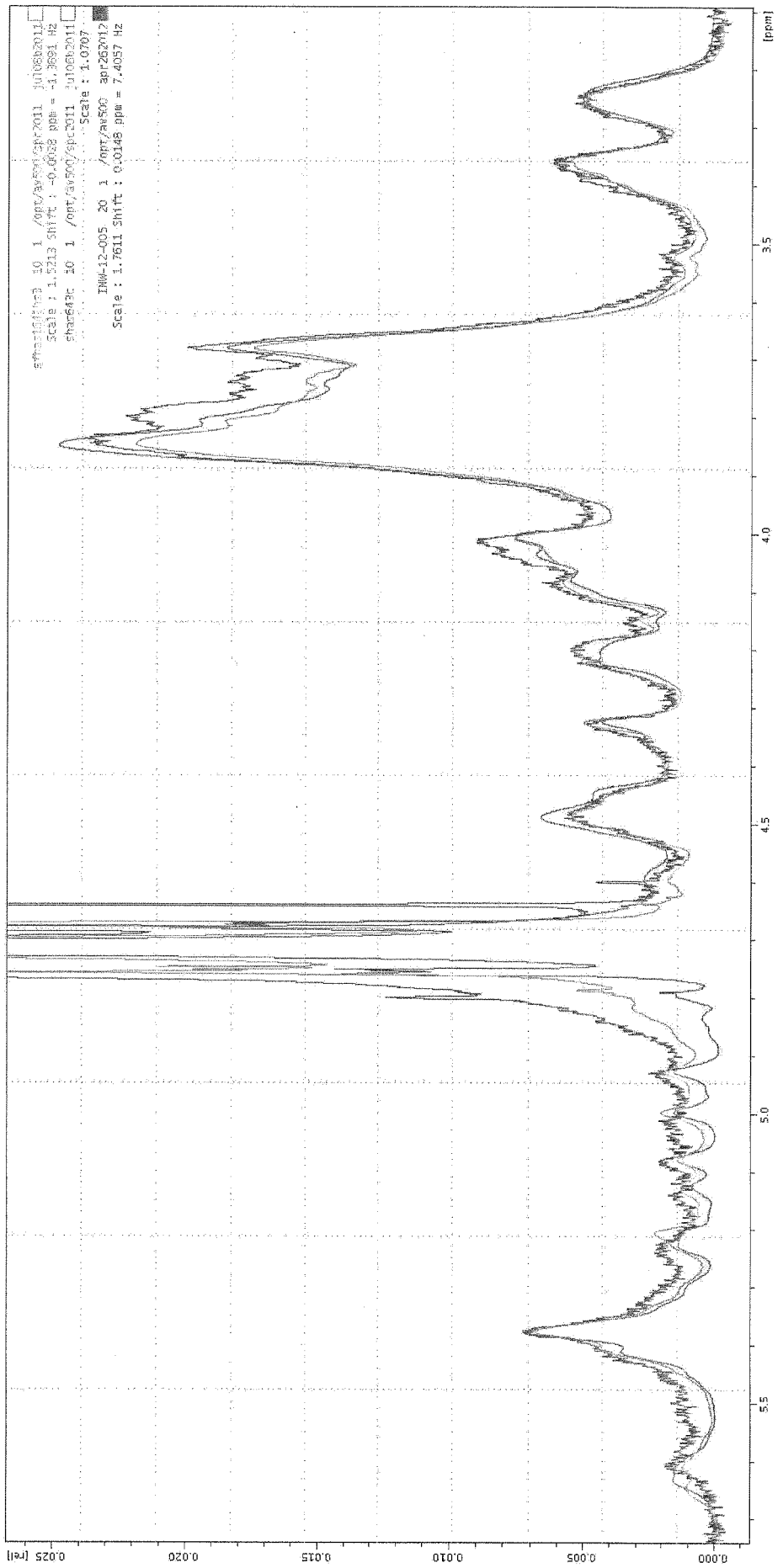



Figure 16

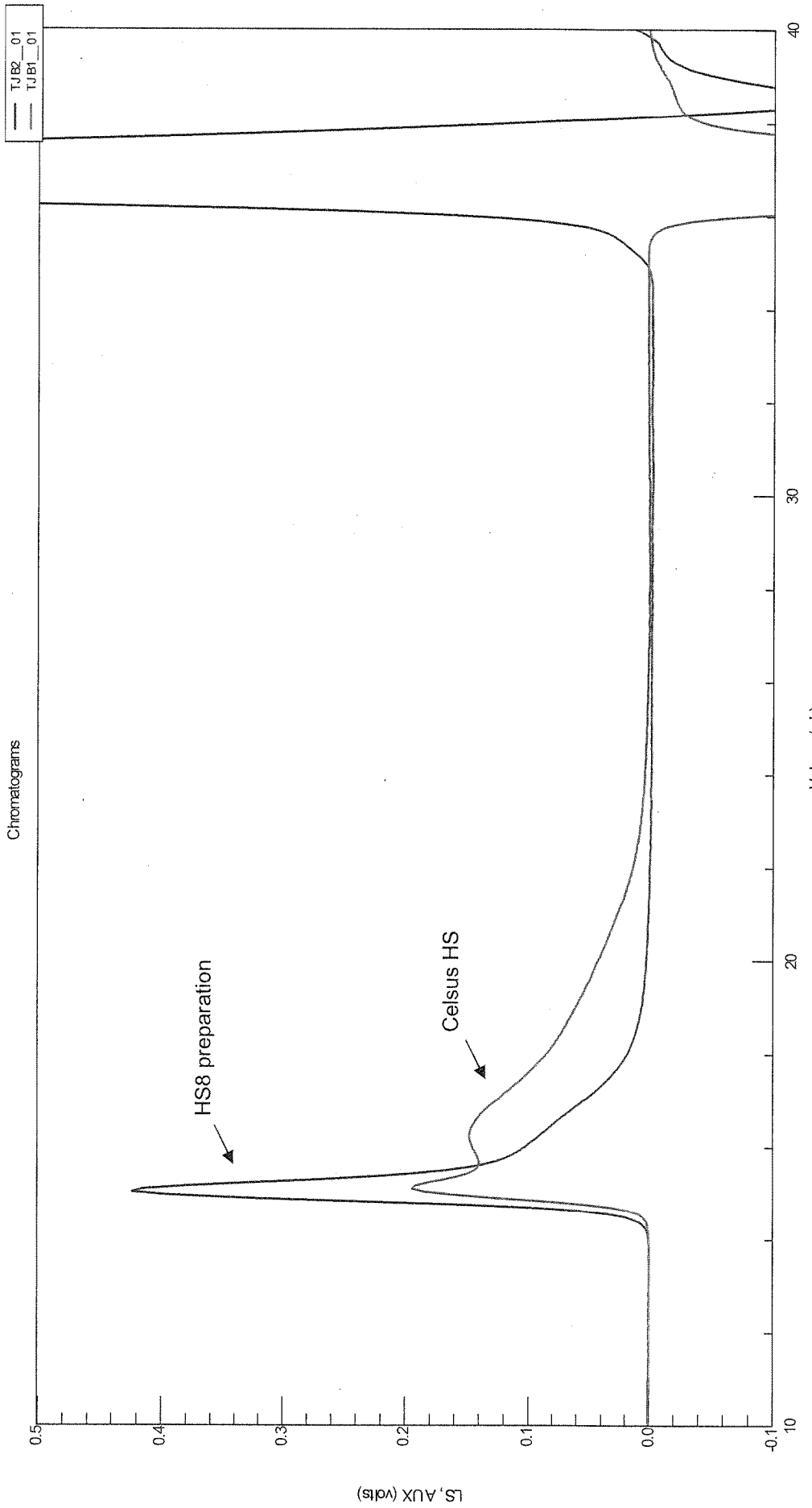

TJB2__01
TJB1__01
Chromatograms
HS8 preparation
Celsus HS
LS, AUX (volts)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
10
20
30
40
Volume (mL)

Figure 17

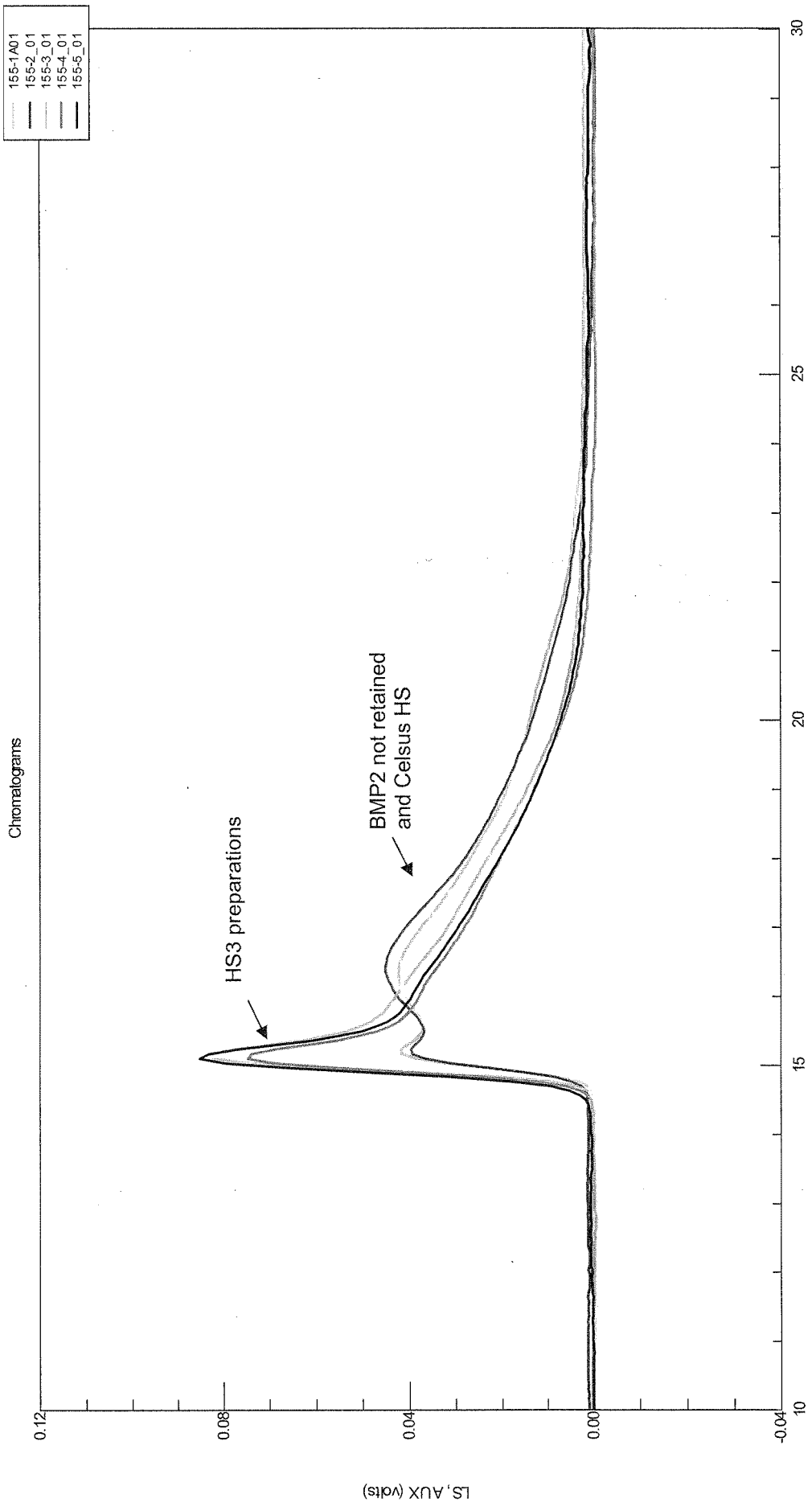

155-1A01
155-2_01
155-3_01
155-4_01
155-5_01
Chromatograms
HS3 preparations
BMP2 not retained
and Celsus HS
LS, AUX (volts)
0.12
0.08
0.04
0.00
-0.04
10
15
20
25
30
Volume (mL)

Figure 18

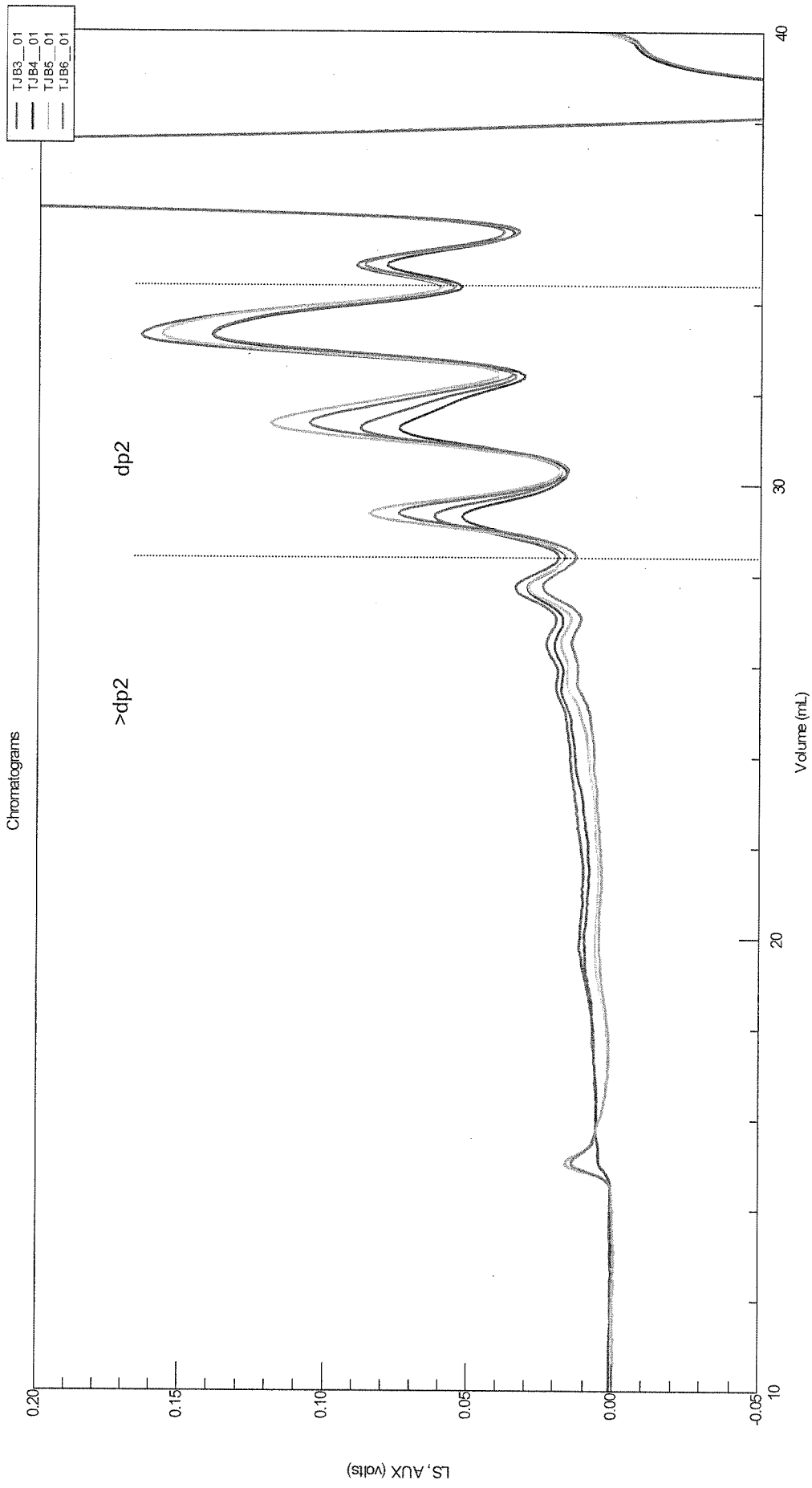

TJB3__01
TJB4__01
TJB5__01
TJB6__01
dp2
>dp2
Chromatograms
Volume (mL)
LS, AUX (volts)
0.20
0.15
0.10
0.05
0.00
-0.05
10
20
30
40

Figure 19

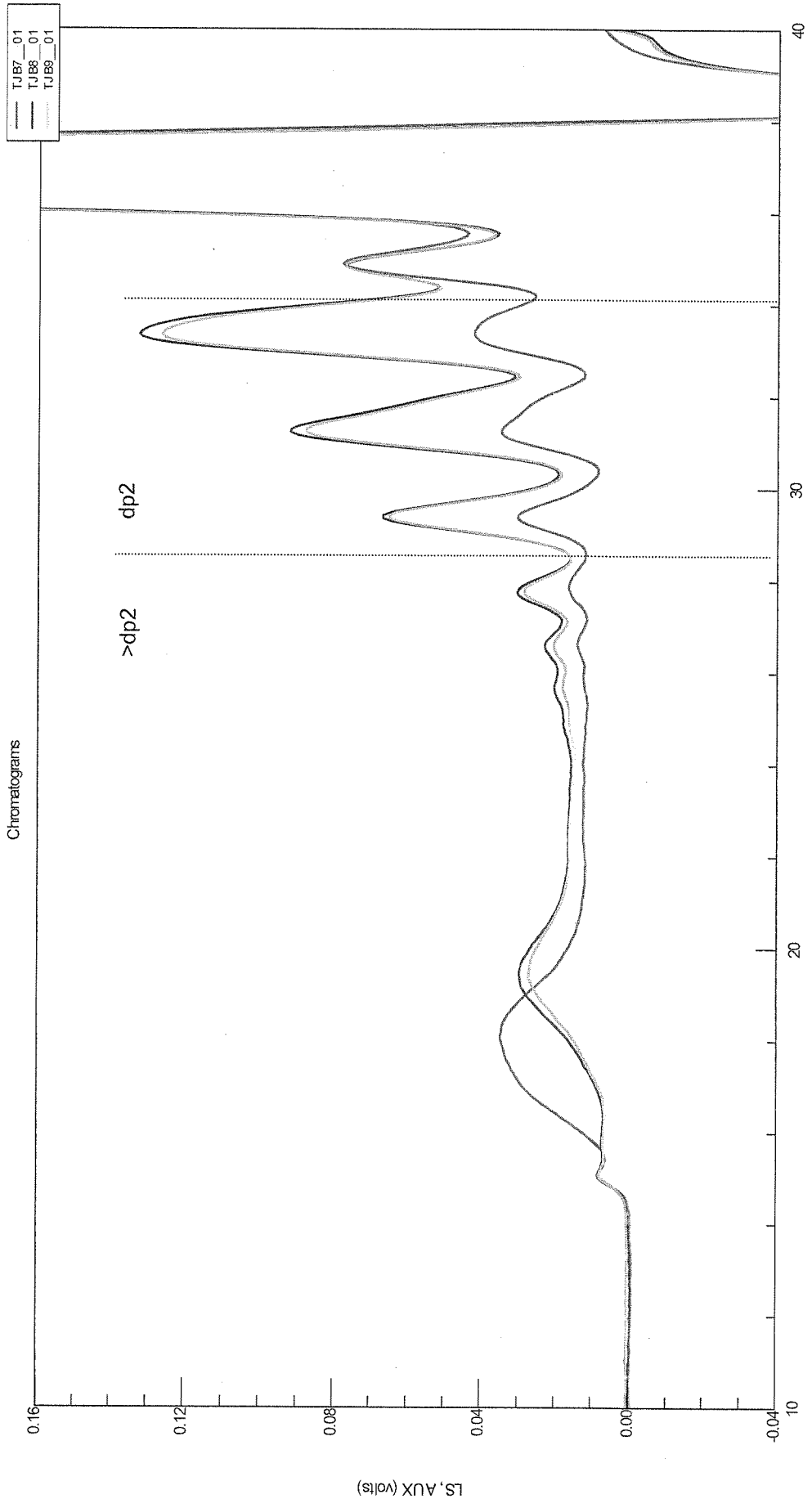

TJB7__01
TJB8__01
TJB9__01
dp2
>dp2
Chromatograms
Volume (mL)
LS, AUX (volts)
0.16
0.12
0.08
0.04
0.00
-0.04
10
20
30
40

Figure 20

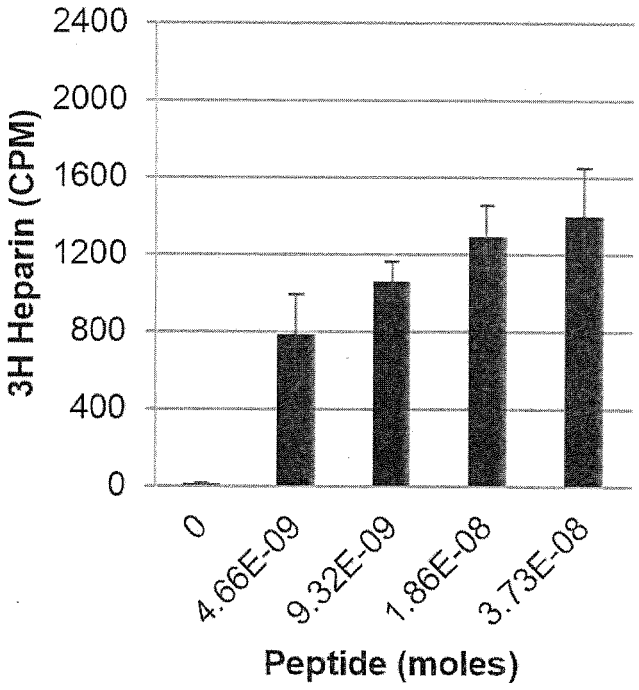

2400
2000
1600
1200
800
400
0
3H Heparin (CPM)
0
4.66E-09
9.32E-09
1.86E-08
3.73E-08
Peptide (moles)

Figure 21

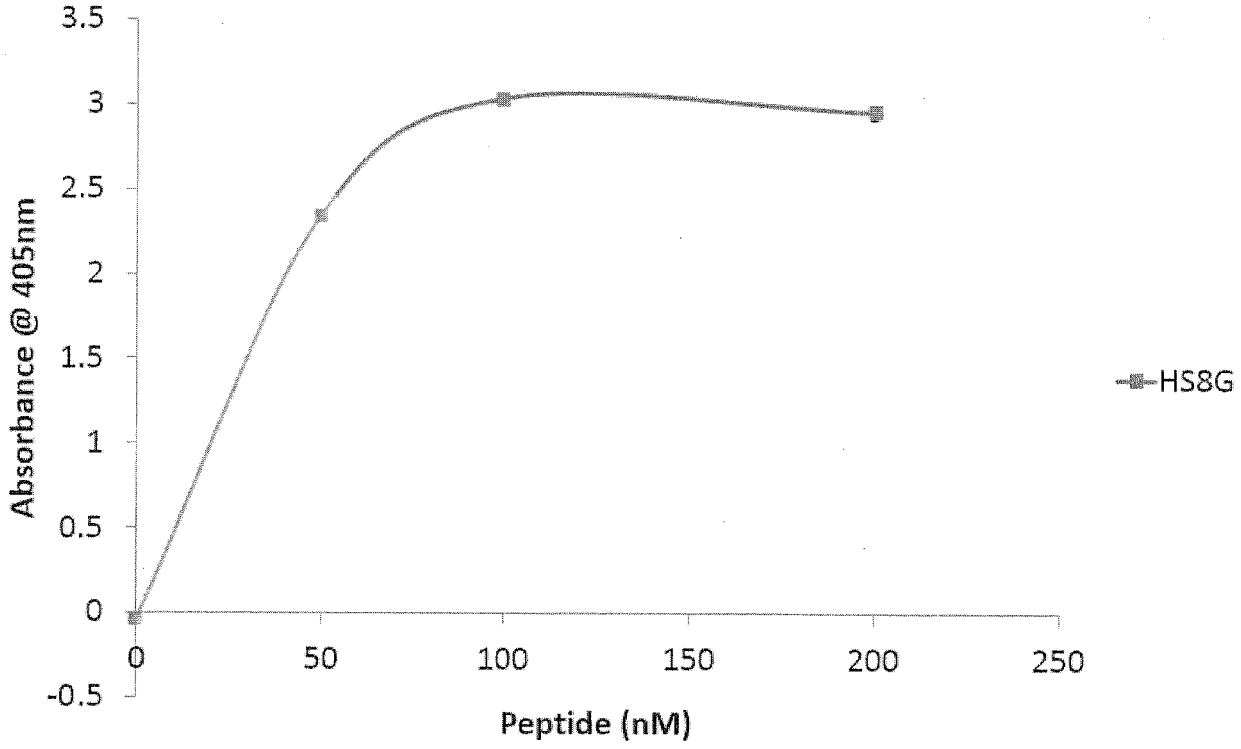



Figure 22

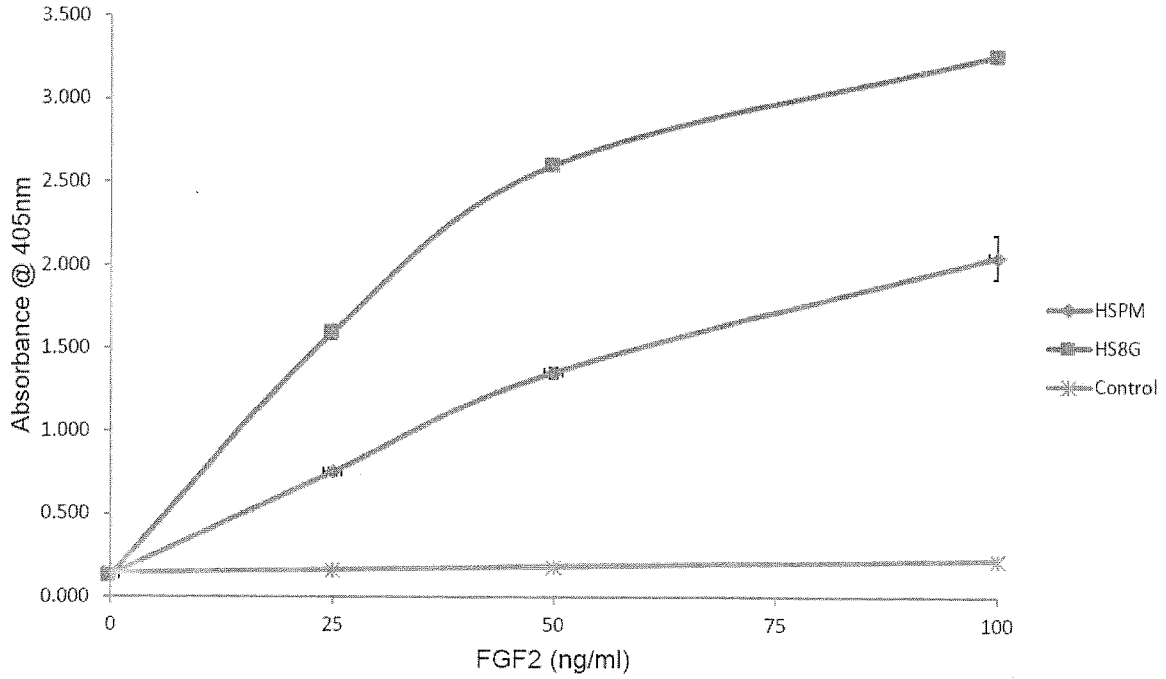



Figure 23

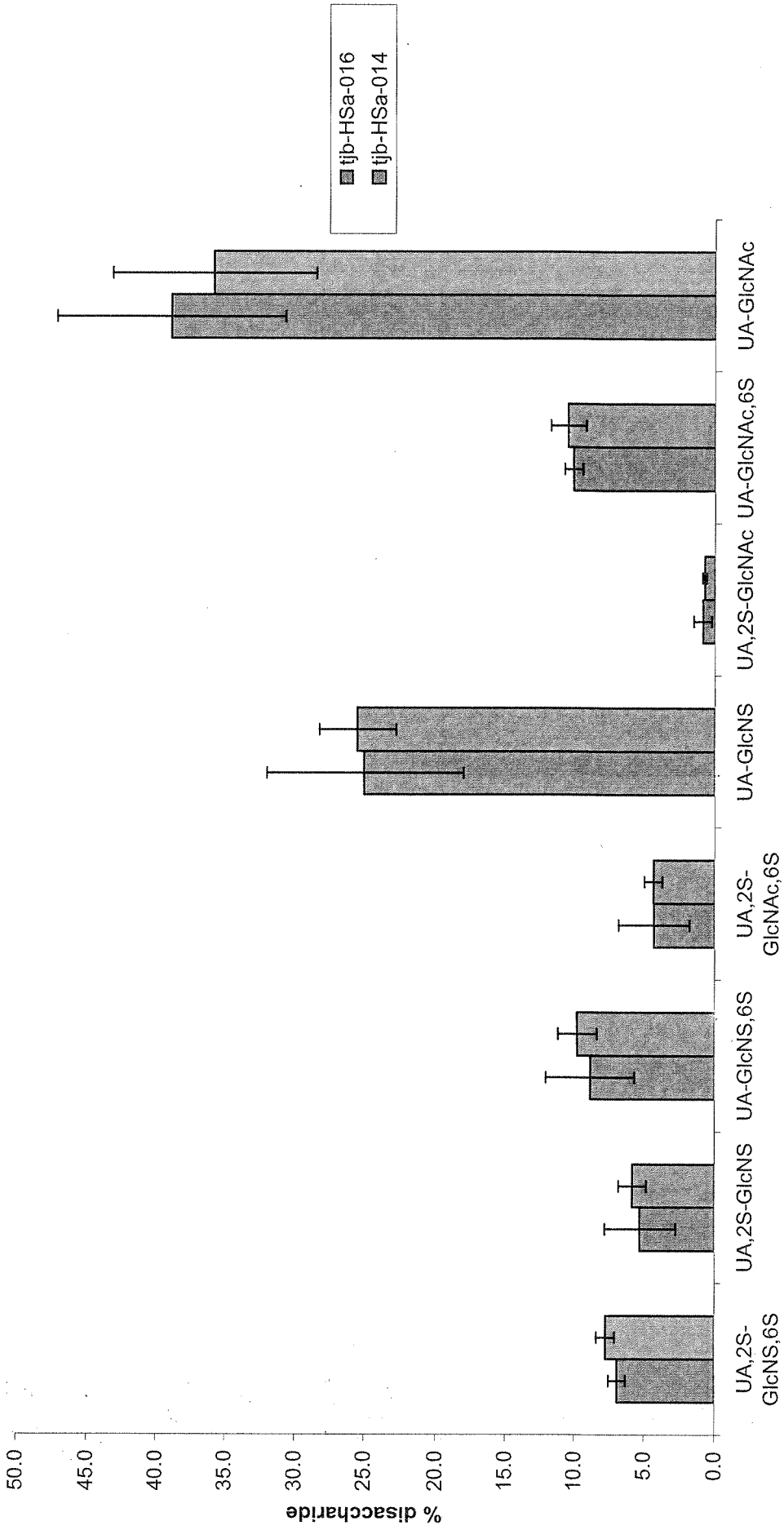

tjb-HSa-016
tjb-HSa-014
% disaccharide
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

Figure 24

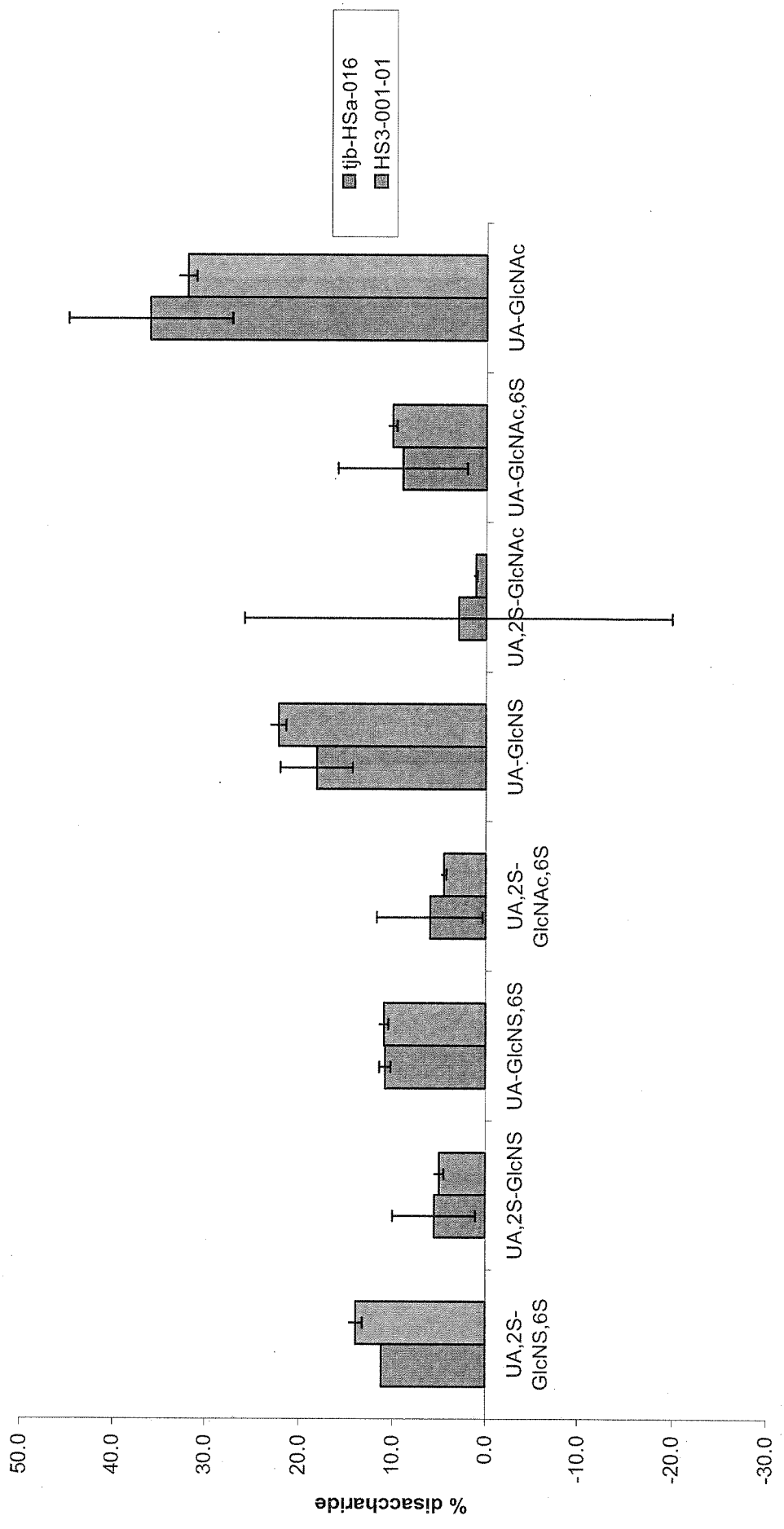

fjb-HSa-016
HS3-001-01
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
-30.0
% disaccharide
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

Figure 25

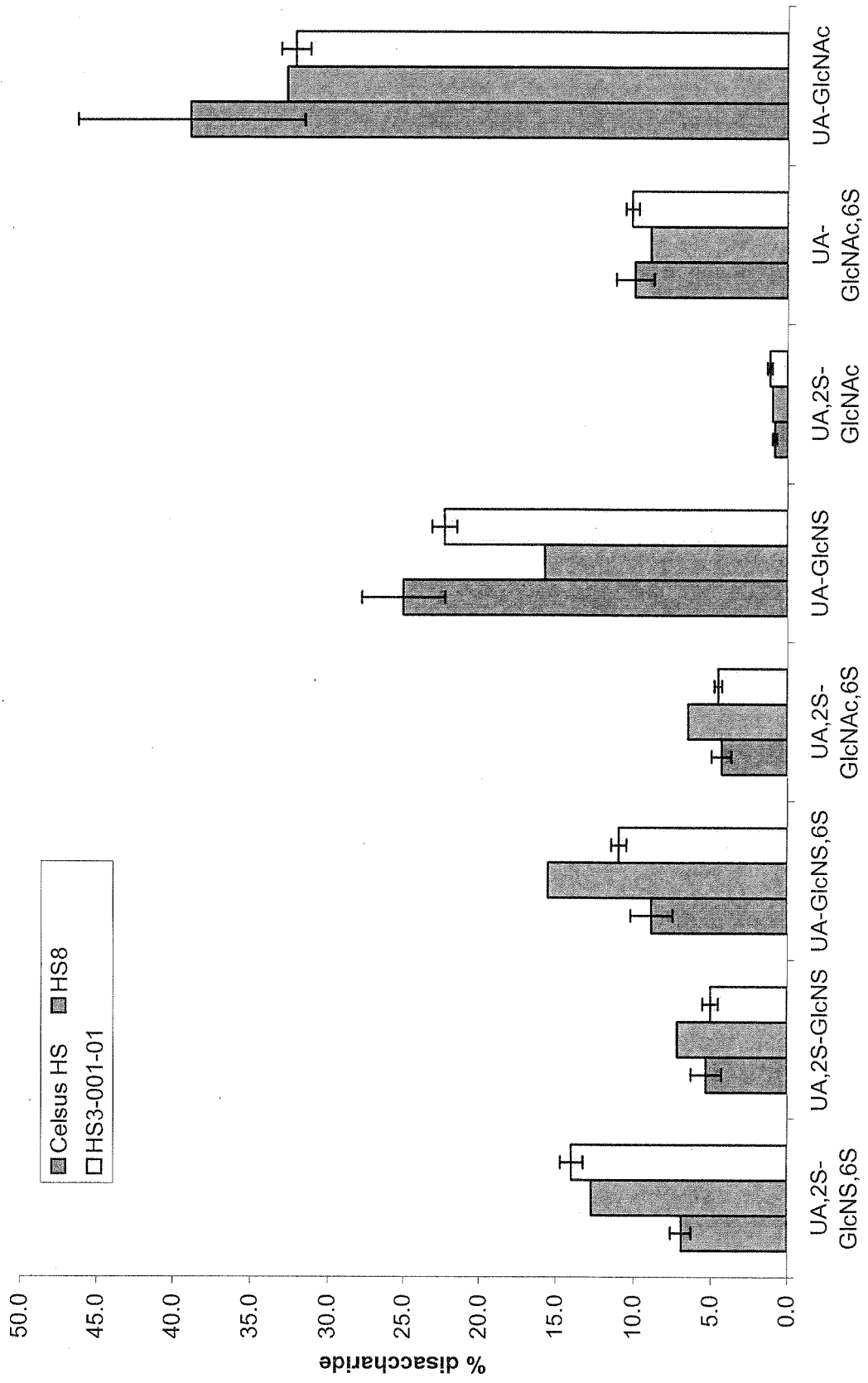

Celsus HS
HS8
HS3-001-01
% disaccharide
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

Figure 26

| Disaccharide | Celsus HS | HS3 (848/HS3/001) | HS8 |
|---|---|---|---|
| ΔHexUA,2S-GlcNS,6S | 8.3 | 14.8 | 12.7 |
| ΔHexUA,2S-GlcNS | 5.1 | 4.9 | 7.2 |
| ΔHexUA-GlcNS,6S | 9.1 | 11.1 | 15.5 |
| ΔHexUA,2S-GlcNAc,6S | 3.3 | 4.8 | 6.5 |
| ΔHexUA-GlcNS | 26.9 | 22.2 | 15.7 |
| ΔHexUA,2S-GlcNAc | 1.6 | 1.1 | 1.0 |
| ΔHexUA-GlcNAc,6S | 10.8 | 10.1 | 8.9 |
| ΔHexUA-GlcNAc | 34.9 | 31.0 | 32.5 |
| **Total** | 100.0 | 100.0 | 100.0 |

Mean average of duplicate analyses of duplicate hydrolysates
Normalised weight percentage disaccharide

Figure 27

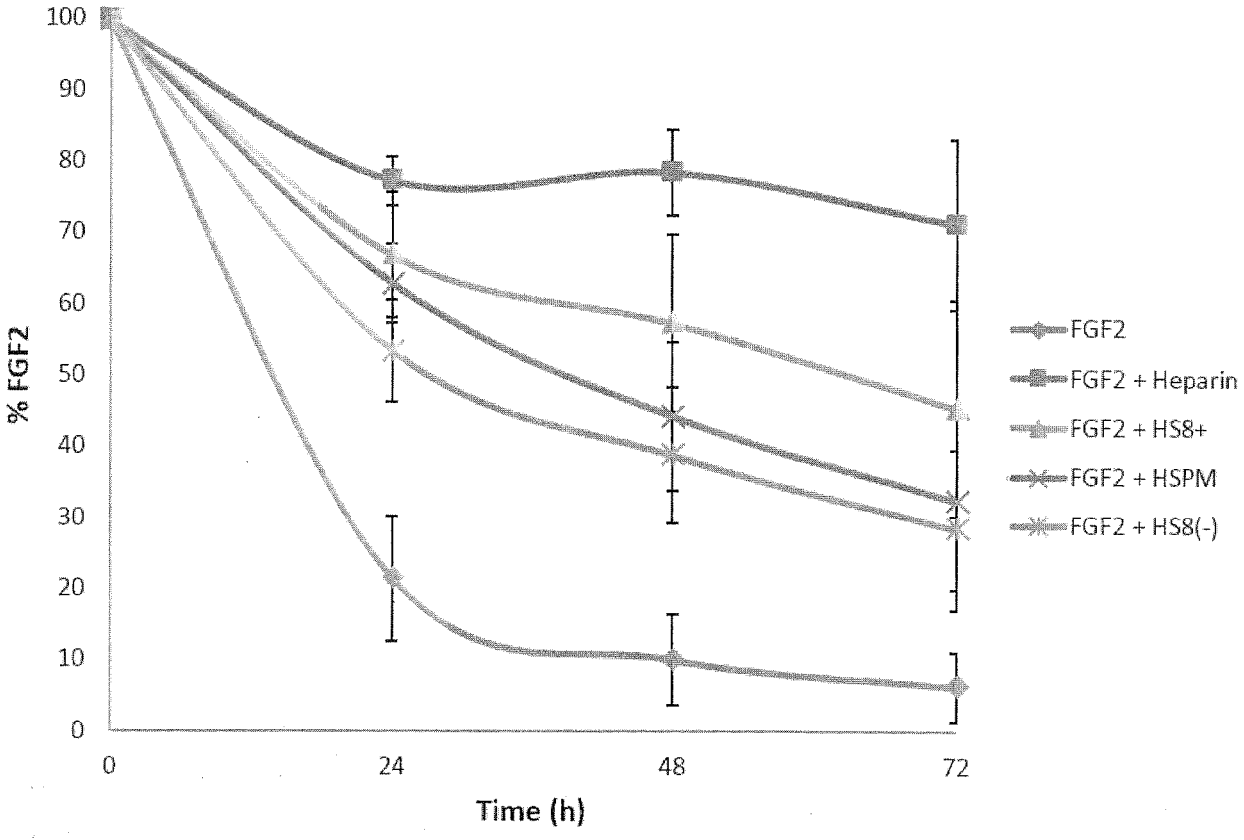



Figure 28

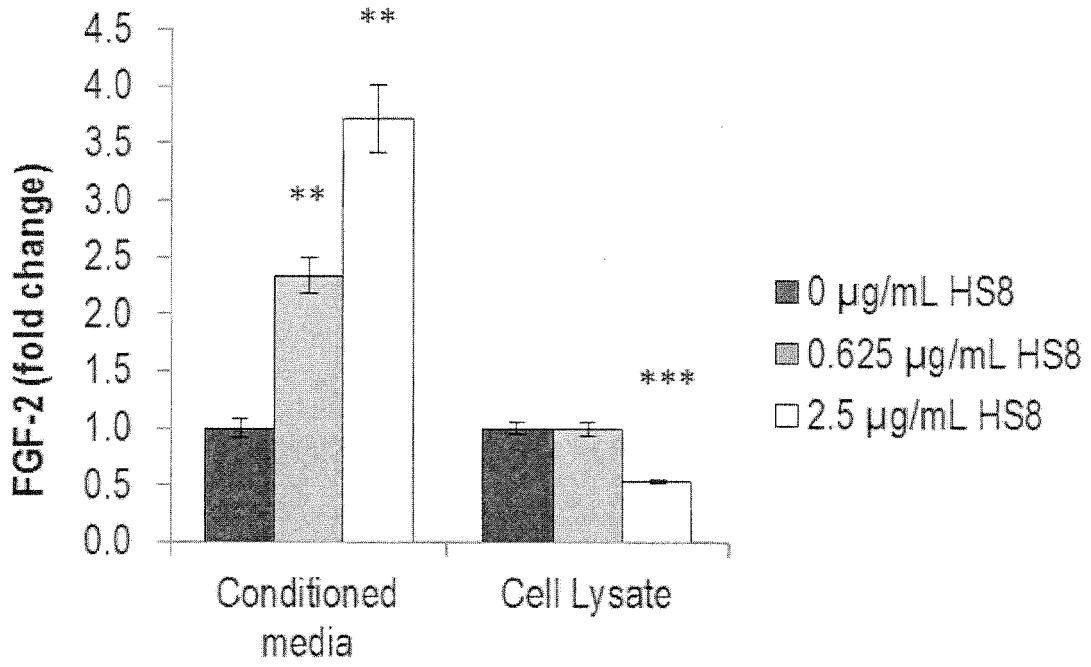



Figure 29

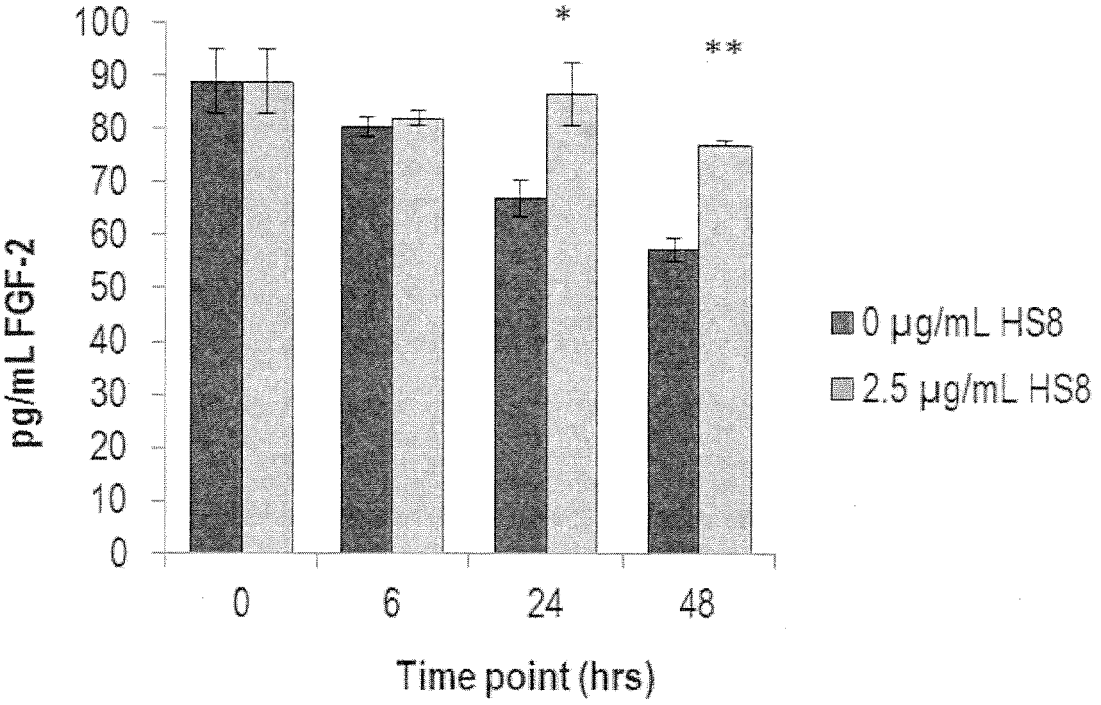



Figure 30

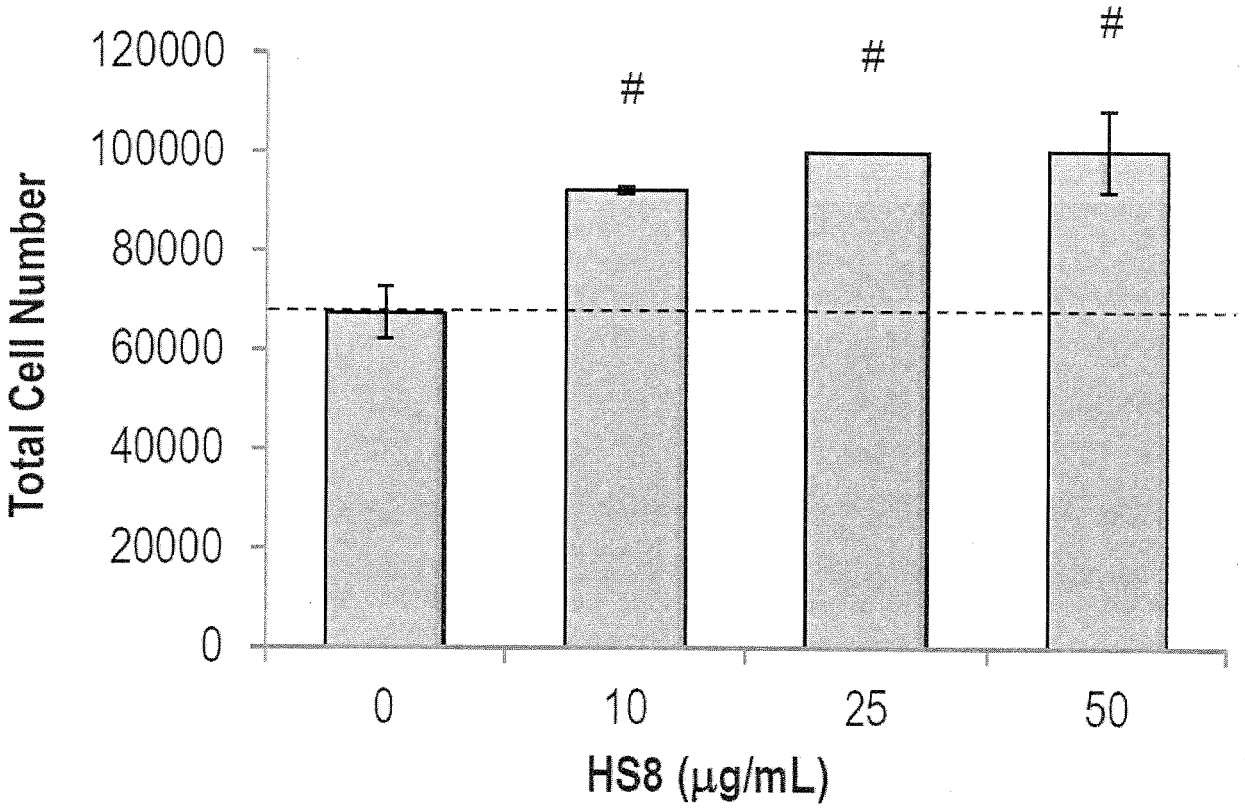

120000
100000
80000
60000
40000
20000
0
Total Cell Number
#
#
#
0
10
25
50
HS8 (µg/mL)

Figure 31

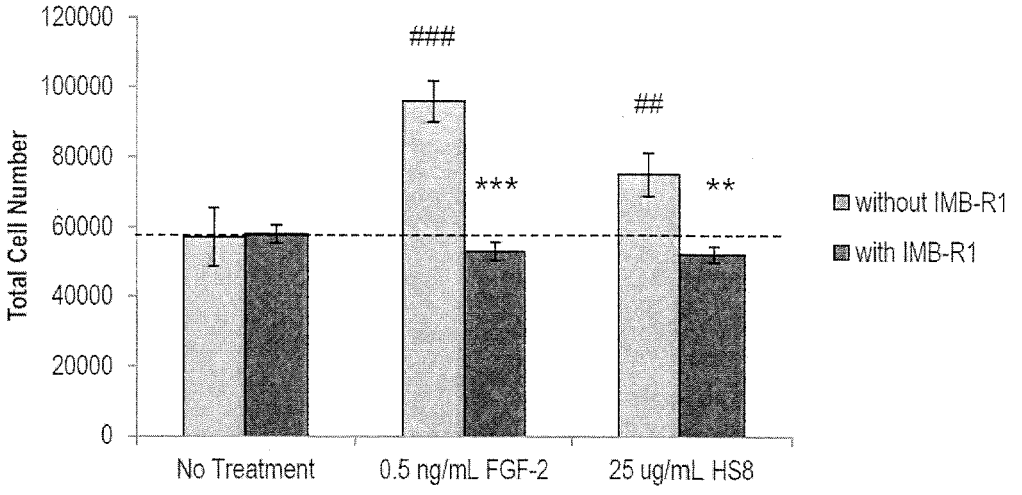



Figure 32A

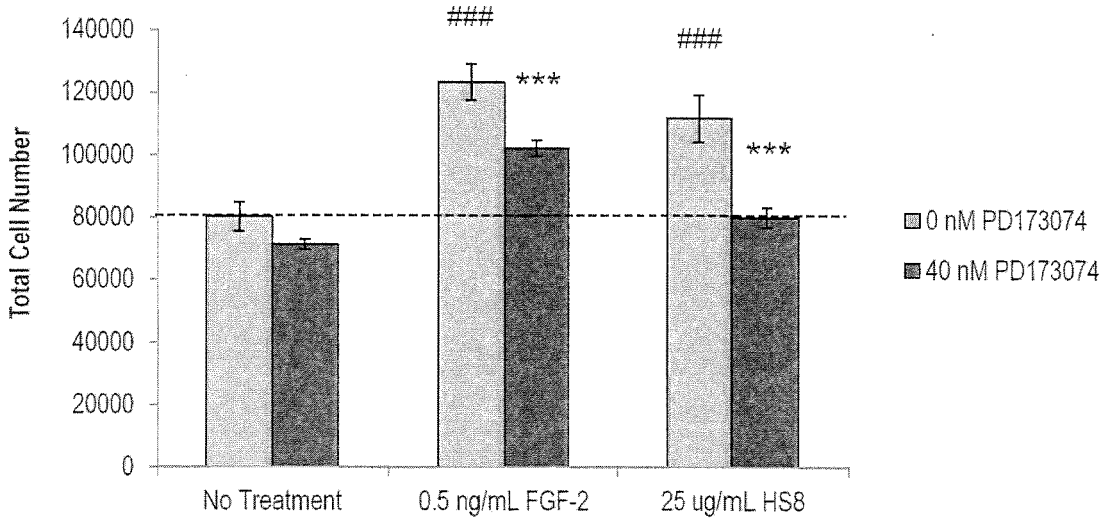



Figure 32B

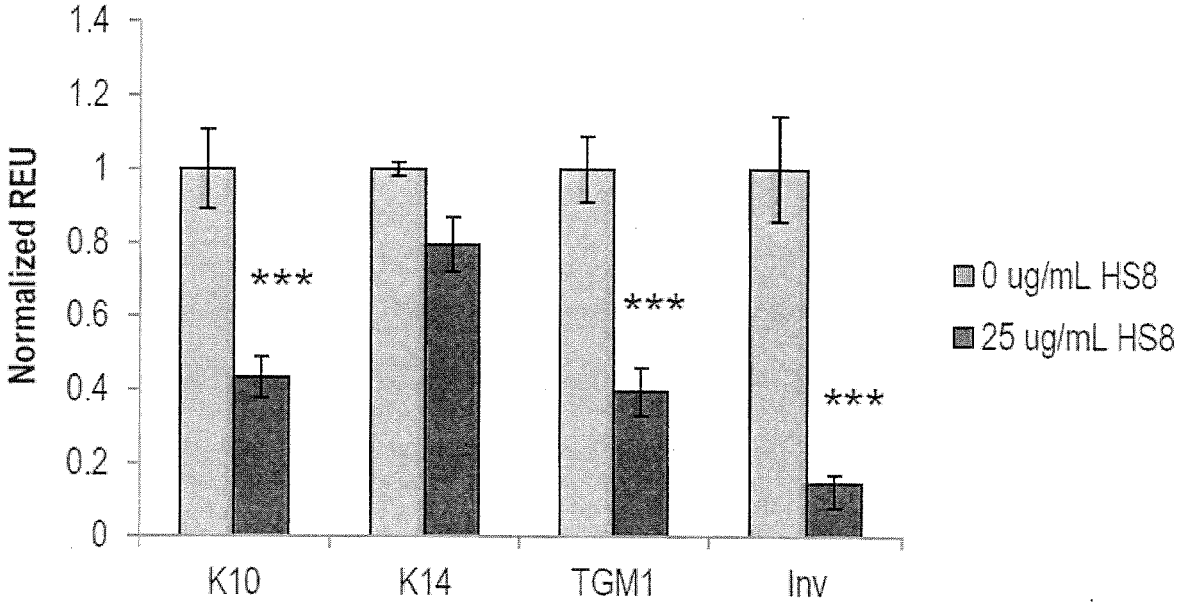



Figure 33

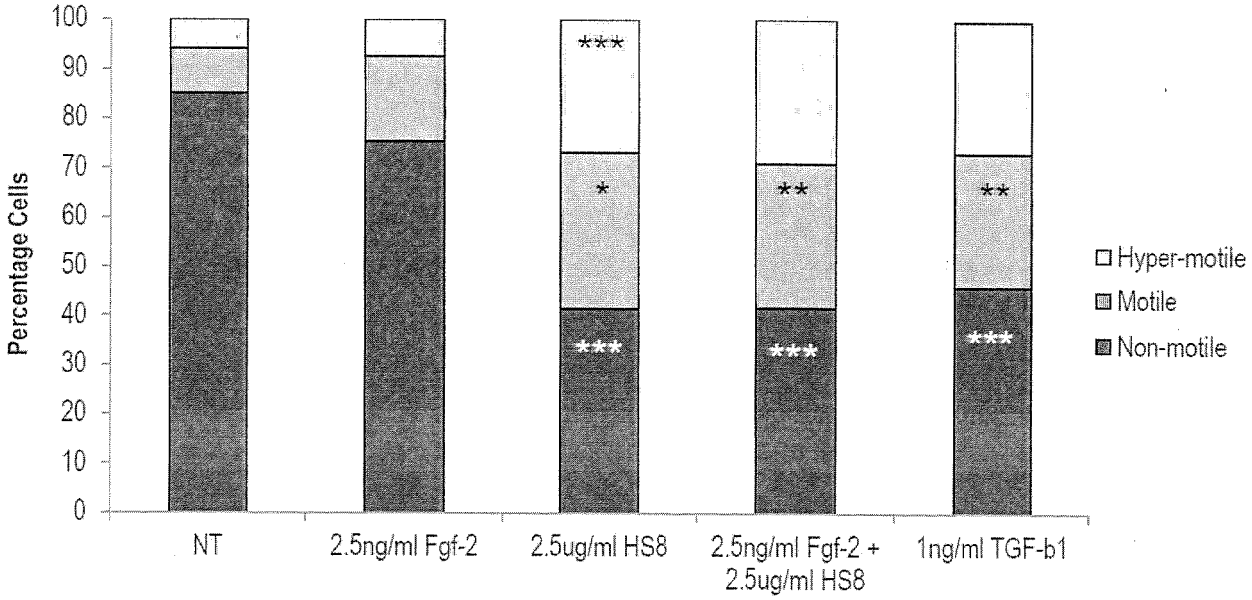



Figure 34

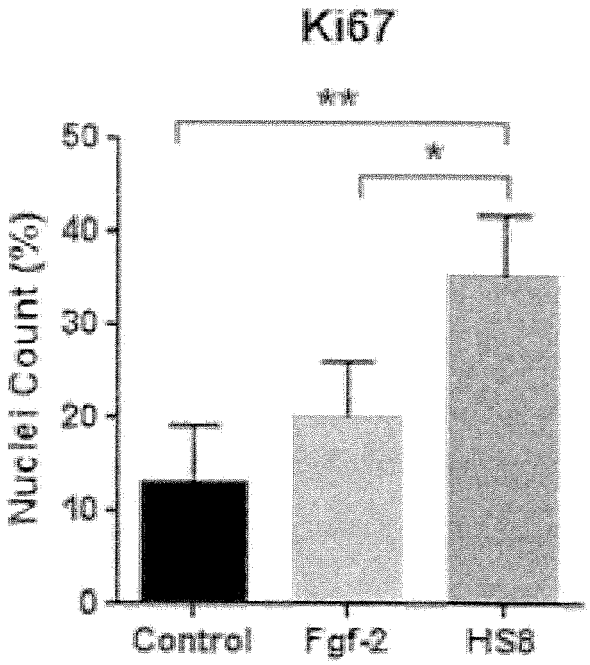



Figure 35A

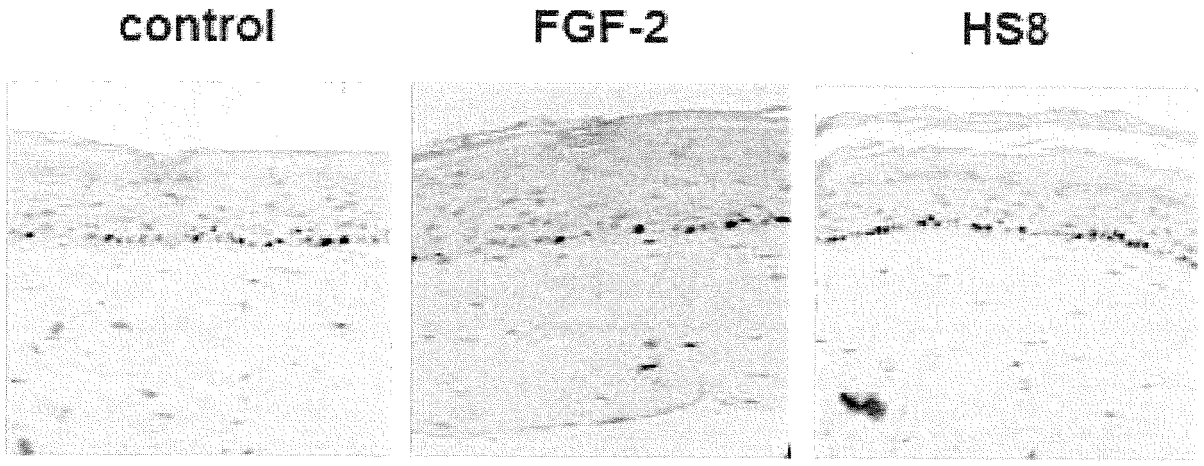



Figure 35B

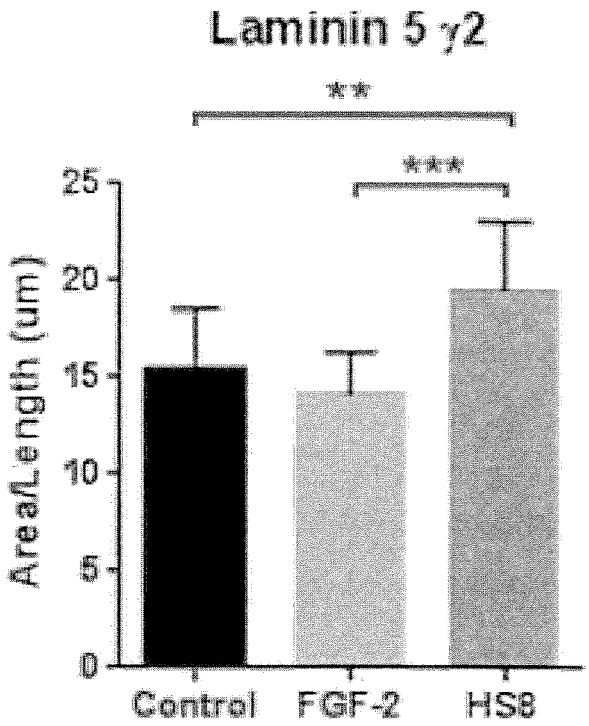



Figure 36A

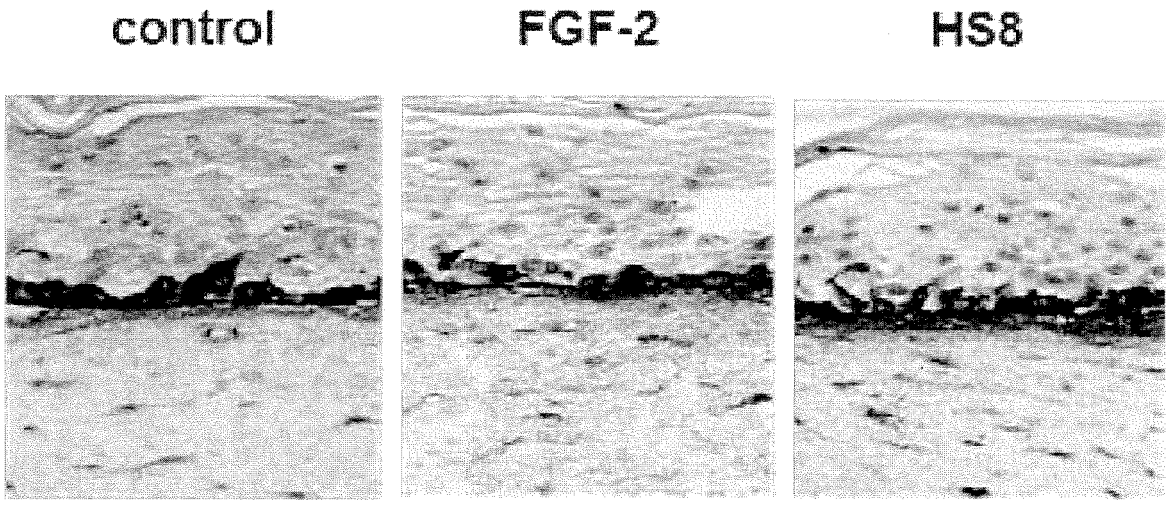



Figure 36B

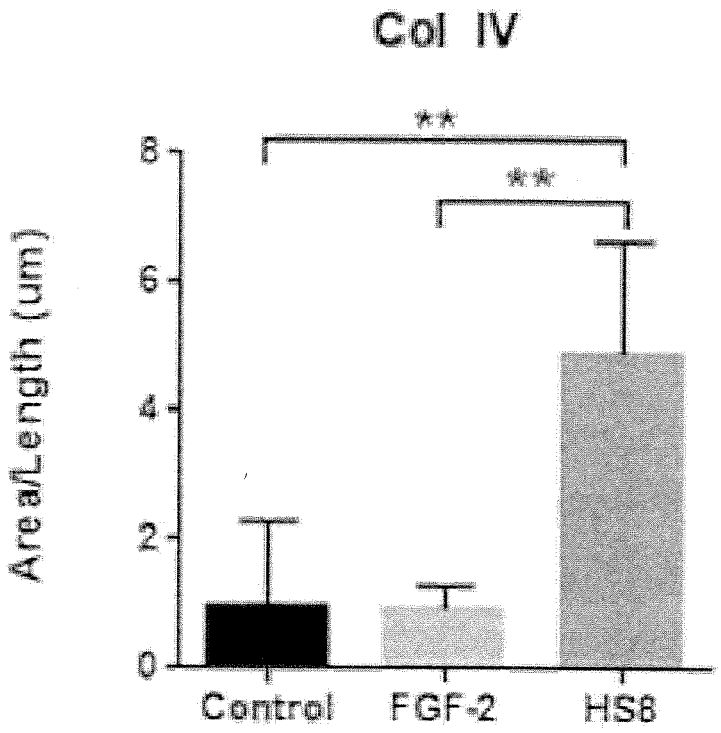



Figure 37A

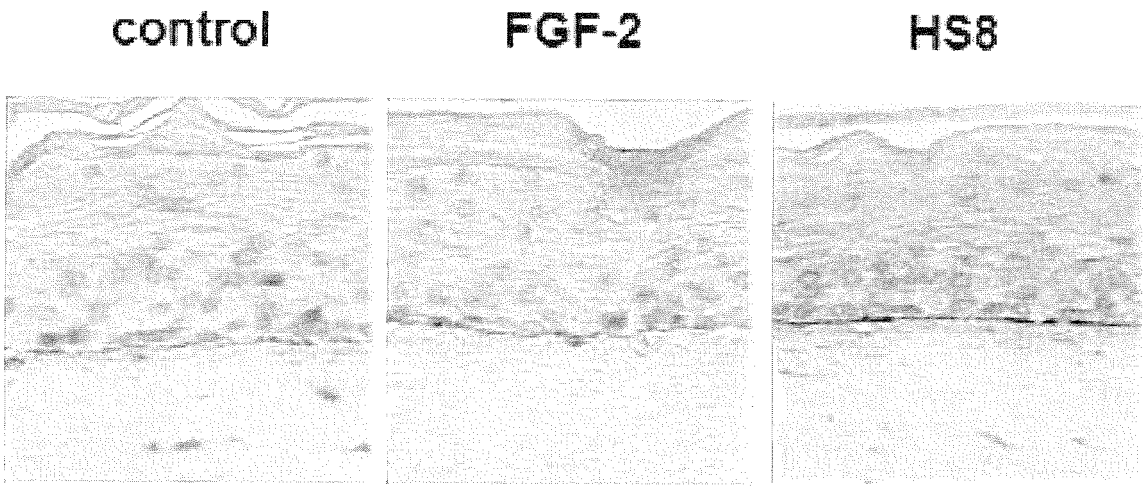



Figure 37B