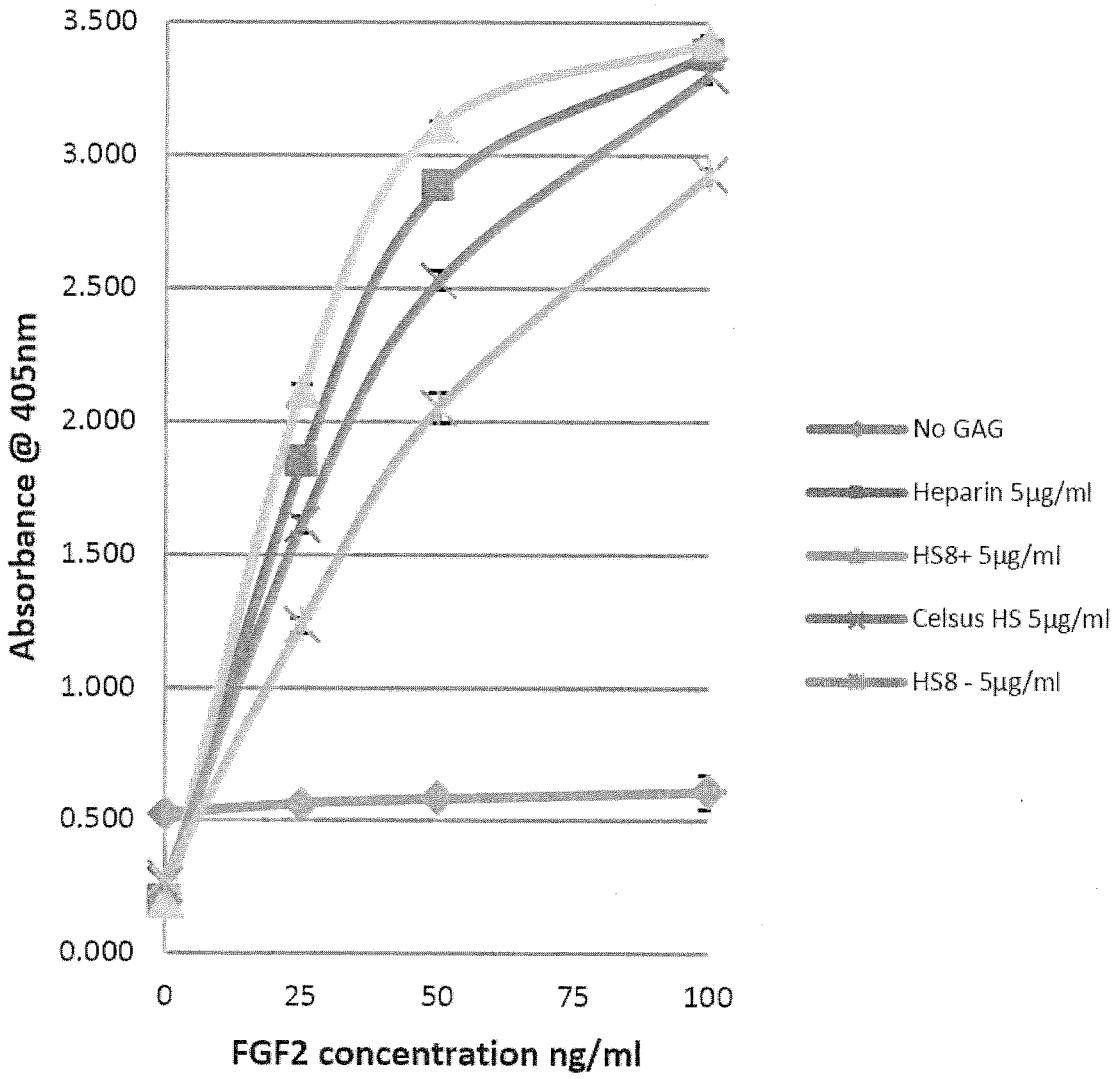
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
No GAG
Heparin 5µg/ml
HS8+ 5µg/ml
Celsus HS 5µg/ml
HS8 - 5µg/ml

Figure 1

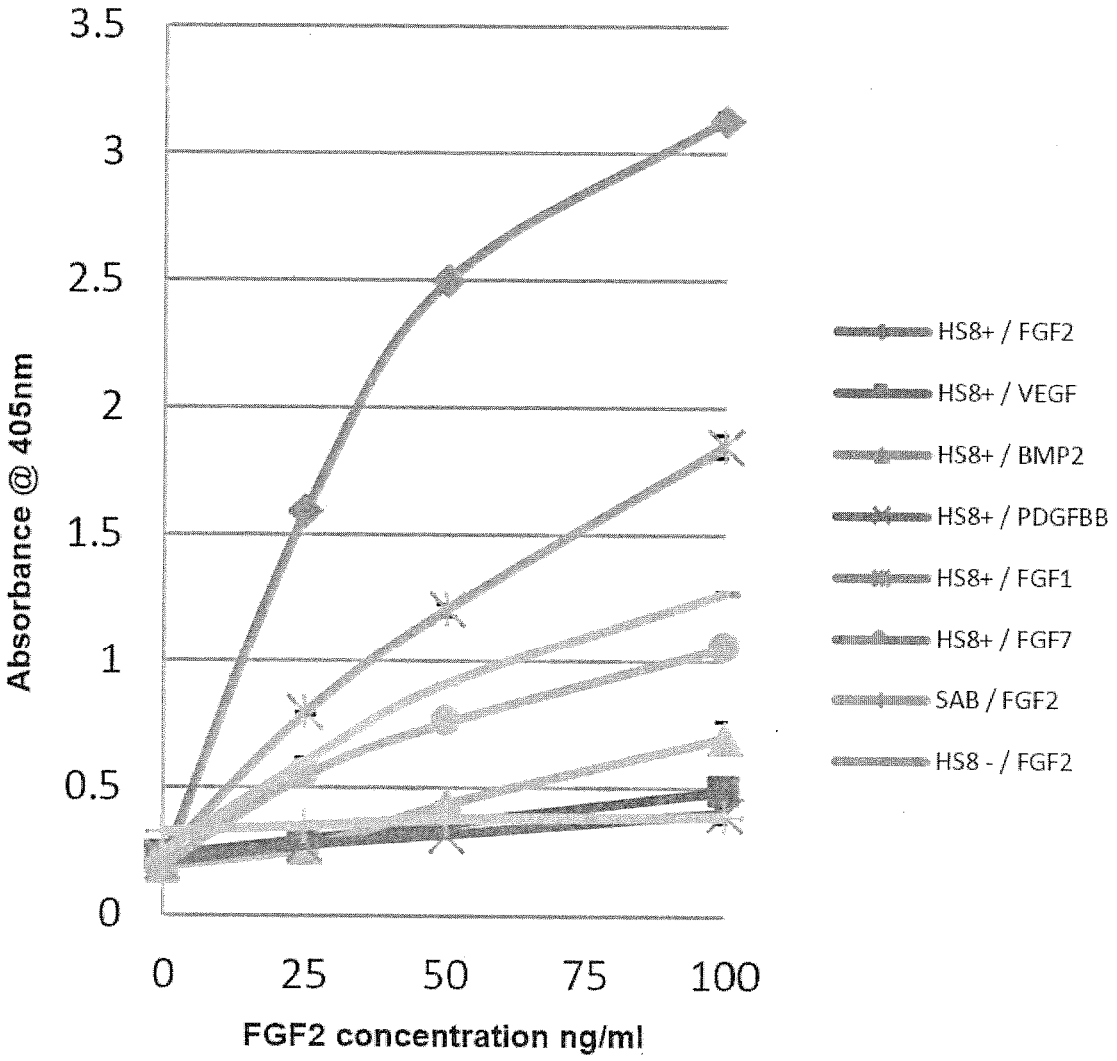
3.5
3
2.5
2
1.5
1
0.5
0
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
HS8+ / FGF2
HS8+ / VEGF
HS8+ / BMP2
HS8+ / PDGFBB
HS8+ / FGF1
HS8+ / FGF7
SAB / FGF2
HS8 - / FGF2

Figure 2

	Residue/ Size	Sequence	Reference
1	**248-254/ 7aa**	^{248}YKRSRYT^{254} -paper 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

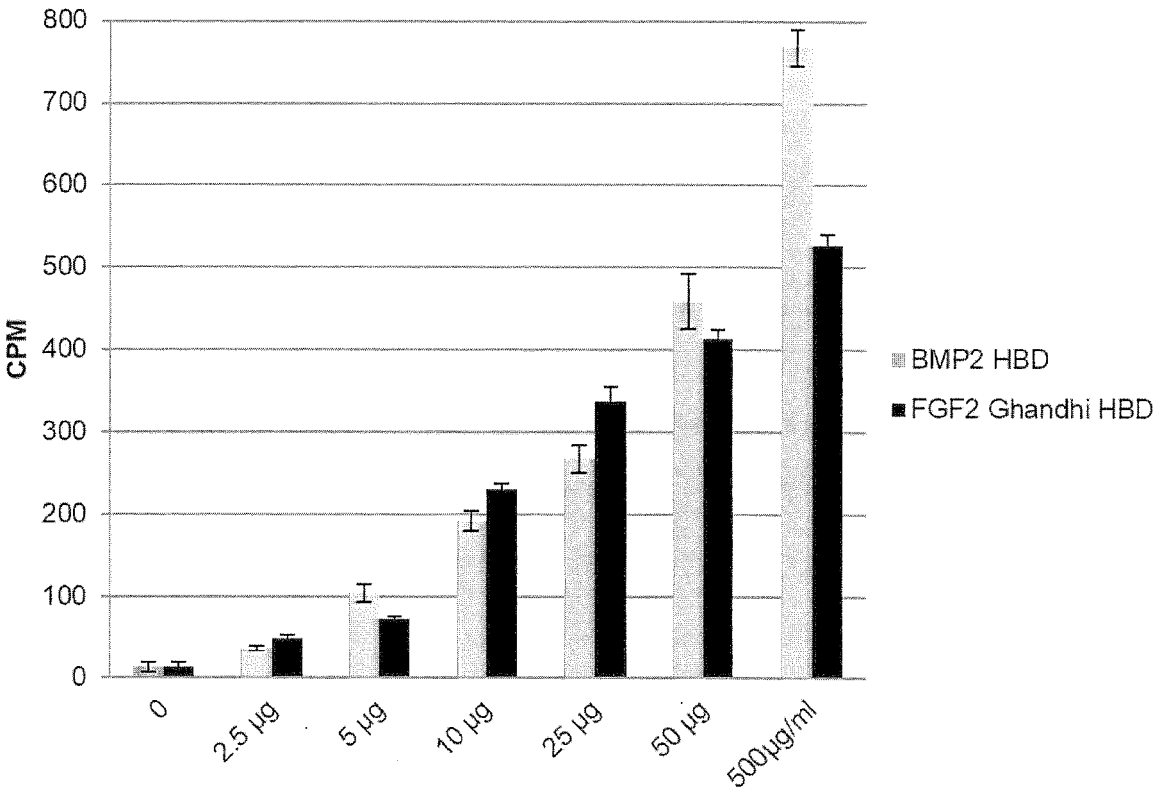
800
700
600
500
400
300
200
100
0
CPM
0
2.5 µg
5 µg
10 µg
25 µg
50 µg
500µg/ml
BMP2 HBD
FGF2 Ghandhi HBD

Figure 4A

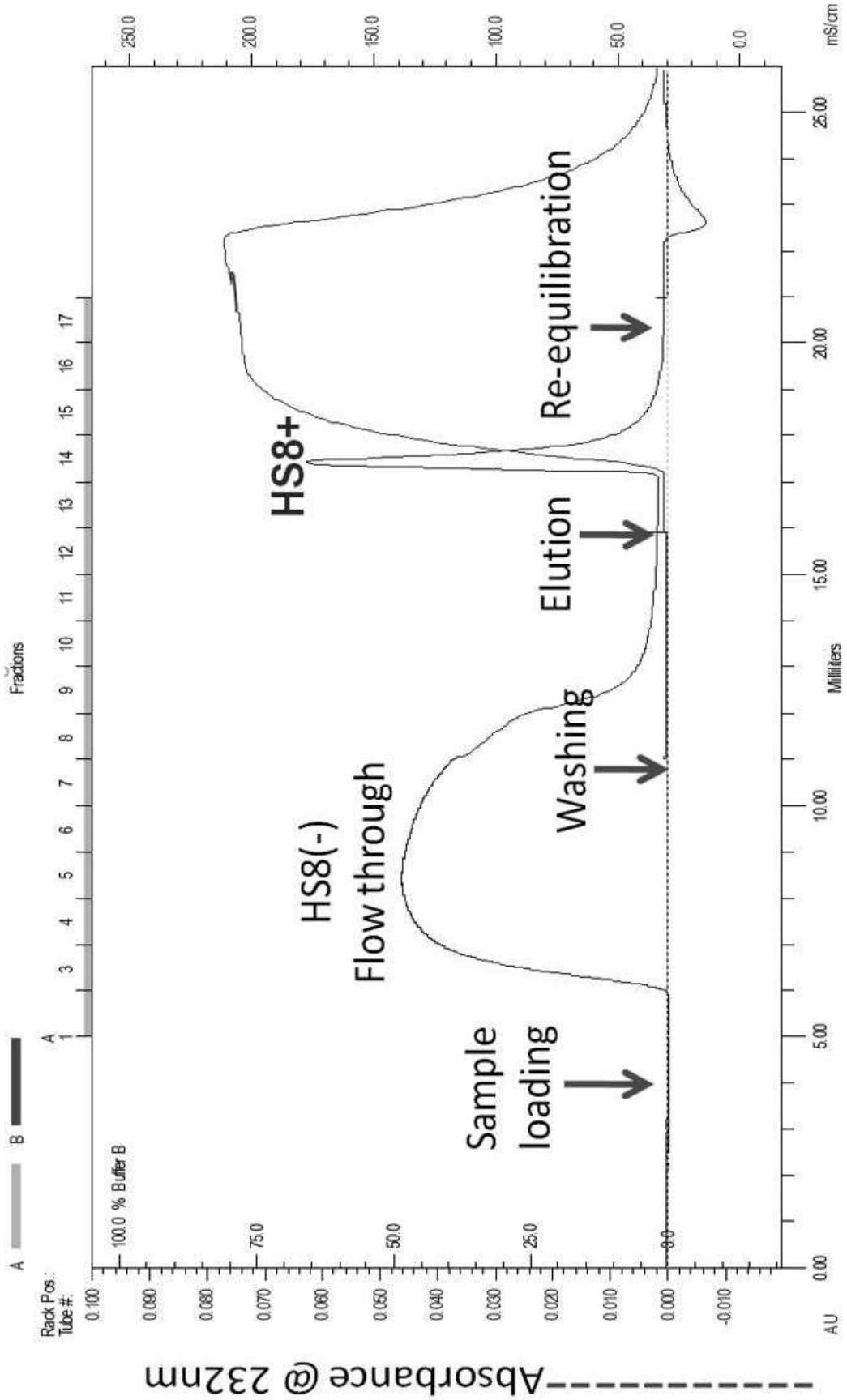
Absorbance @ 232nm
Conductivity
Fractions
Rack Pos.:
Tube #:
A
B
100.0 % Buffer B
AU
mS/cm
Milliliters
Sample loading
HS8(-) Flow through
Washing
Elution
HS8+
Re-equilibration
Fraction number

Figure 4B

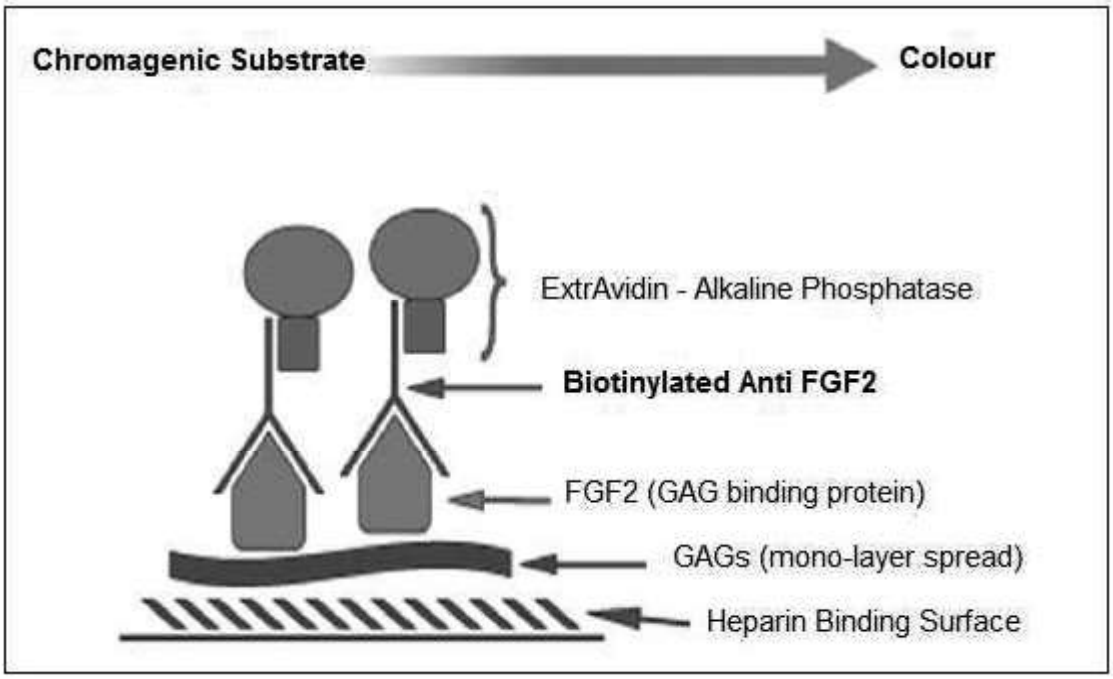
Chromagenic Substrate
Colour
ExtrAvidin - Alkaline Phosphatase
Biotinylated Anti FGF2
FGF2 (GAG binding protein)
GAGs (mono-layer spread)
Heparin Binding Surface

Figure 5

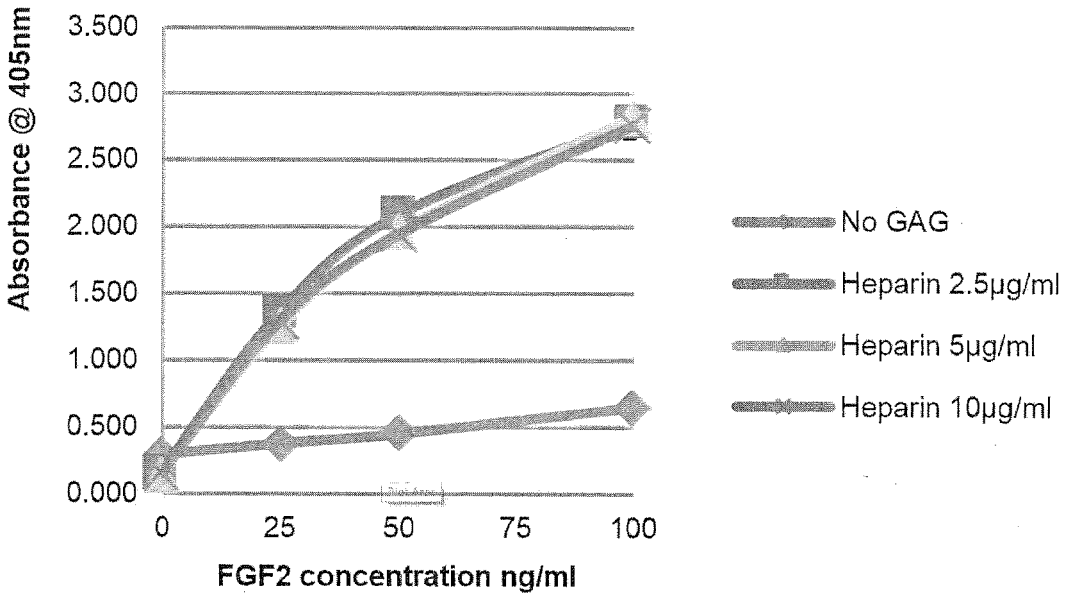

Figure 6A

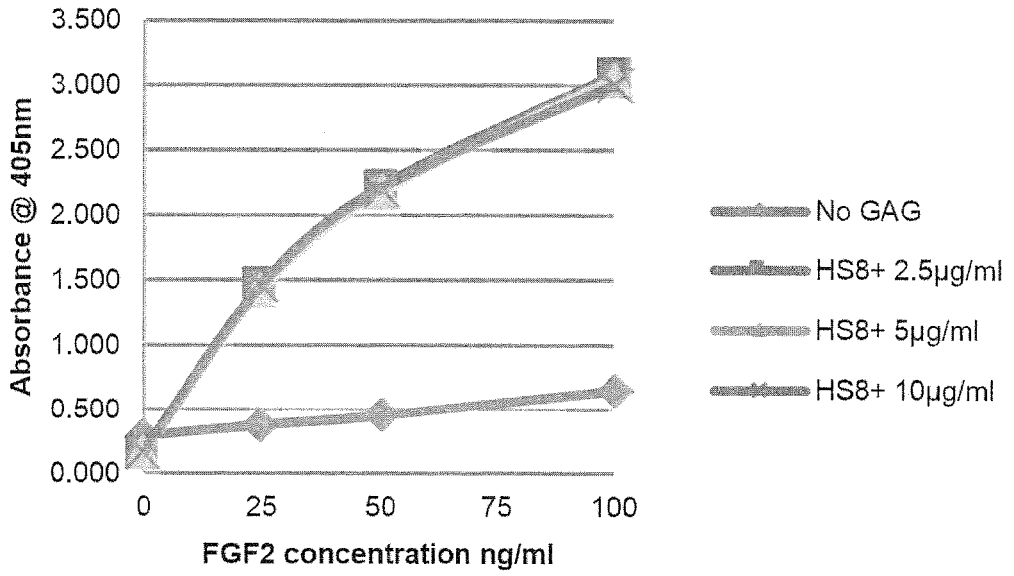

Figure 6B

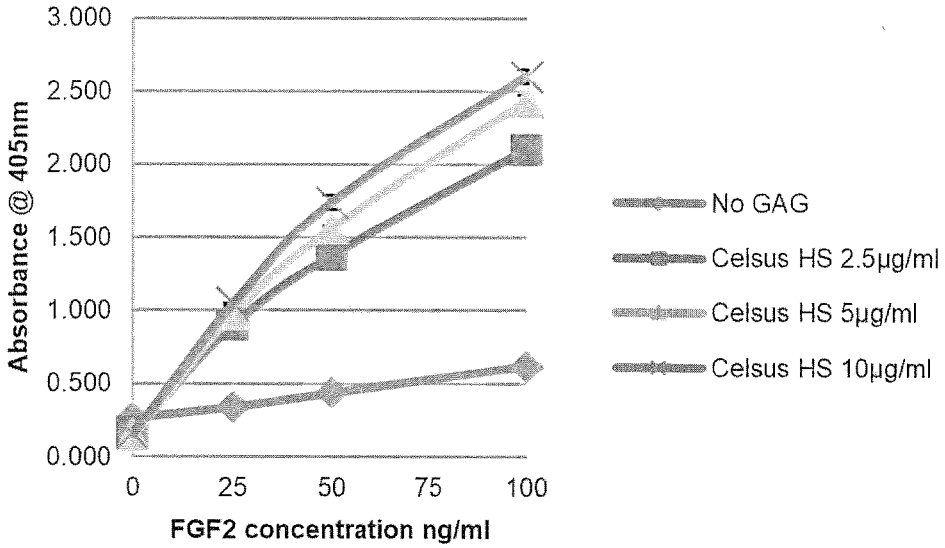

Figure 6C

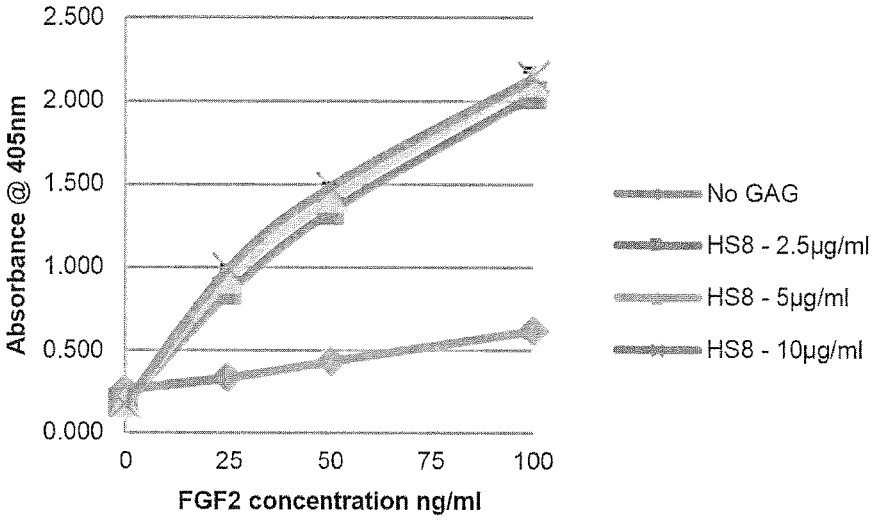

Figure 6D

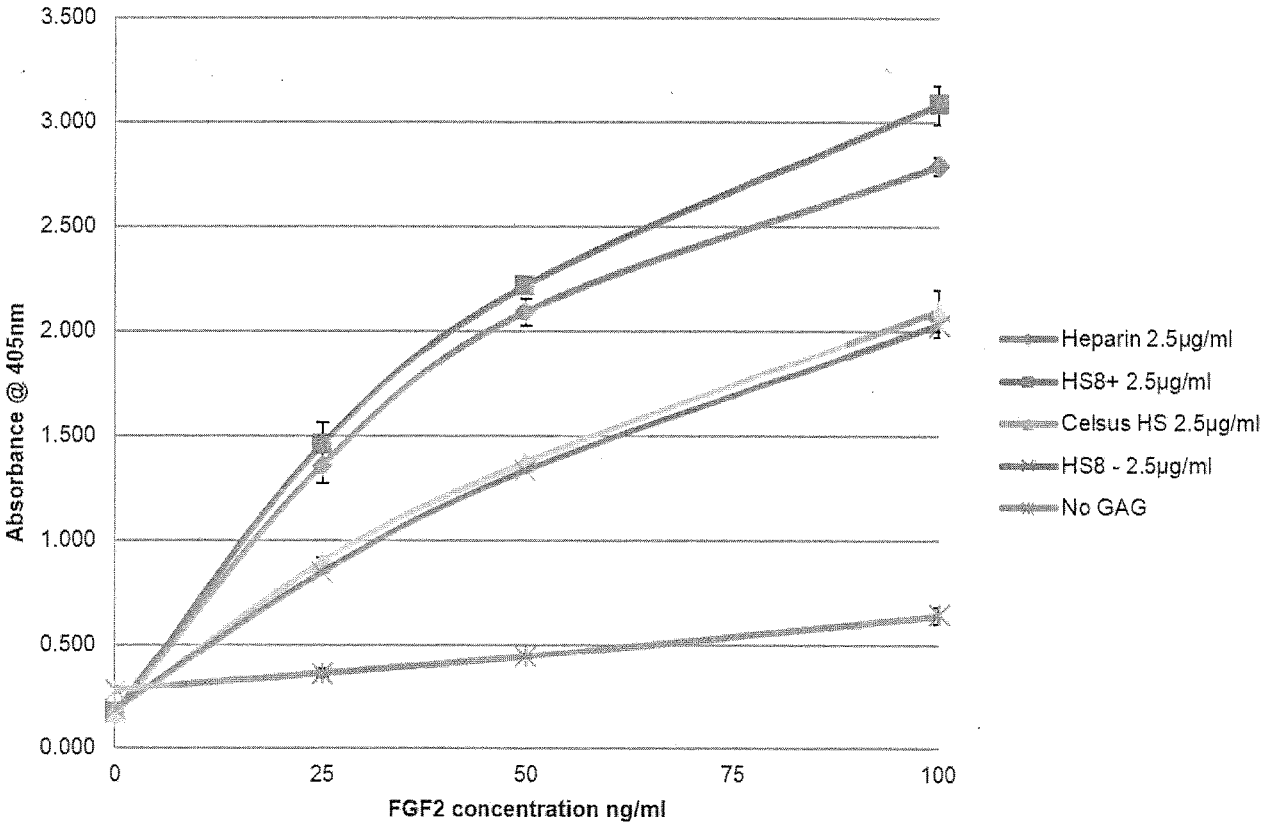
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Absorbance @ 405nm
0
25
50
75
100
FGF2 concentration ng/ml
Heparin 2.5µg/ml
HS8+ 2.5µg/ml
Celsus HS 2.5µg/ml
HS8 - 2.5µg/ml
No GAG

Figure 7

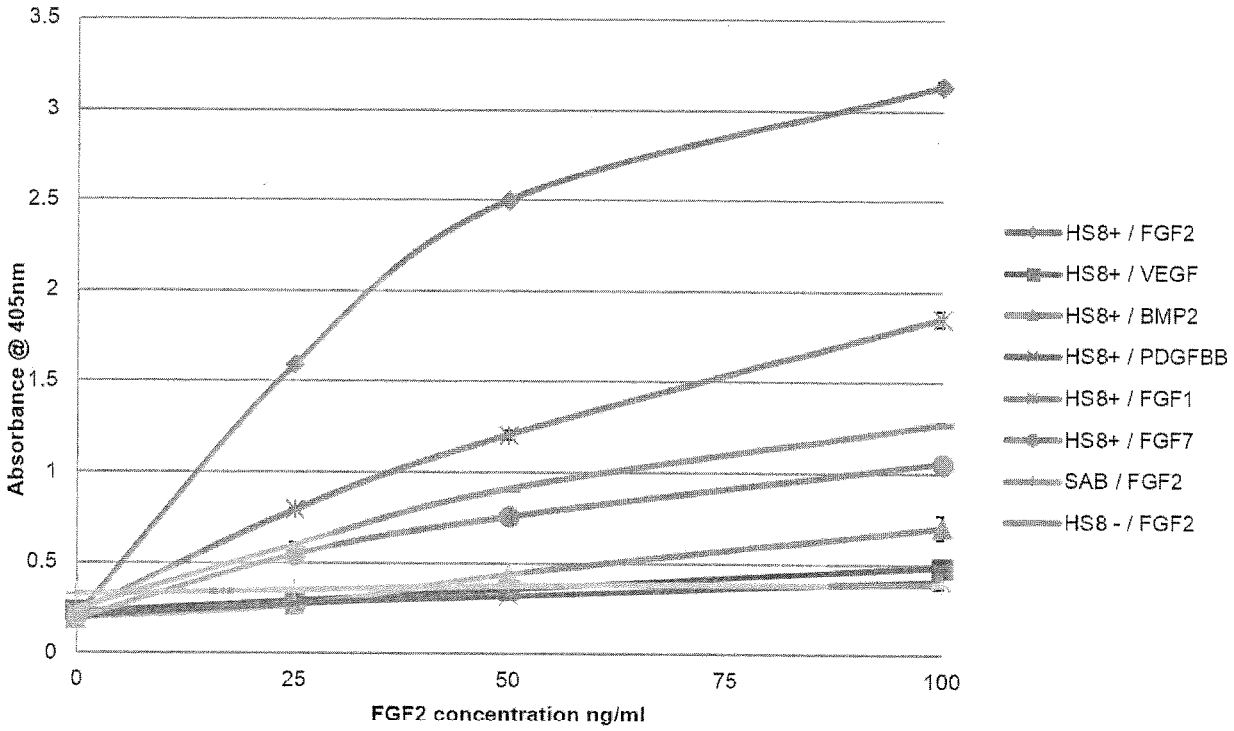

Figure 8A

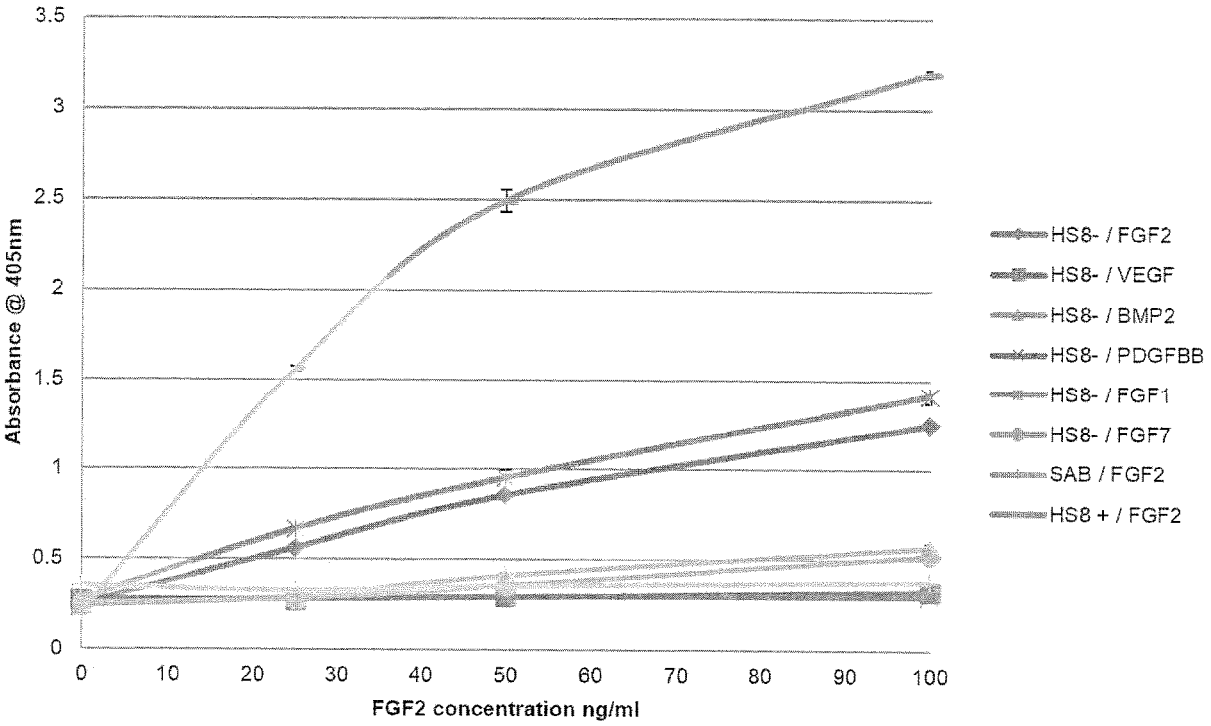

Figure 8B

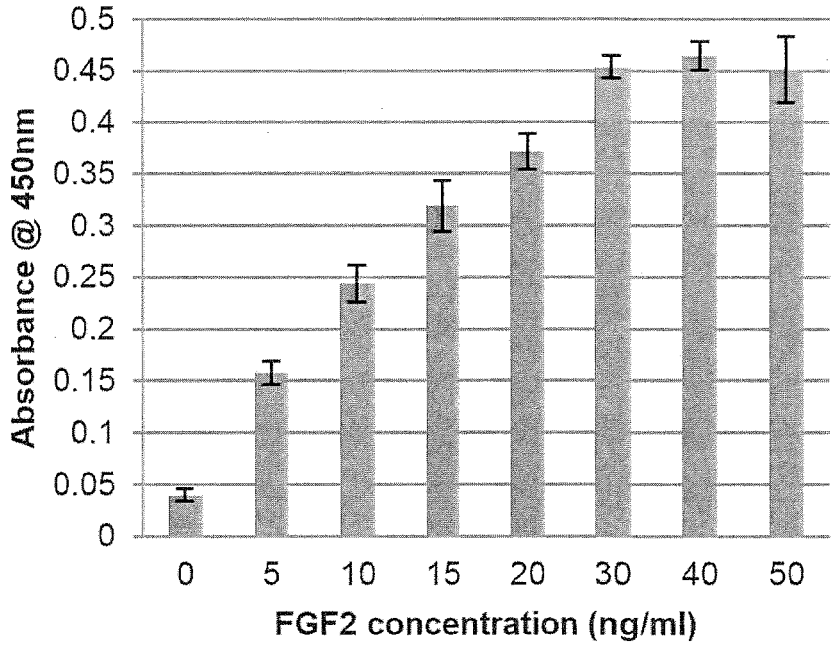

Figure 9A

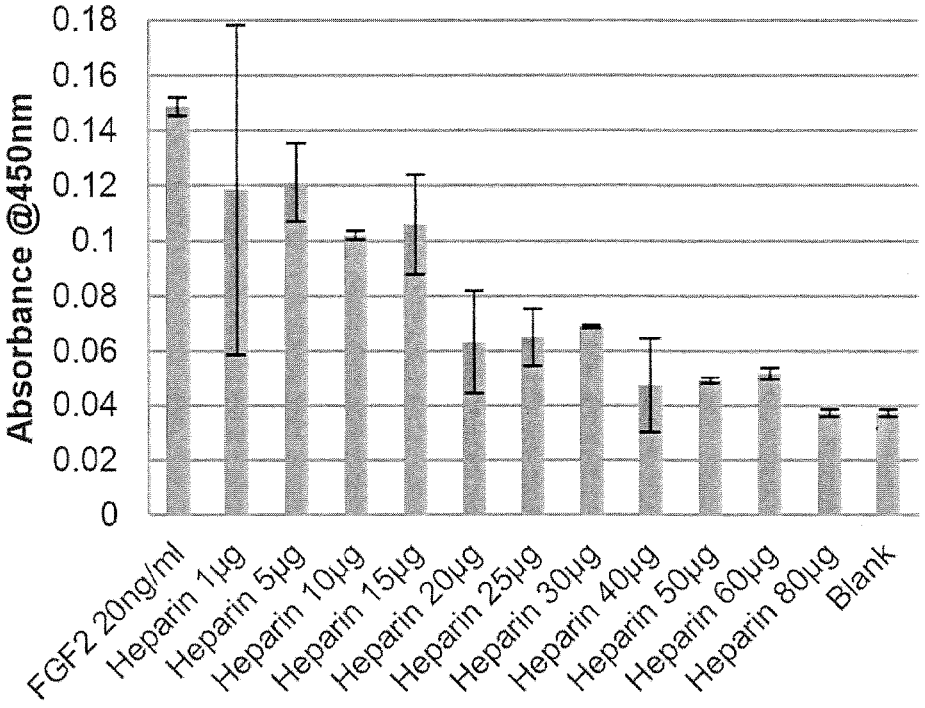

Figure 9B

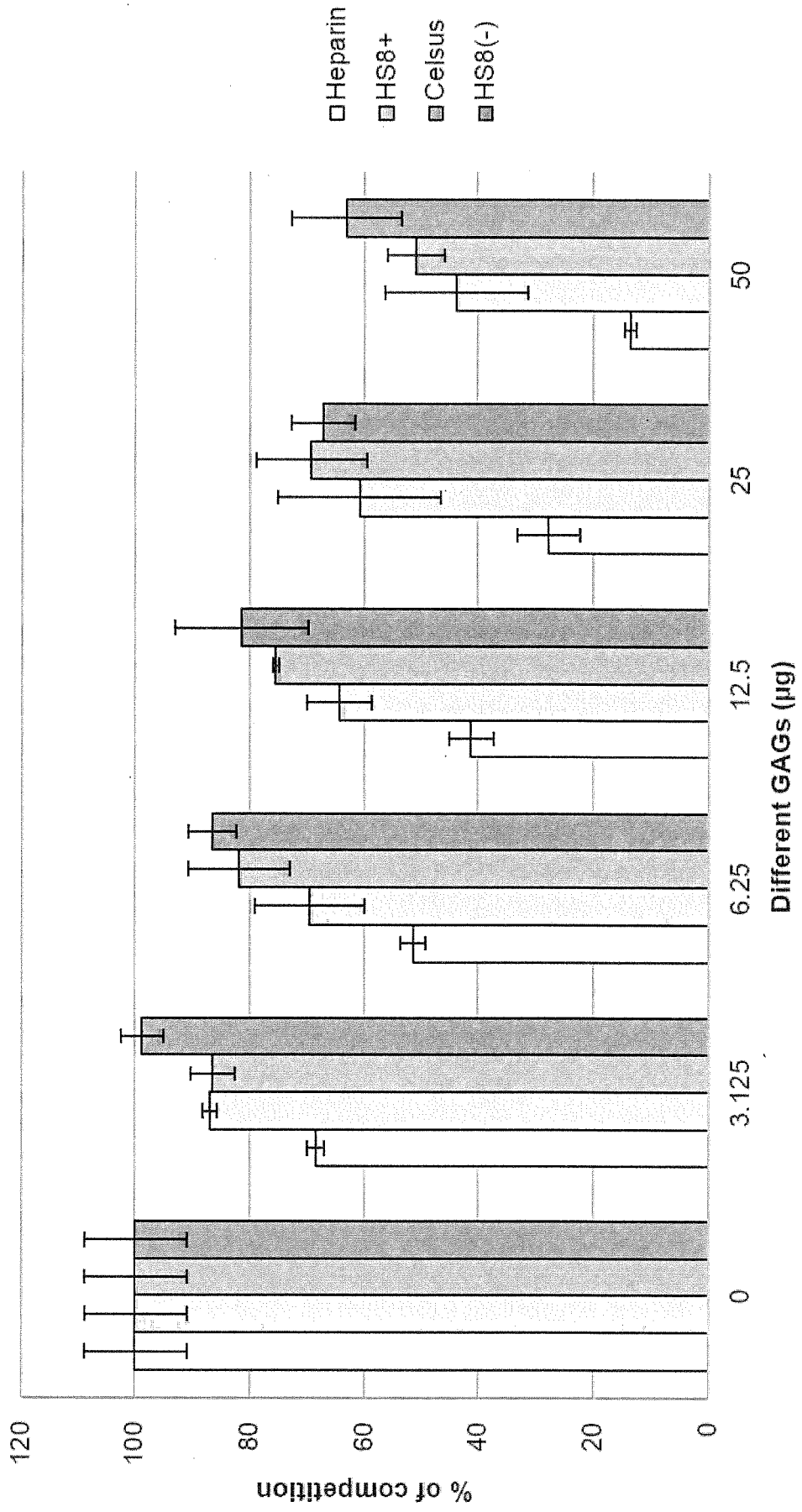
Heparin
HS8+
Celsus
HS8(-)
% of competition
120
100
80
60
40
20
0
0
3.125
6.25
12.5
25
50
Different GAGs (µg)

Figure 9C

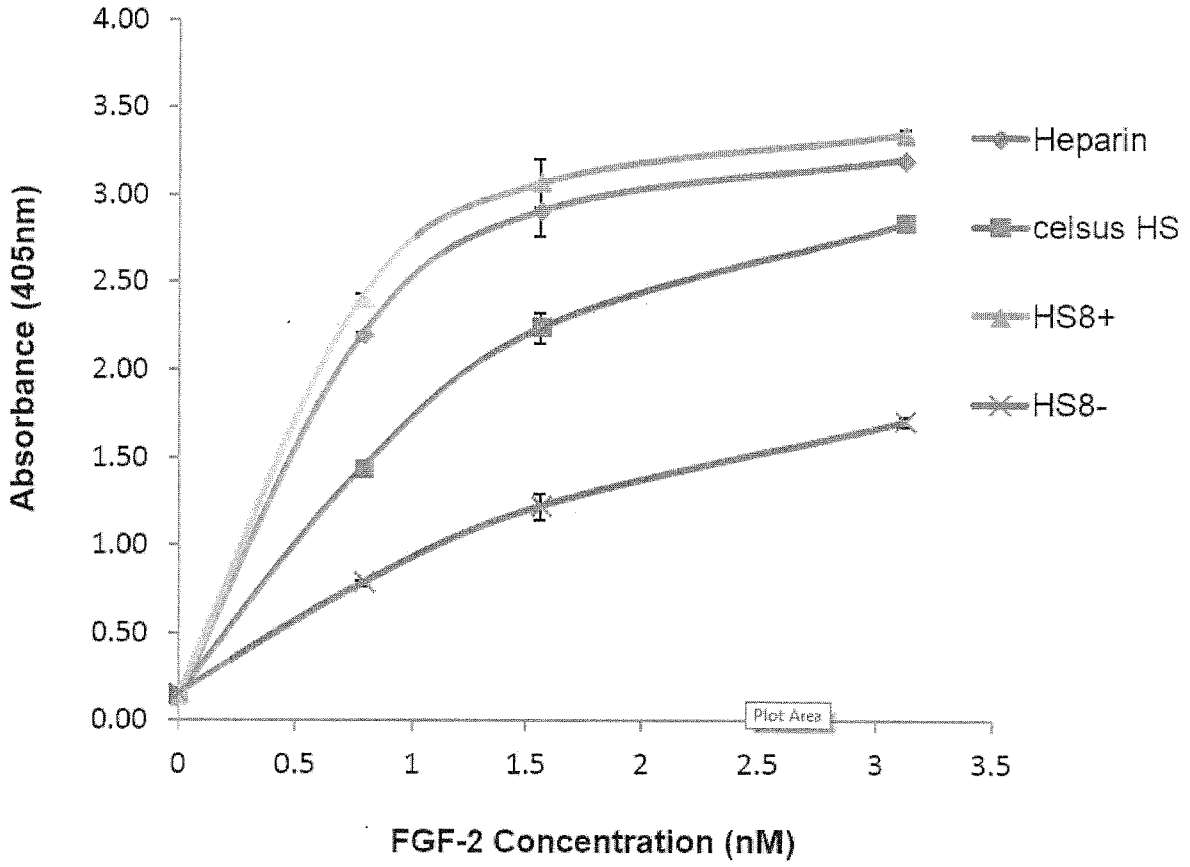
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Absorbance (405nm)
0
0.5
1
1.5
2
2.5
3
3.5
FGF-2 Concentration (nM)
Plot Area
Heparin
celsus HS
HS8+
HS8-

Figure 10

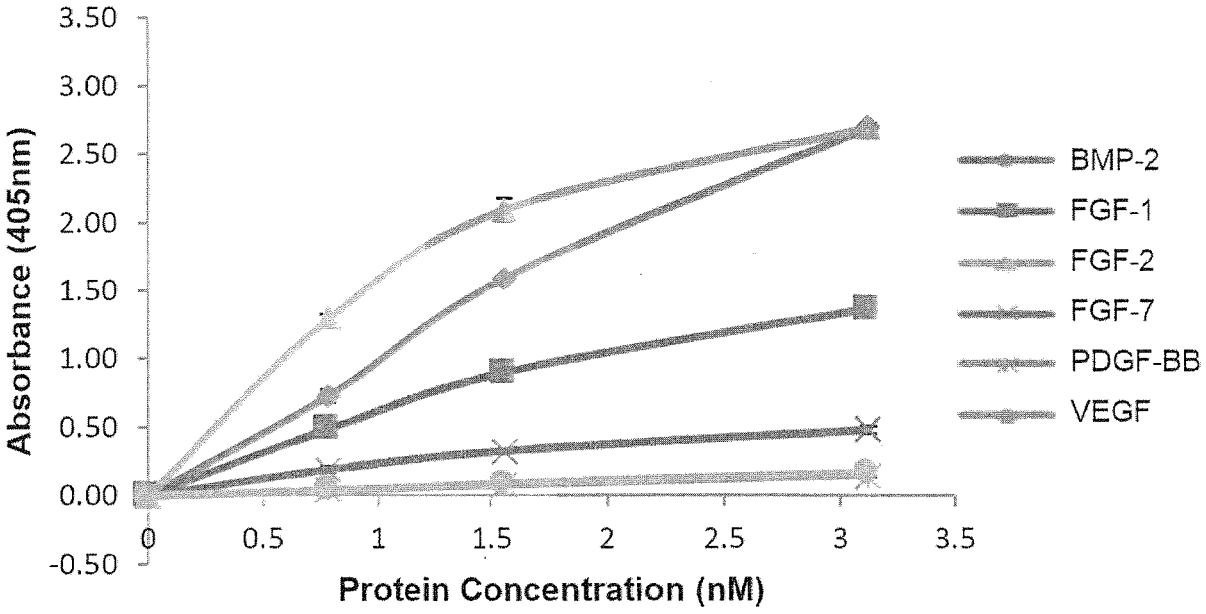

Figure 11A

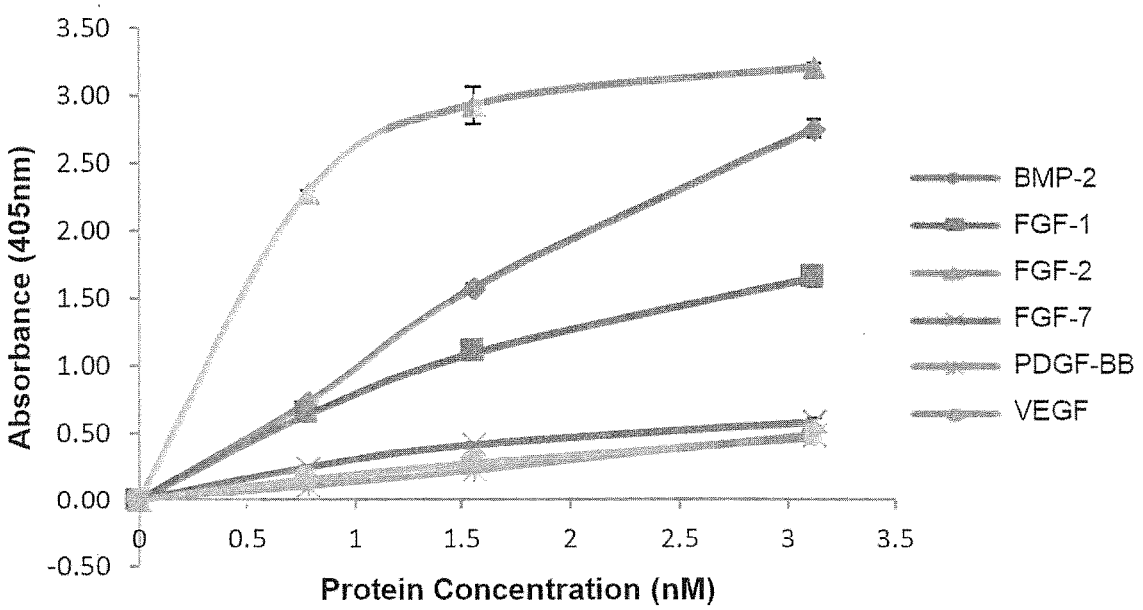

Figure 11B

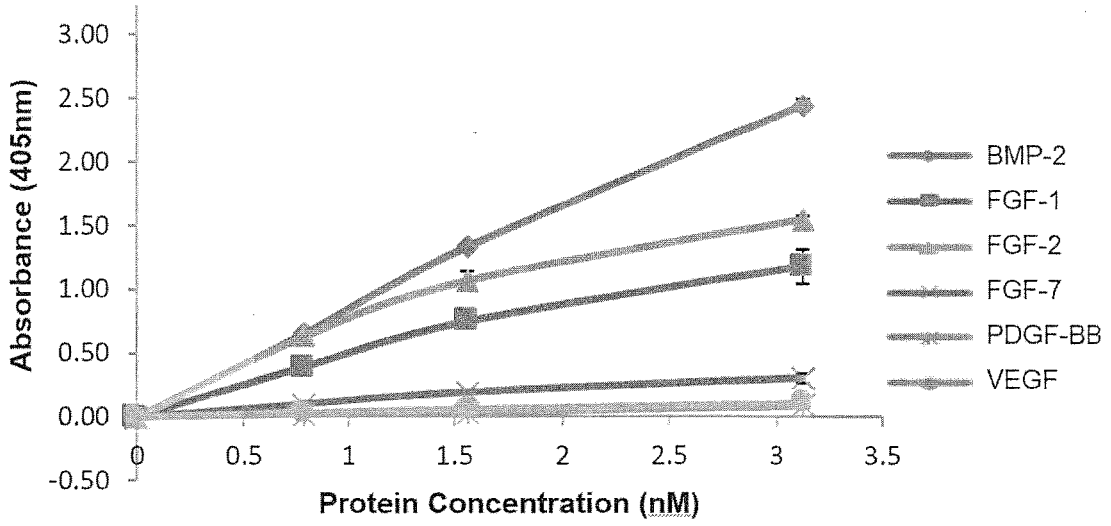

Figure 11C

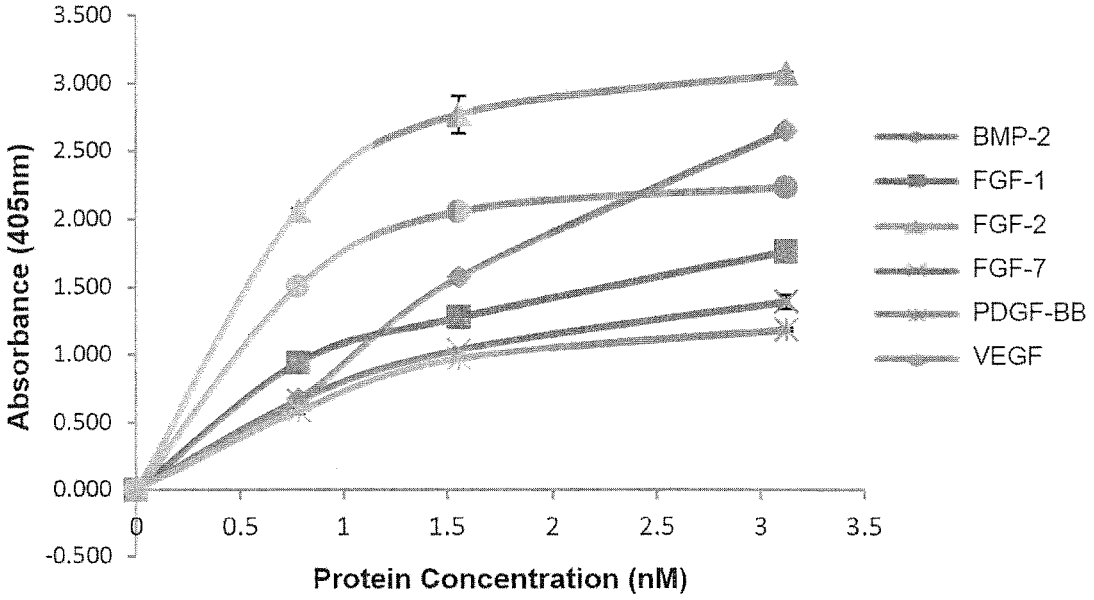

Figure 11D

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

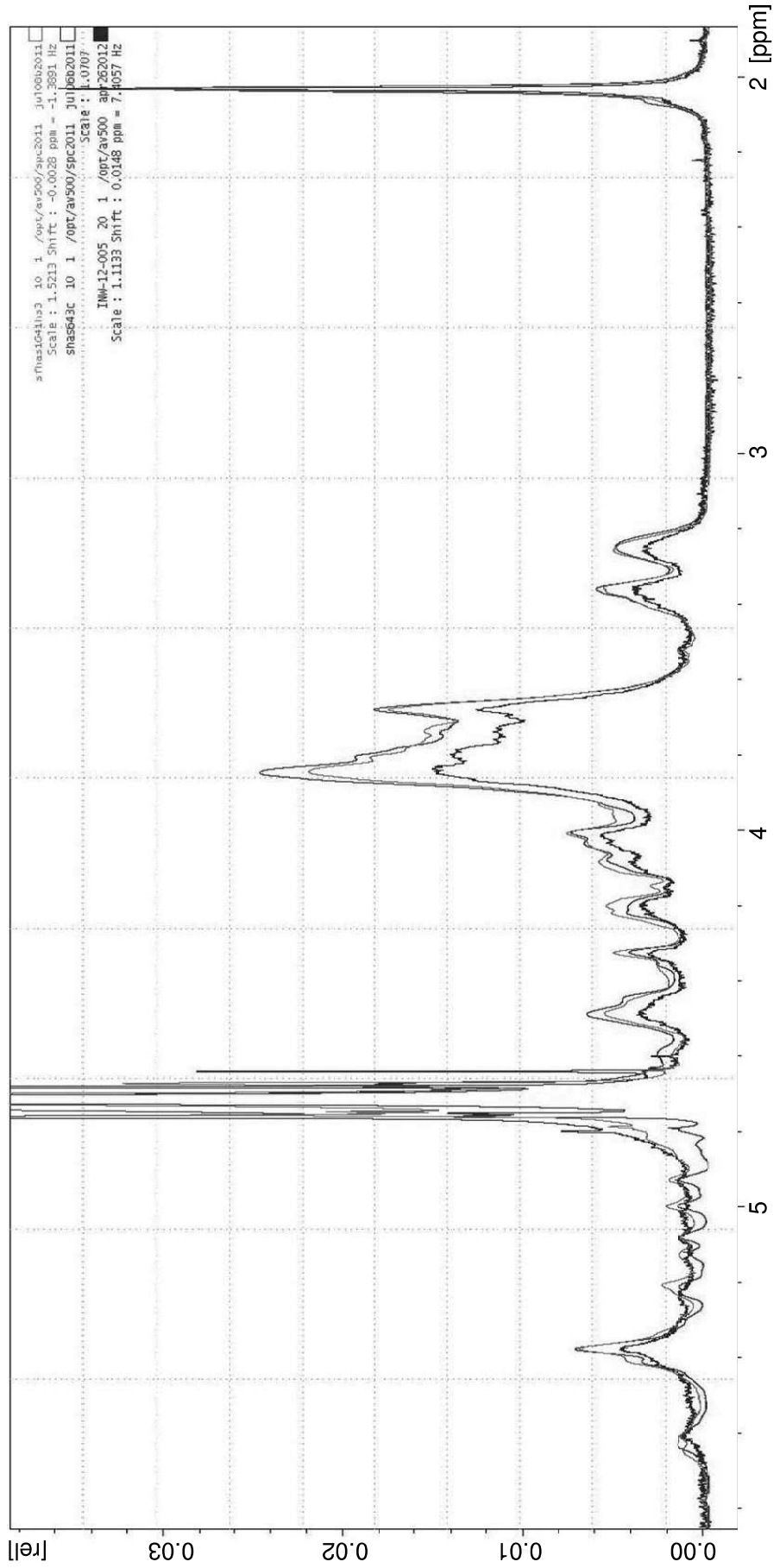
[rel]
0.00
0.01
0.02
0.03
5
4
3
2 [ppm]
IMW-12-005 20 1 /opt/av500 apr262012
Scale : 1.1133 Shift : 0.0148 ppm = 7.4057 Hz
shas643c 10 1 /opt/av500/spc2011 jul060
Scale : 1.0707
Scale : 1.5213 Shift : -0.0028 ppm = -1.3891 Hz

Figure 15

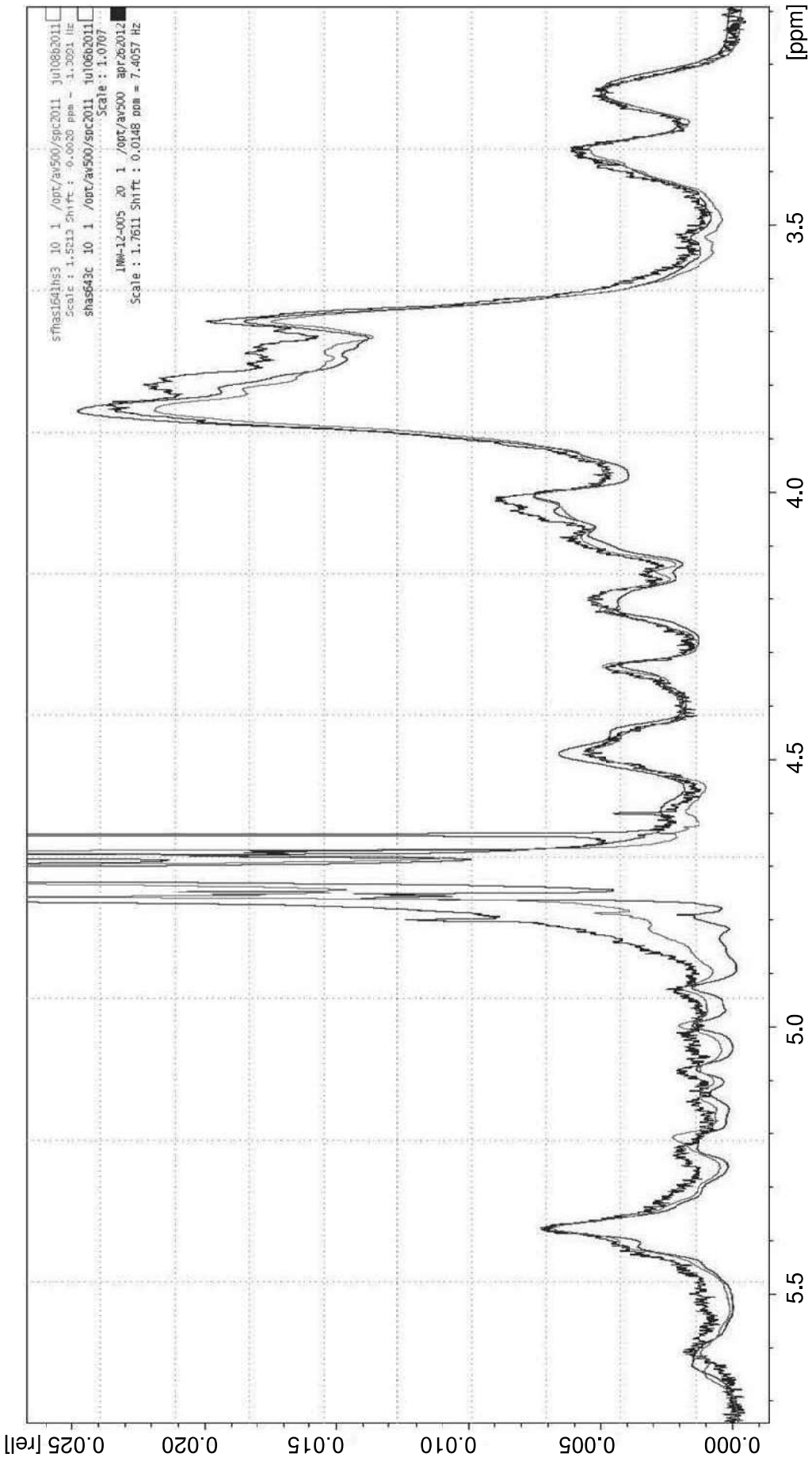

Figure 16

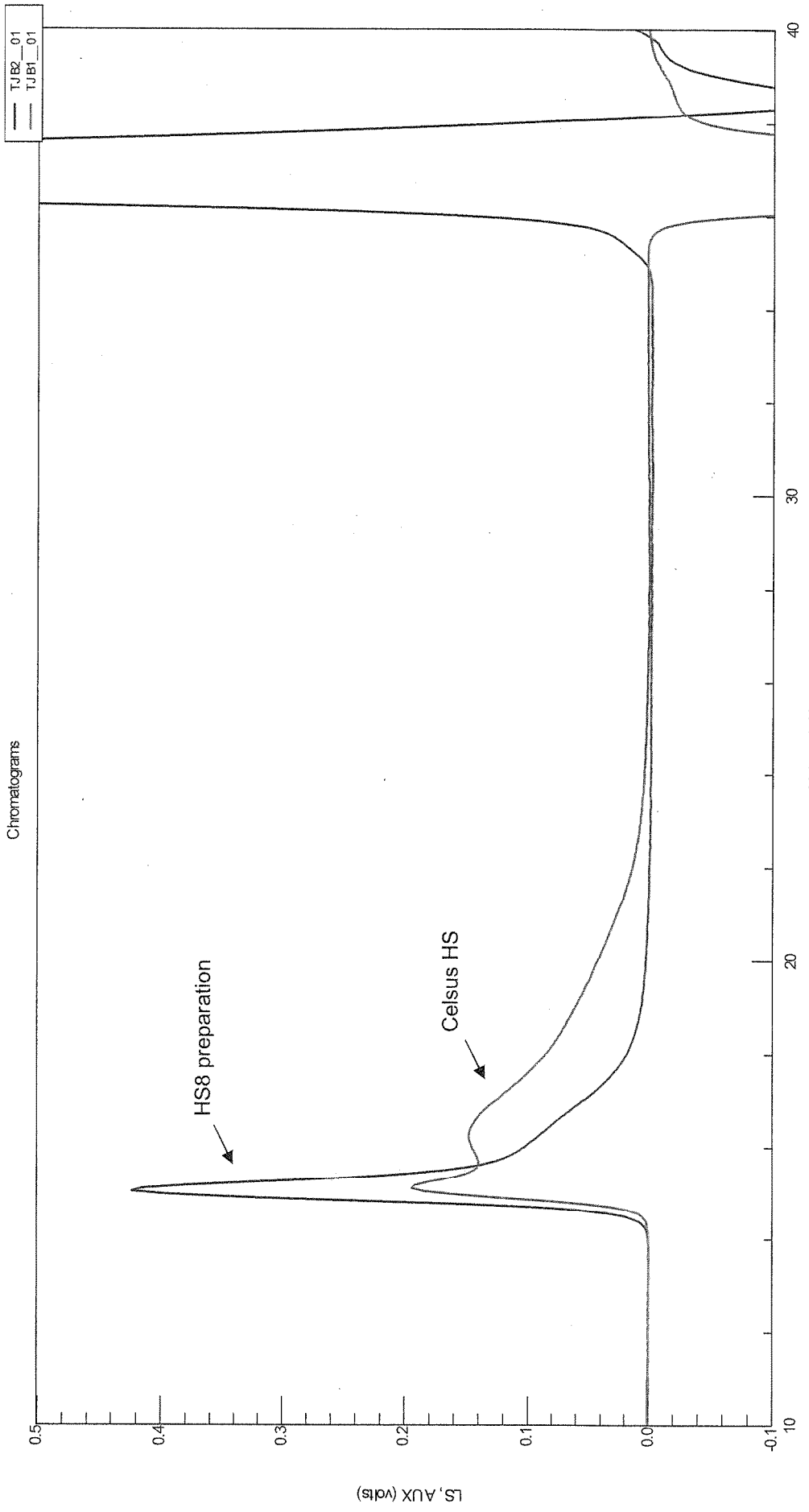
Chromatograms
TJB2__01
TJB1__01
HS8 preparation
Celsus HS
LS, AUX (volts)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
10
20
30
40
Volume (mL)

Figure 17

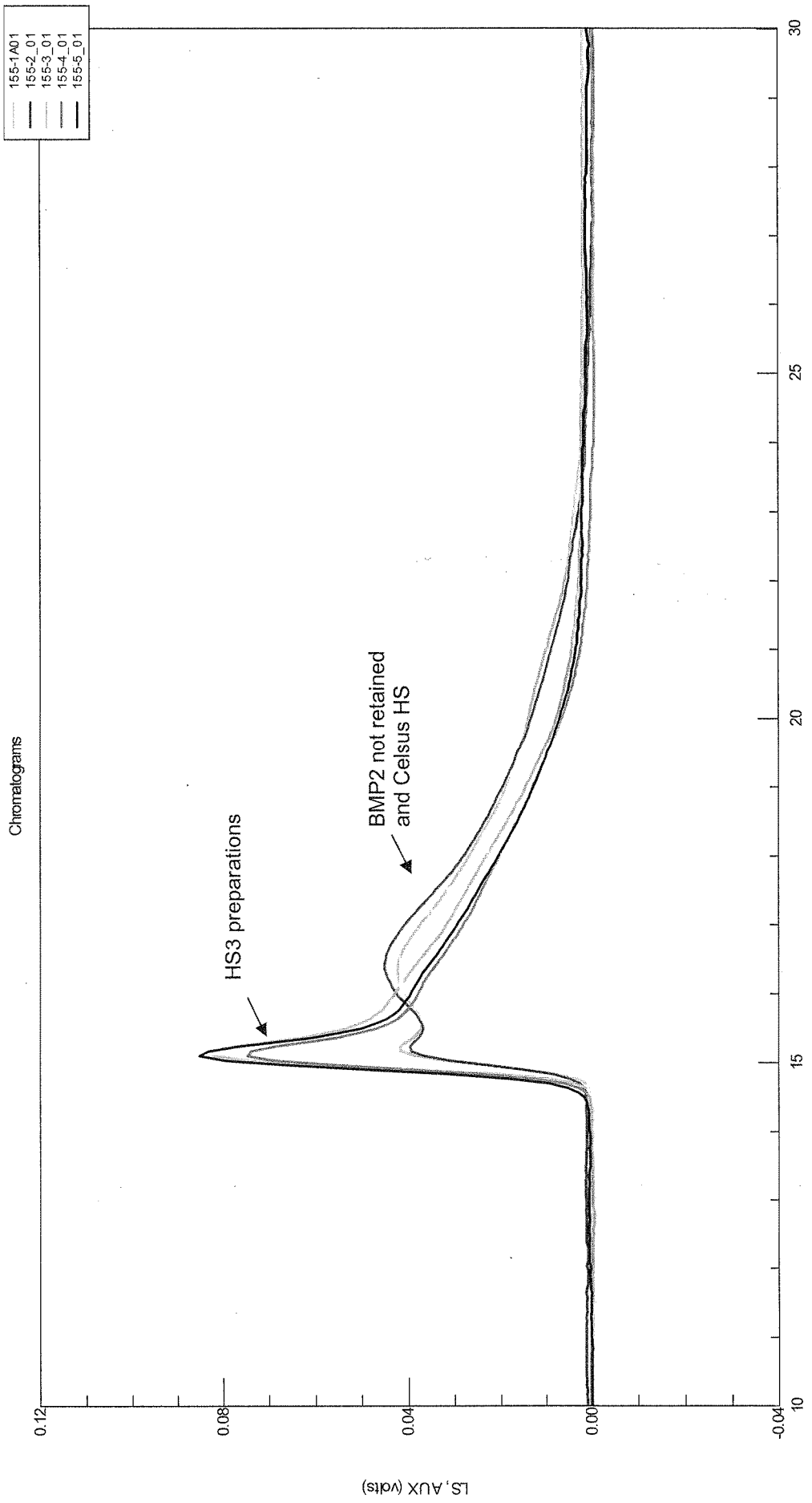
Chromatograms
155-1A01
155-2_01
155-3_01
155-4_01
155-5_01
HS3 preparations
BMP2 not retained
and Celsus HS
LS, AUX (volts)
0.12
0.08
0.04
0.00
-0.04
10
15
20
25
30
Volume (mL)

Figure 18

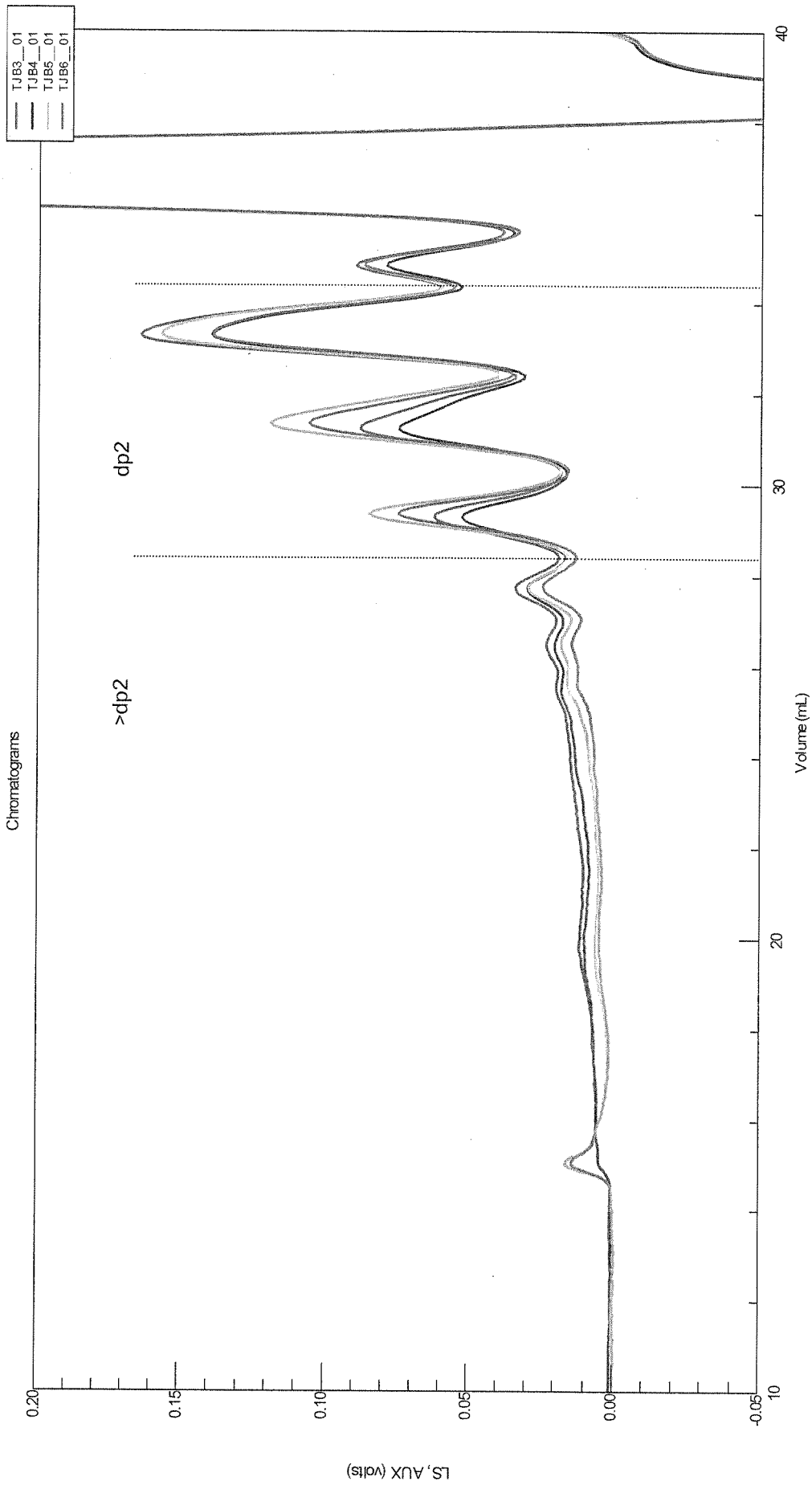
TJB3__01
TJB4__01
TJB5__01
TJB6__01
Chromatograms
dp2
>dp2
LS, AUX (volts)
0.20
0.15
0.10
0.05
0.00
-0.05
10
20
30
40
Volume (mL)

Figure 19

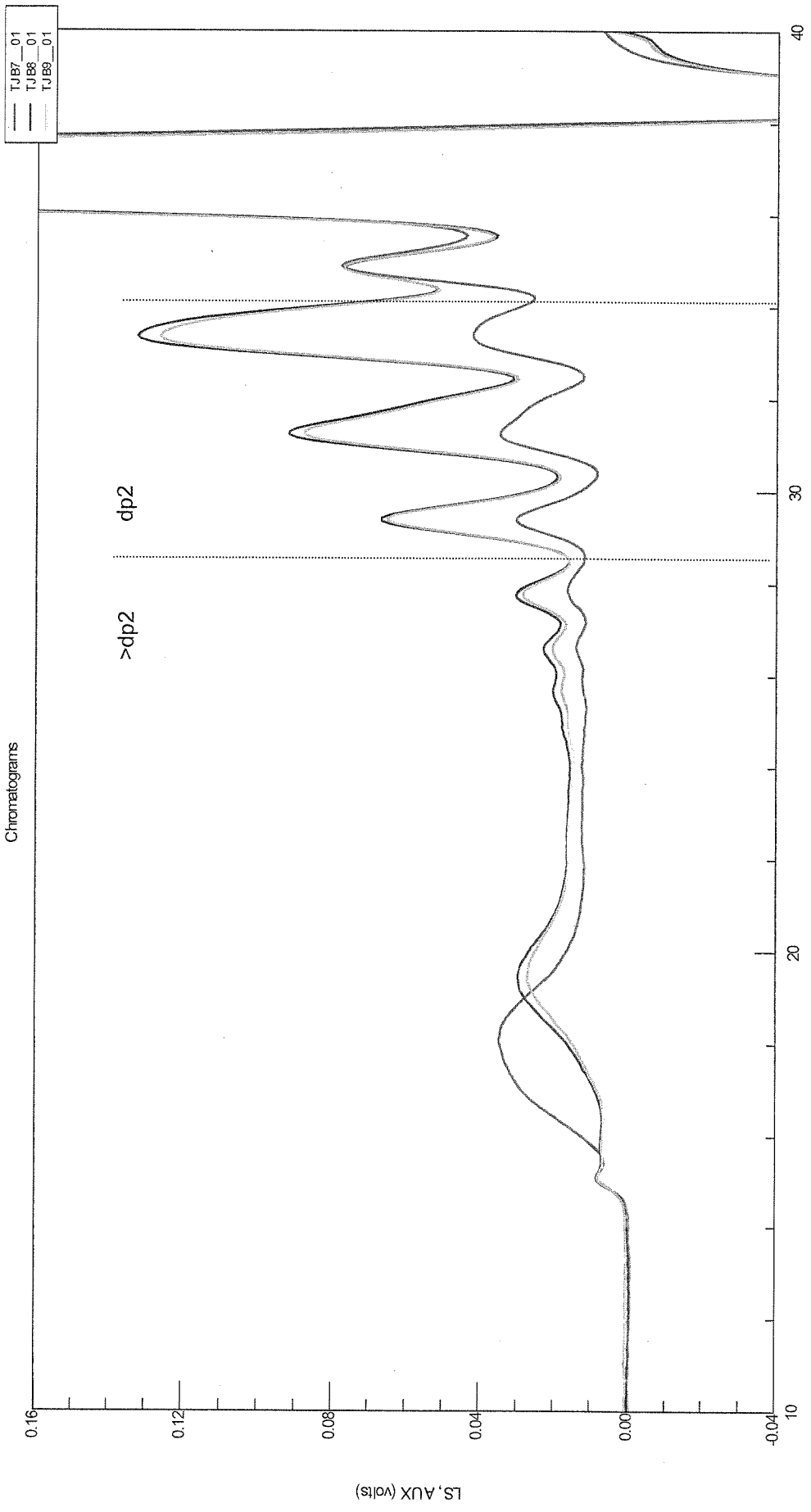
TJB7__01
TJB8__01
TJB9__01
dp2
>dp2
Chromatograms
LS, AUX (volts)
Volume (mL)
0.16
0.12
0.08
0.04
0.00
-0.04
10
20
30
40

Figure 20

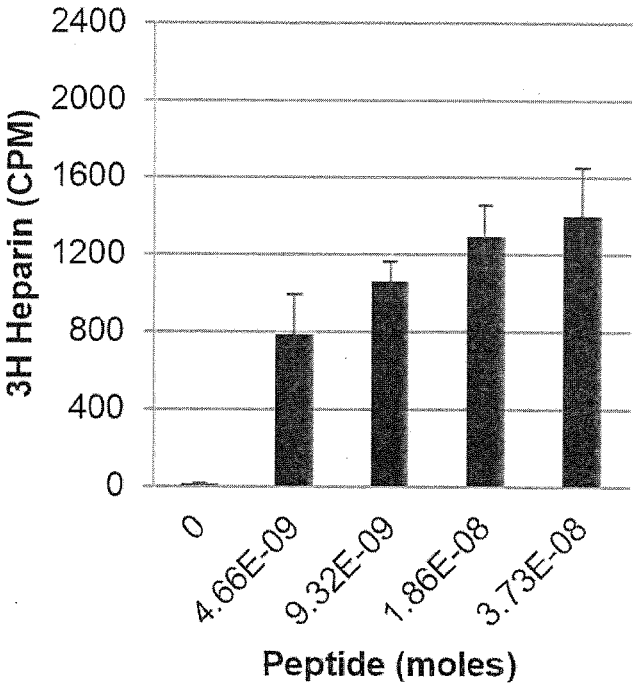
2400
2000
1600
1200
800
400
0
3H Heparin (CPM)
0
4.66E-09
9.32E-09
1.86E-08
3.73E-08
Peptide (moles)

Figure 21

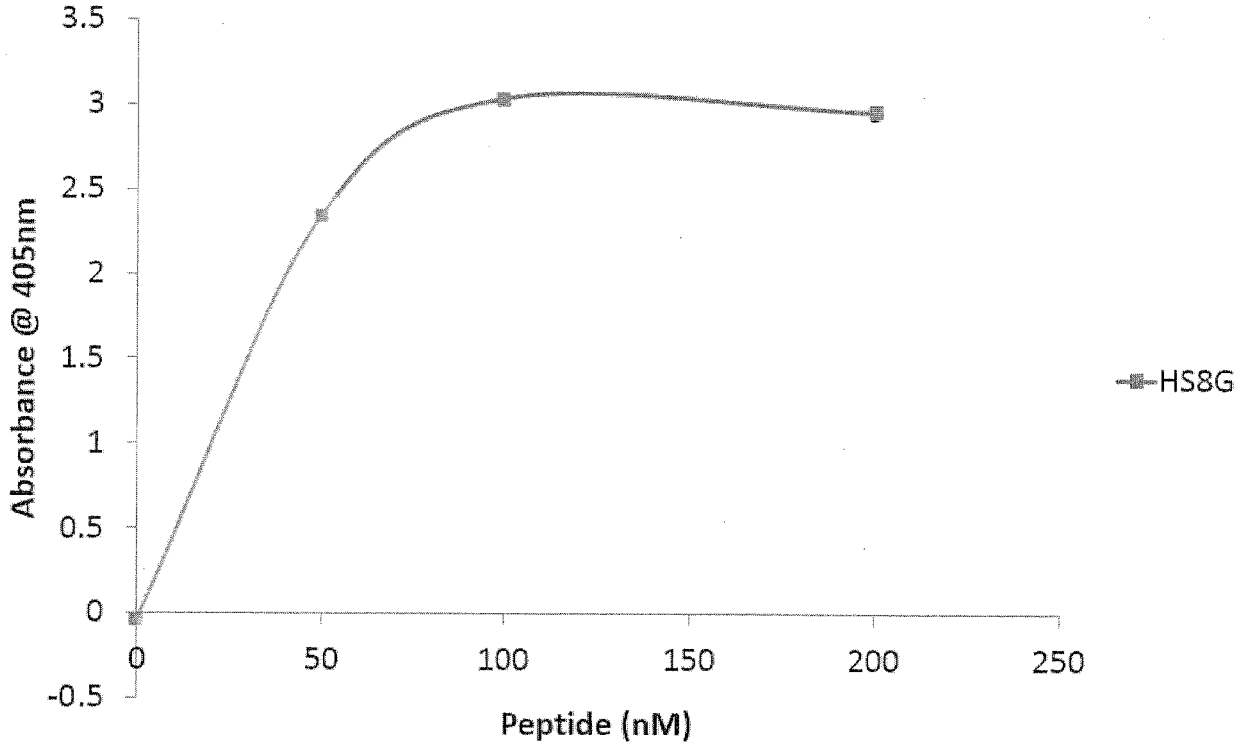

Figure 22

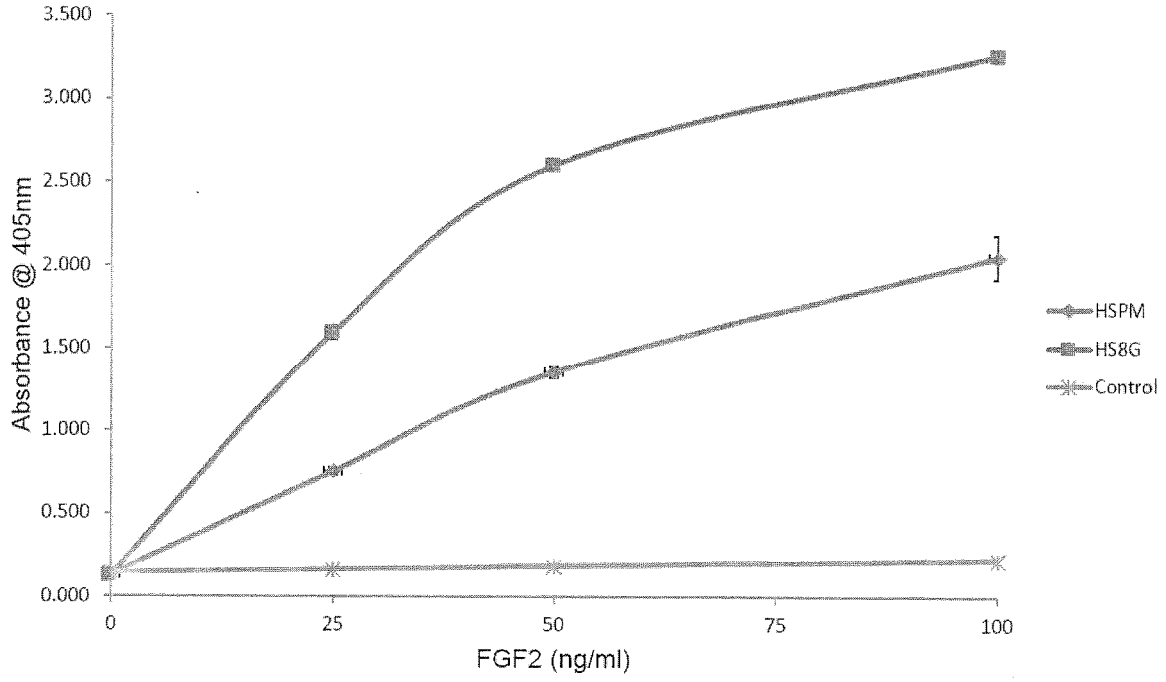

Figure 23

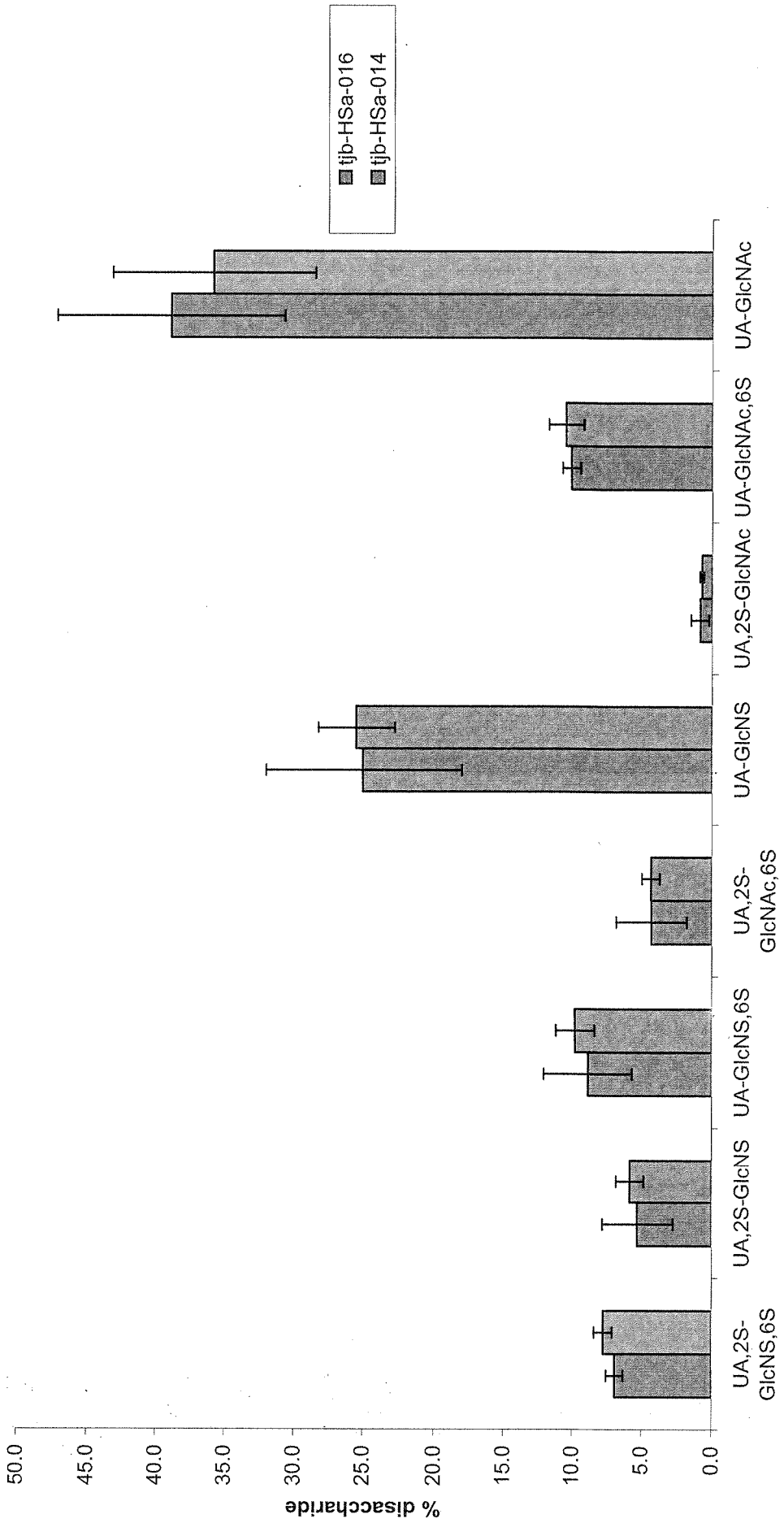
tjb-HSa-016
tjb-HSa-014
% disaccharide
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

Figure 24

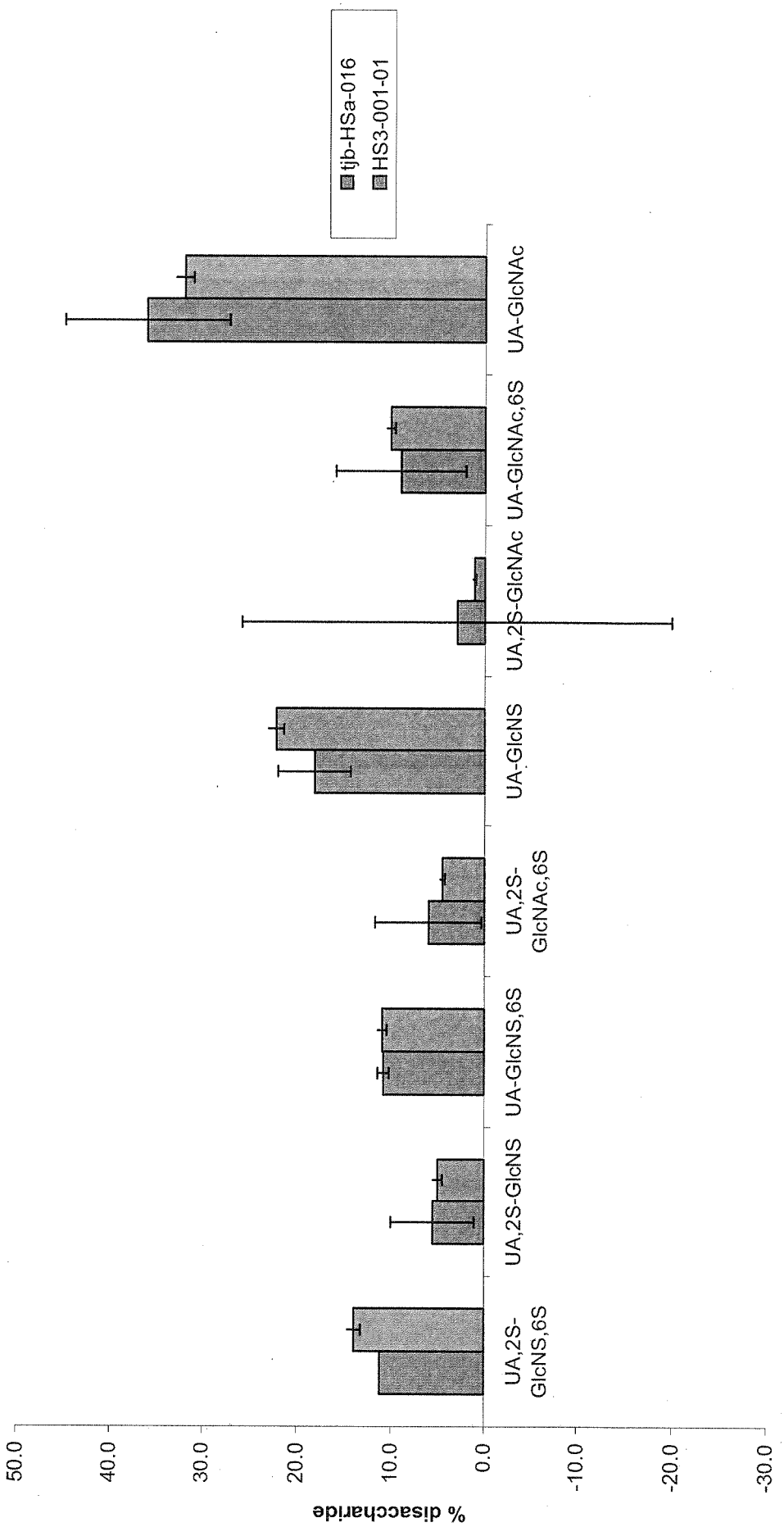
fjb-HSa-016
HS3-001-01
% disaccharide
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
-30.0
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

Figure 25

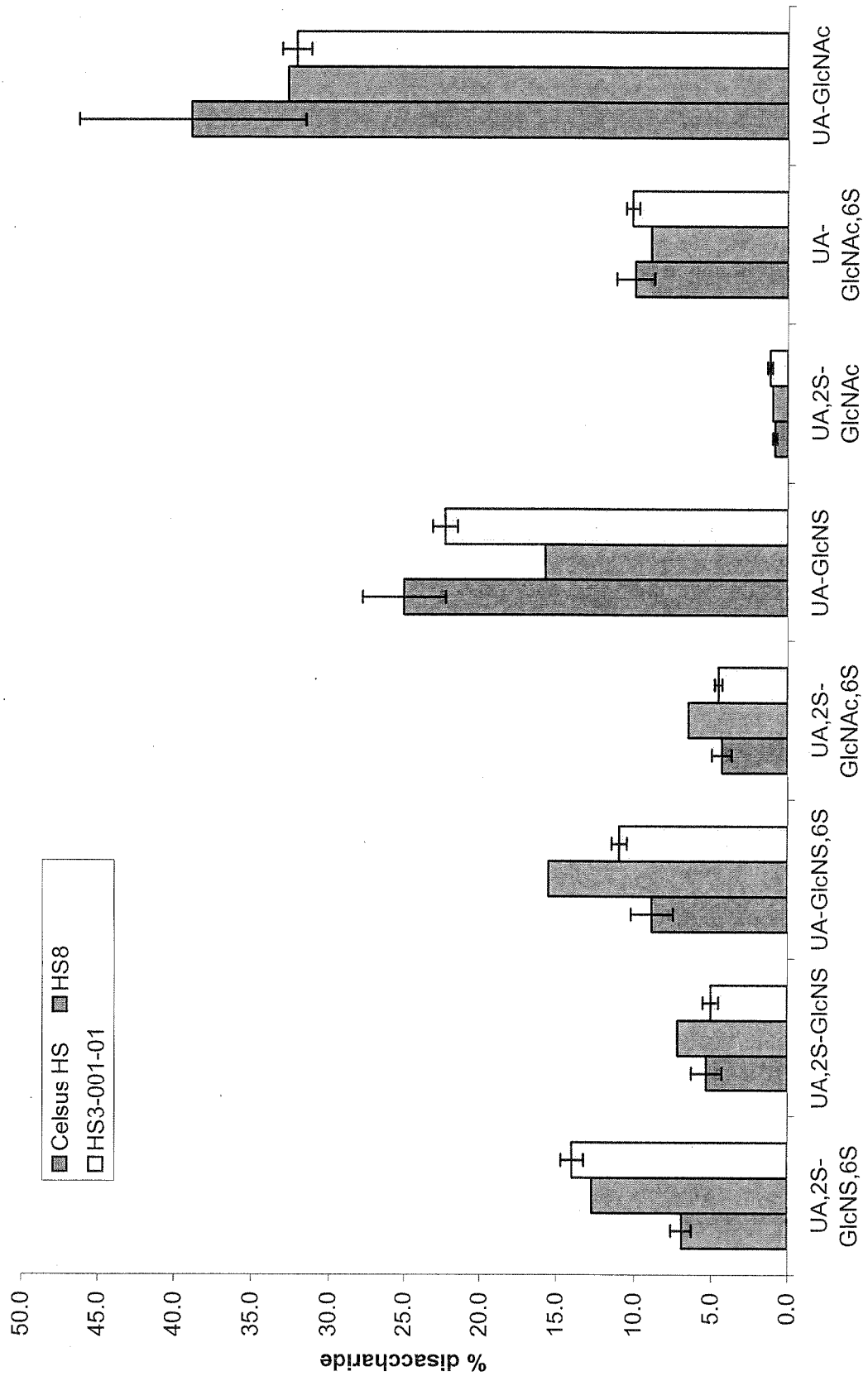
Celsus HS
HS8
HS3-001-01
% disaccharide
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
UA,2S-GlcNS,6S
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
UA-GlcNAc
Heparin disaccharides

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

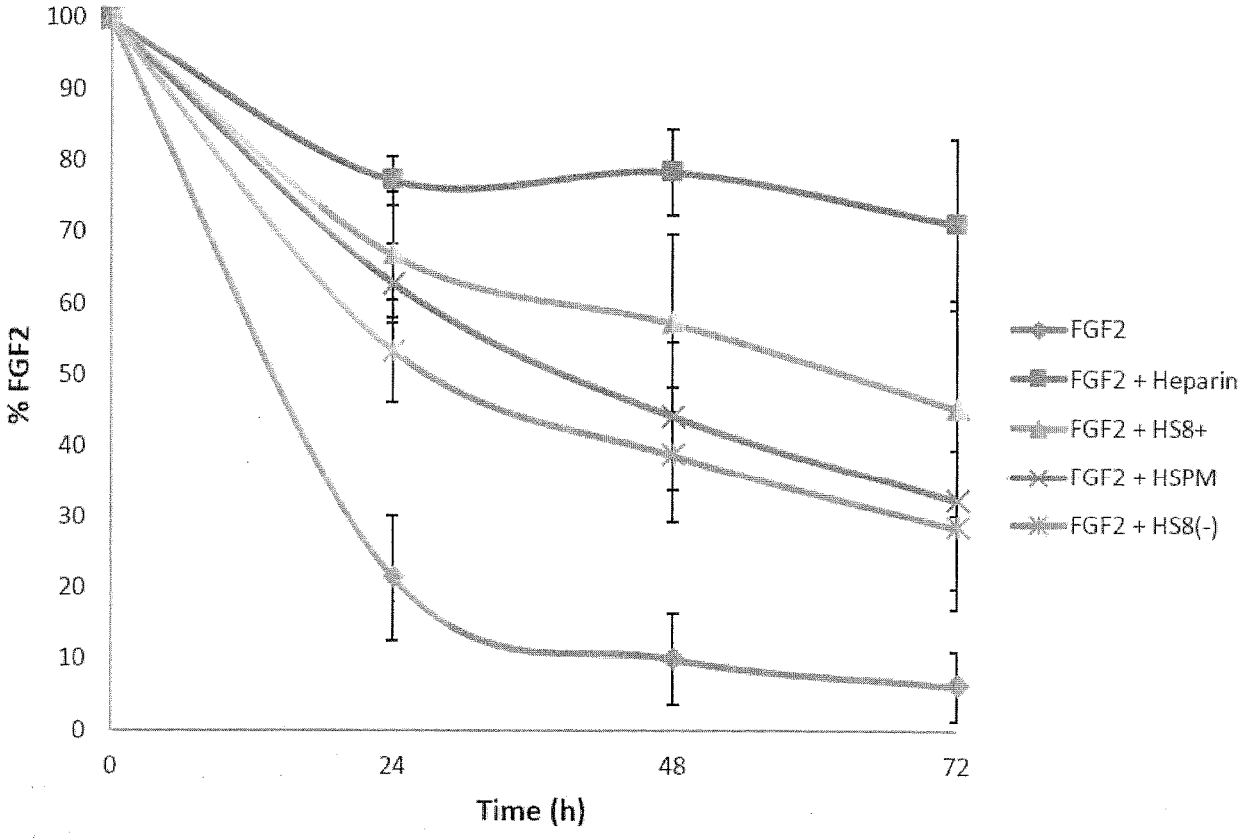

Figure 28

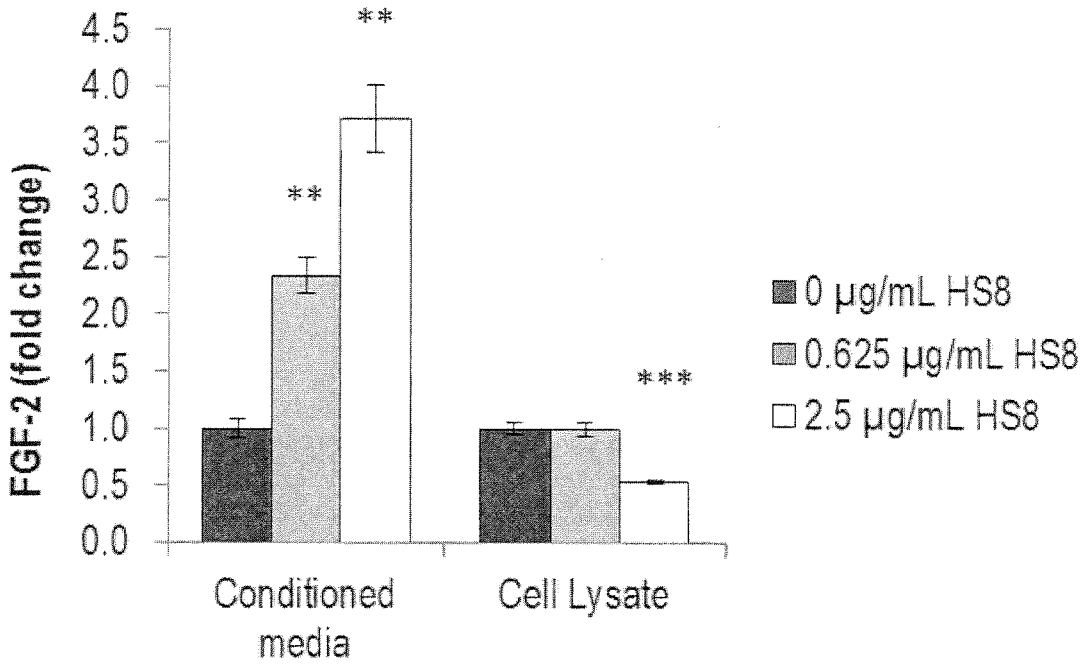

Figure 29

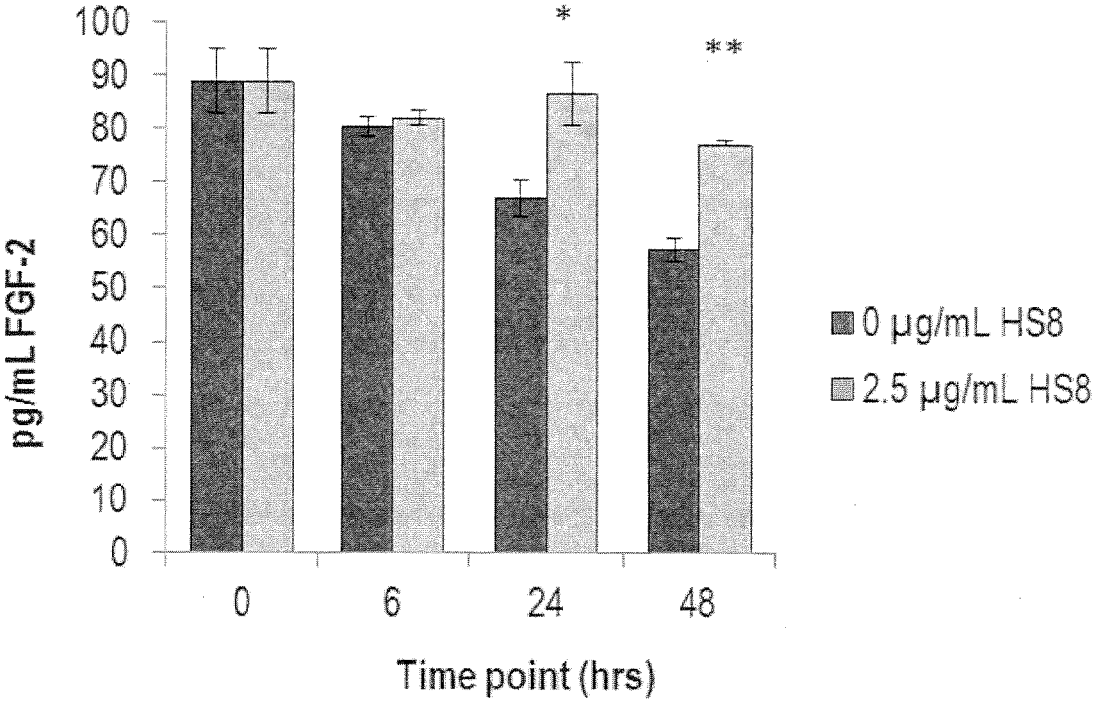

Figure 30

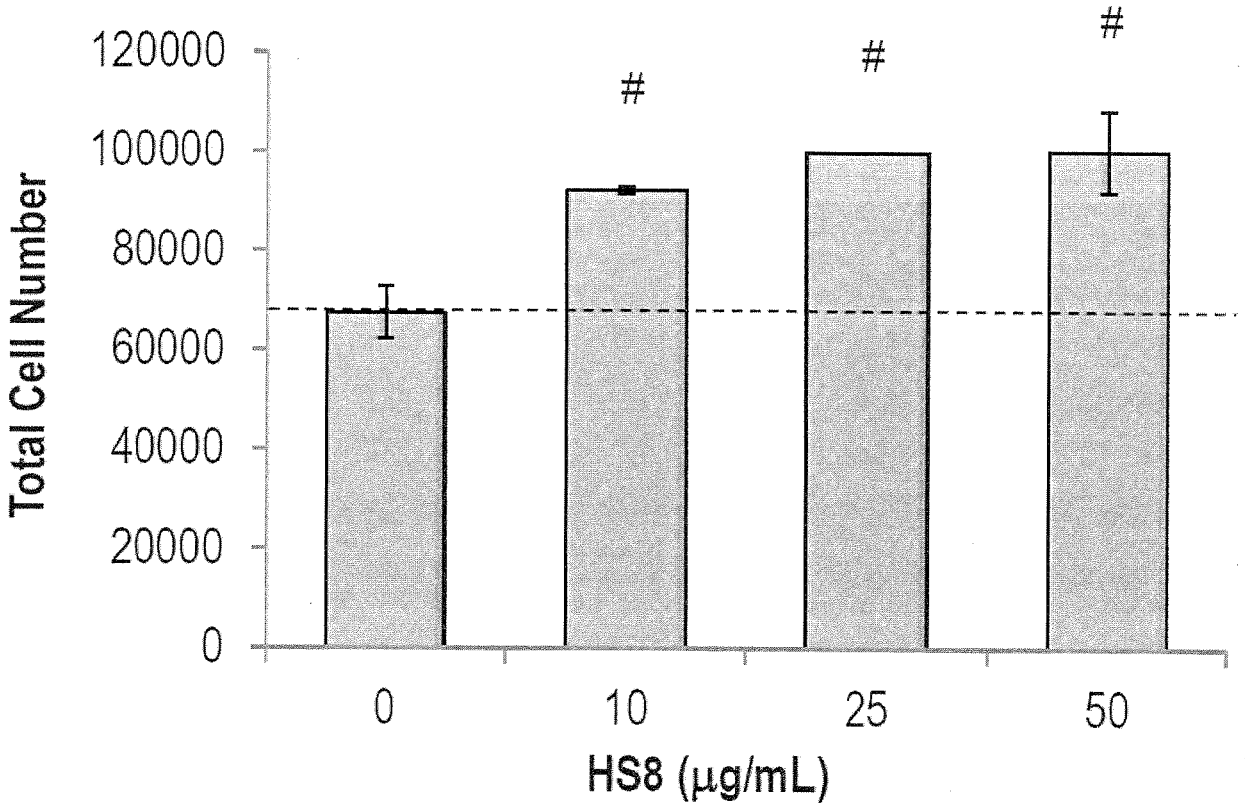
120000
100000
80000
60000
40000
20000
0
Total Cell Number
#
#
#
0
10
25
50
HS8 (µg/mL)

Figure 31

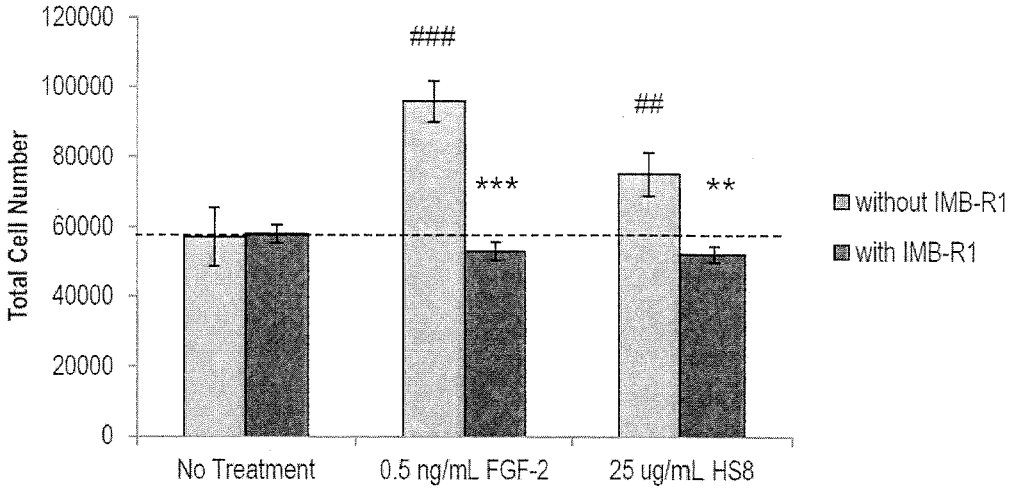

Figure 32A

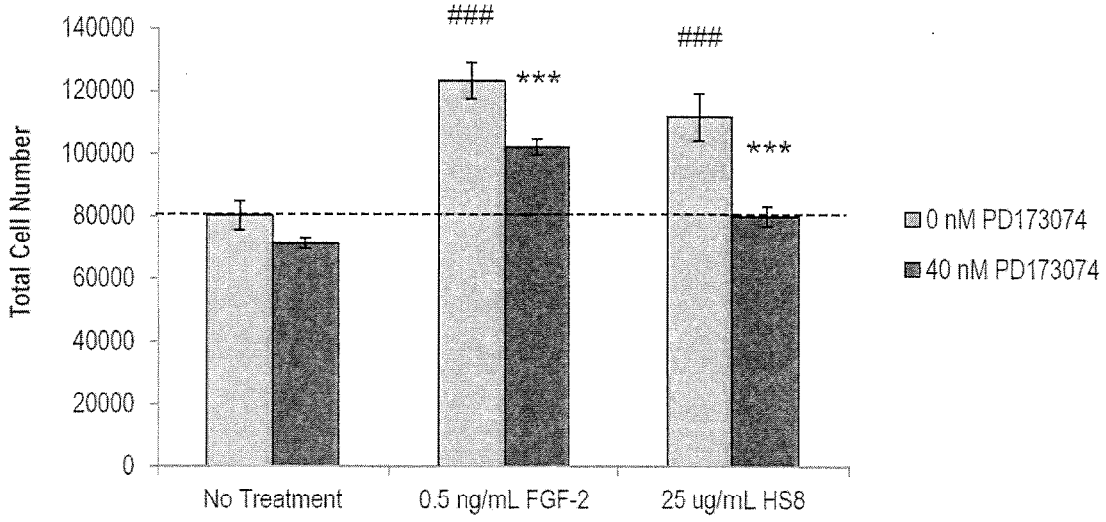

Figure 32B

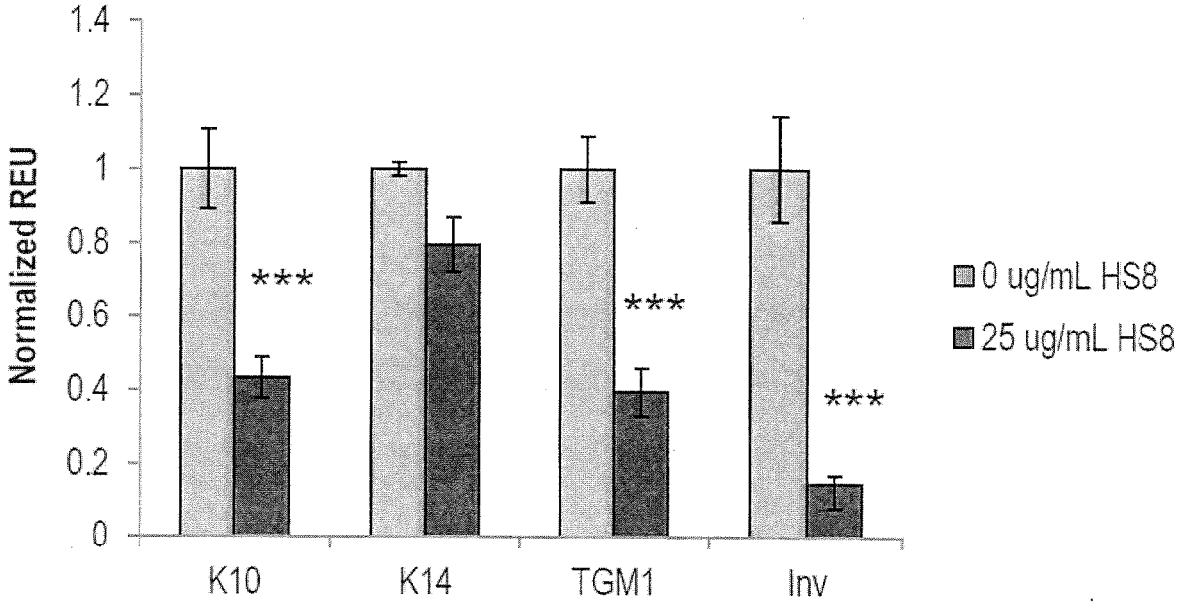

Figure 33

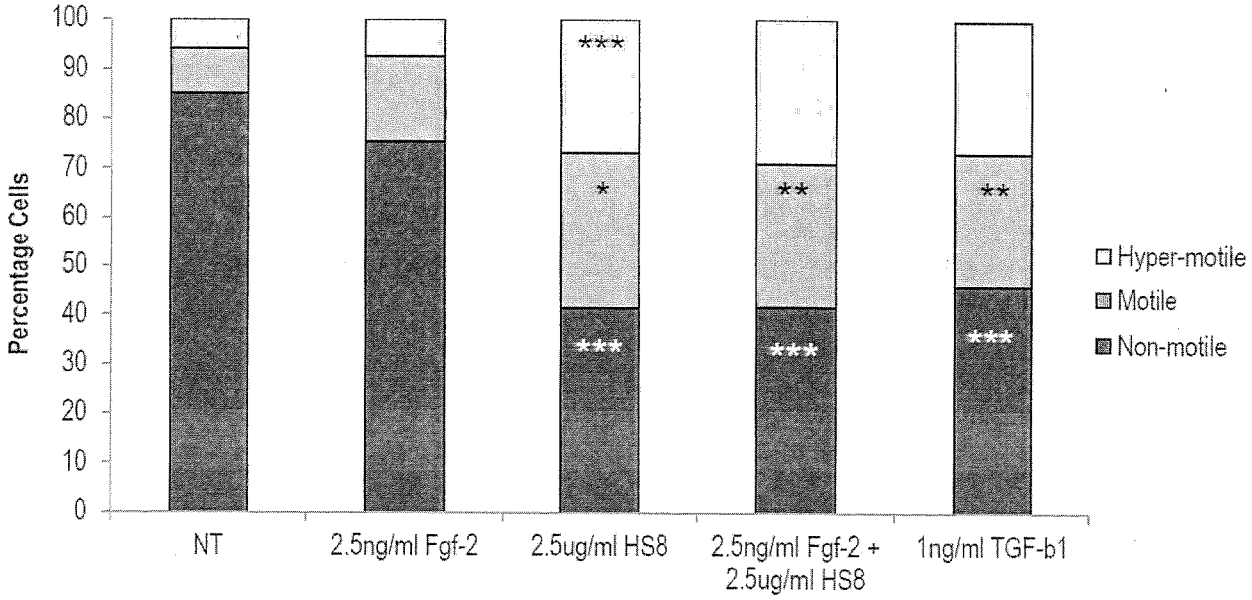

Figure 34

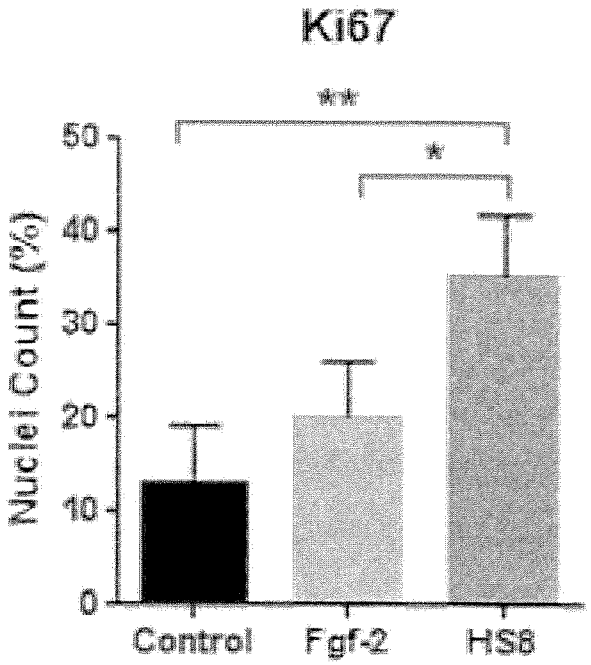

Figure 35A

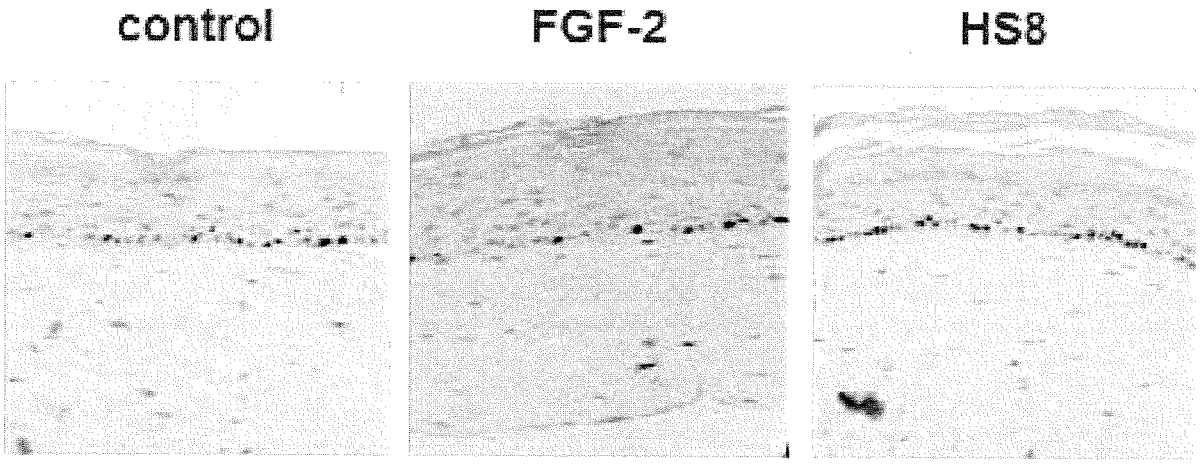

Figure 35B

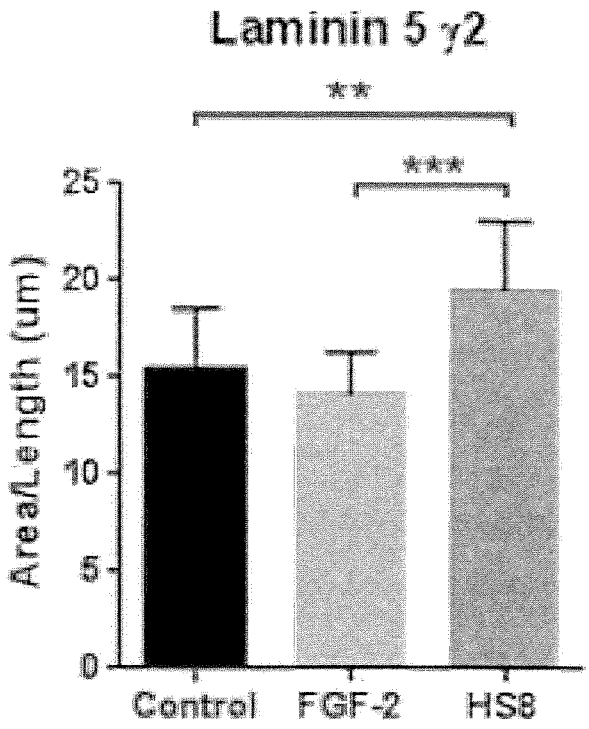

Figure 36A

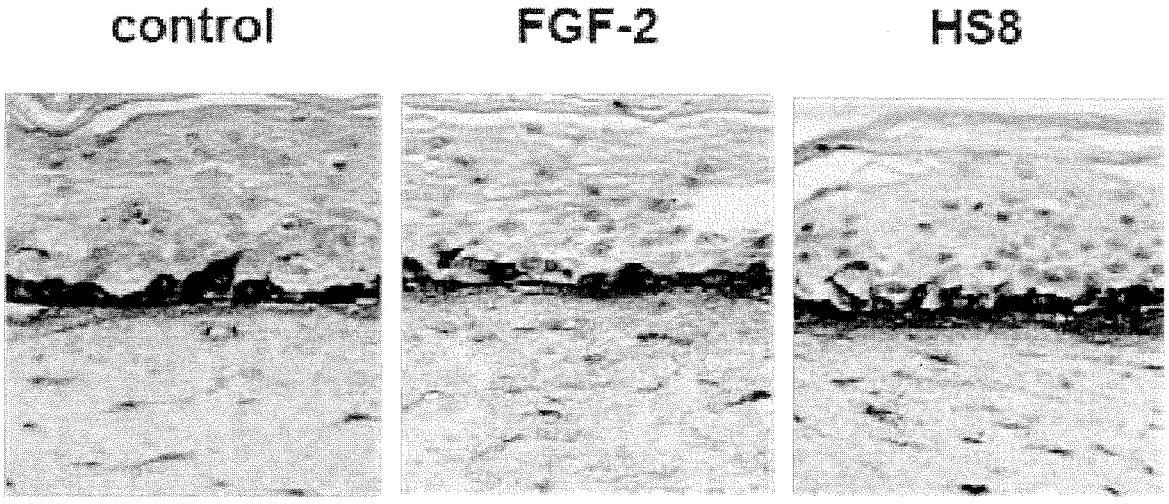

Figure 36B

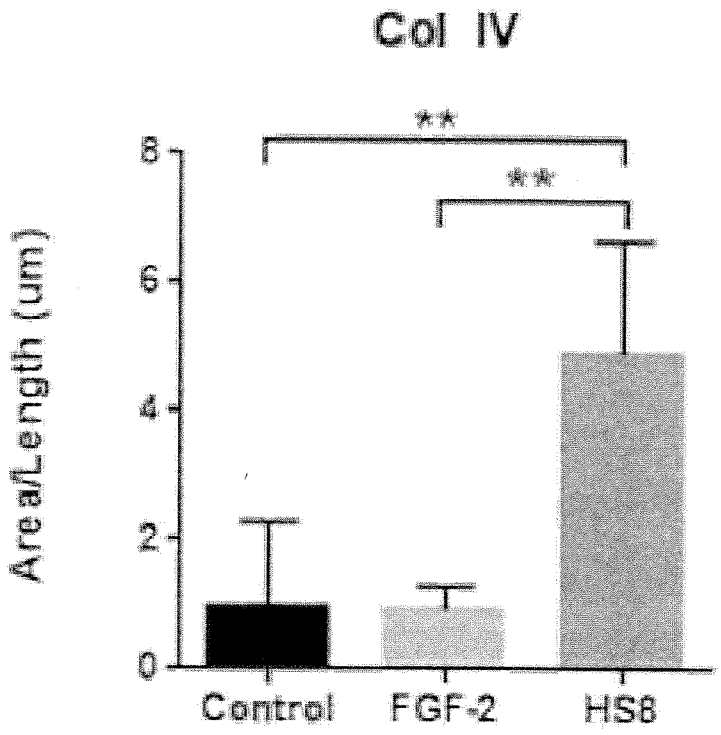

Figure 37A

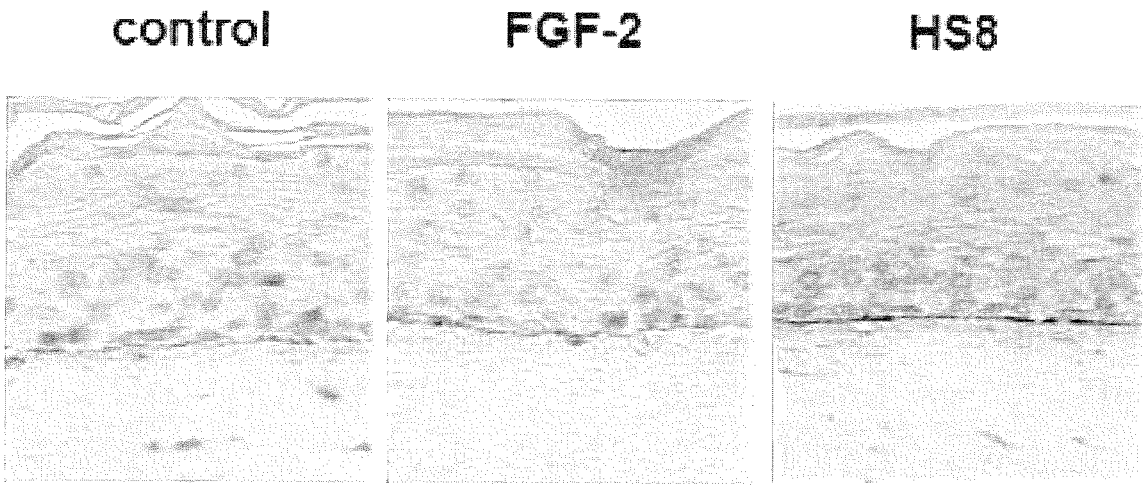

Figure 37B