FIGURE 1

HUMAN SULFATASE MODIFYING FACTOR 1 (SUMF1) POLYNUCLEOTIDE SEQUENCE (SEQ ID NO:1)

ATGGCTGCGCCCGCACTAGGGCTGGTGTGTGGACGTTGCCCTGAGCTGGGTCTCGTCCTCTTGCTGCTGCTGCTC
TCGCTGCTGTGTGGAGCGGCAGGGAGCCAGGAGGCCGGGACCGGTGCGGGCGCGGGGTCCCTTGCGGGTTCTTGC
GGCTGCGGCACGCCCCAGCGGCCTGGCGCCCATGGCAGTTCGGCAGCCGCTCACCGATACTCGCGGGAGGCTAAC
GCTCCGGGCCCCGTACCCGGAGAGCGGCAACTCGCGCACTCAAAGATGGTCCCCATCCCTGCTGGAGTATTTACA
ATGGGCACAGATGATCCTCAGATAAAGCAGGATGGGGAAGCACCTGCGAGGAGAGTTACTATTGATGCCTTTTAC
ATGGATGCCTATGAAGTCAGTAATACTGAATTTGAGAAGTTTGTGAACTCAACTGGCTATTTGACAGAGGCTGAG
AAGTTTGGCGACTCCTTTGTCTTTGAAGGCATGTTGAGTGAGCAAGTGAAGACCAATATTCAACAGGCAGTTGCA
GCTGCTCCCTGGTGGTTACCTGTGAAAGGCGCTAACTGGAGACACCCAGAAGGGCCTGACTCTACTATTCTGCAC
AGGCCGGATCATCCAGTTCTCCATGTGTCCTGGAATGATGCGGTTGCCTACTGCACTTGGGCAGGGAAGCGGCTG
CCCACGGAAGCTGAGTGGGAATACAGCTGTCGAGGAGGCCTGCATAATAGACTTTTCCCCTGGGGCAACAAACTG
CAGCCCAAAGGCCAGCATTATGCCAACATTTGGCAGGGCGAGTTTCCGGTGACCAACACTGGTGAGGATGGCTTC
CAAGGAACTGCGCCTGTTGATGCCTTCCCTCCCAATGGTTATGGCTTATACAACATAGTGGGGAACGCATGGGAA
TGGACTTCAGACTGGTGGACTGTTCATCATTCTGTTGAAGAAACGCTTAACCCAAAAGGTCCCCCTTCTGGGAAA
GACCGAGTGAAGAAAGGTGGATCCTACATGTGCCATAGGTCTTATTGTTACAGGTATCGCTGTGCTGCTCGGAGC
CAGAACACACCTGATAGCTCTGCTTCGAATCTGGGATTCCGCTGTGCAGCCGACCGCCTGCCCACCATGGACTGA

FIGURE 2

HUMAN SULFATASE MODIFYING FACTOR 1 (SUMF1) POLYPEPTIDE SEQUENCE (SEQ ID NO:2)

MAAPALGLVCGRCPELGLVLLLLLLSLLCGAAGSQEAGTGAGAGSLAGSCGCGTPQRPGAHGSSAAAHRYSREAN
APGPVPGERQLAHSKMVPIPAGVFTMGTDDPQIKQDGEAPARRVTIDAFYMDAYEVSNTEFEKFVNSTGYLTEAE
KFGDSFVFEGMLSEQVKTNIQQAVAAAPWWLPVKGANWRHPEGPDSTILHRPDHPVLHVSWNDAVAYCTWAGKRL
PTEAEWEYSCRGGLHNRLFPWGNKLQPKGQHYANIWQGEFPVTNTGEDGFQGTAPVDAFPPNGYGLYNIVGNAWE
WTSDWWTVHHSVEETLNPKGPPSGKDRVKKGGSYMCHRSYCYRYRCAARSQNTPDSSASNLGFRCAADRLPTMD

FIGURE 3

HUMAN N-ACETYLGALACTOSAMINE-6_SULFATASE (GALNS) POLYNUCLEOTIDE SEQUENCE (SEQ ID NO:3)

ATGGCGGCGGTTGTCGCGGCGACGAGGTGGTGGCAGCTGTTGCTGGTGCTCAGCGCCGCGGGGATGGGGGCCTCG
GGCGCCCCGCAGCCCCCCAACATCCTGCTCCTGCTCATGGACGACATGGGATGGGGTGACCTCGGGGTGTATGGA
GAGCCCTCCAGAGAGACCCCGAATTTGGACCGGATGGCTGCAGAAGGGCTGCTTTTCCCAAACTTCTATTCTGCC
AACCCTCTGTGCTCGCCATCGAGGGCGGCACTGCTCACAGGACGGCTACCCATCCGCAATGGCTTCTACACCACC
AACGCCCATGCCAGAAACGCCTACACACCGCAGGAGATTGTGGGCGGCATCCCAGACTCGGAGCAGCTCCTGCCG
GAGCTTCTGAAGAAGGCCGGCTACGTCAGCAAGATTGTCGGCAAGTGGCATCTGGGTCACAGGCCCCAGTTCCAC
CCCCTGAAGCACGGATTTGATGAGTGGTTTGGATCCCCCAACTGCCACTTTGGACCTTATGACAACAAGGCCAGG
CCCAACATCCCTGTGTACAGGGACTGGGAGATGGTTGGCAGATATTATGAAGAATTTCCTATTAATCTGAAGACG
GGGGAAGCCAACCTCACCCAGATCTACCTGCAGGAAGCCCTGGACTTCATTAAGAGACAGGCACGGCACCACCCC
TTTTTCCTCTACTGGGCTGTCGACGCCACGCACGCACCCGTCTATGCCTCCAAACCCTTCTTGGGCACCAGTCAG
CGAGGGCGGTATGGAGACGCCGTCCGGGAGATTGATGACAGCATTGGGAAGATACTGGAGCTCCTCCAAGACCTG
CACGTCGCGGACAACACCTTCGTCTTCTTCACGTCGGACAACGGCGCTGCCCTCATTTCCGCCCCCGAACAAGGT
GGCAGCAACGGCCCCTTTCTGTGTGGGAAGCAGACCACGTTTGAAGGAGGGATGAGGGAGCCTGCCCTCGCATGG
TGGCCAGGGCACGTCACTGCAGGCCAGGTGAGCCACCAGCTGGGCAGCATCATGGACCTCTTCACCACCAGCCTG
GCCCTTGCGGGCCTGACGCCGCCCAGCGACAGGGCCATTGATGGCCTCAACCTCCTCCCCACCCTCCTGCAGGGC
CGGCTGATGGACAGGCCTATCTTCTATTACCGTGGCGACACGCTGATGGCGGCCACCCTCGGGCAGCACAAGGCT
CACTTCTGGACCTGGACCAACTCCTGGGAGAACTTCAGACAGGGCATTGATTTCTGCCCTGGGCAGAACGTTTCA
GGGGTCACAACTCACAATCTGGAAGACCACACGAAGCTGCCCCTGATCTTCCACCTGGGACGGGACCCAGGGGAG
AGGTTCCCCCTCAGCTTTGCCAGCGCCGAGTACCAGGAGGCCCTCAGCAGGATCACCTCGGTCGTCCAGCAGCAC
CAGGAGGCCTTGGTCCCCGCGCAGCCCCAGCTCAACGTGTGCAACTGGGCGGTCATGAACTGGGCACCTCCGGGC
TGTGAAAAGTTAGGGAAGTGTCTGACACCTCCAGAATCCATTCCCAAGAAGTGCCTCTGGTCCCACTAG

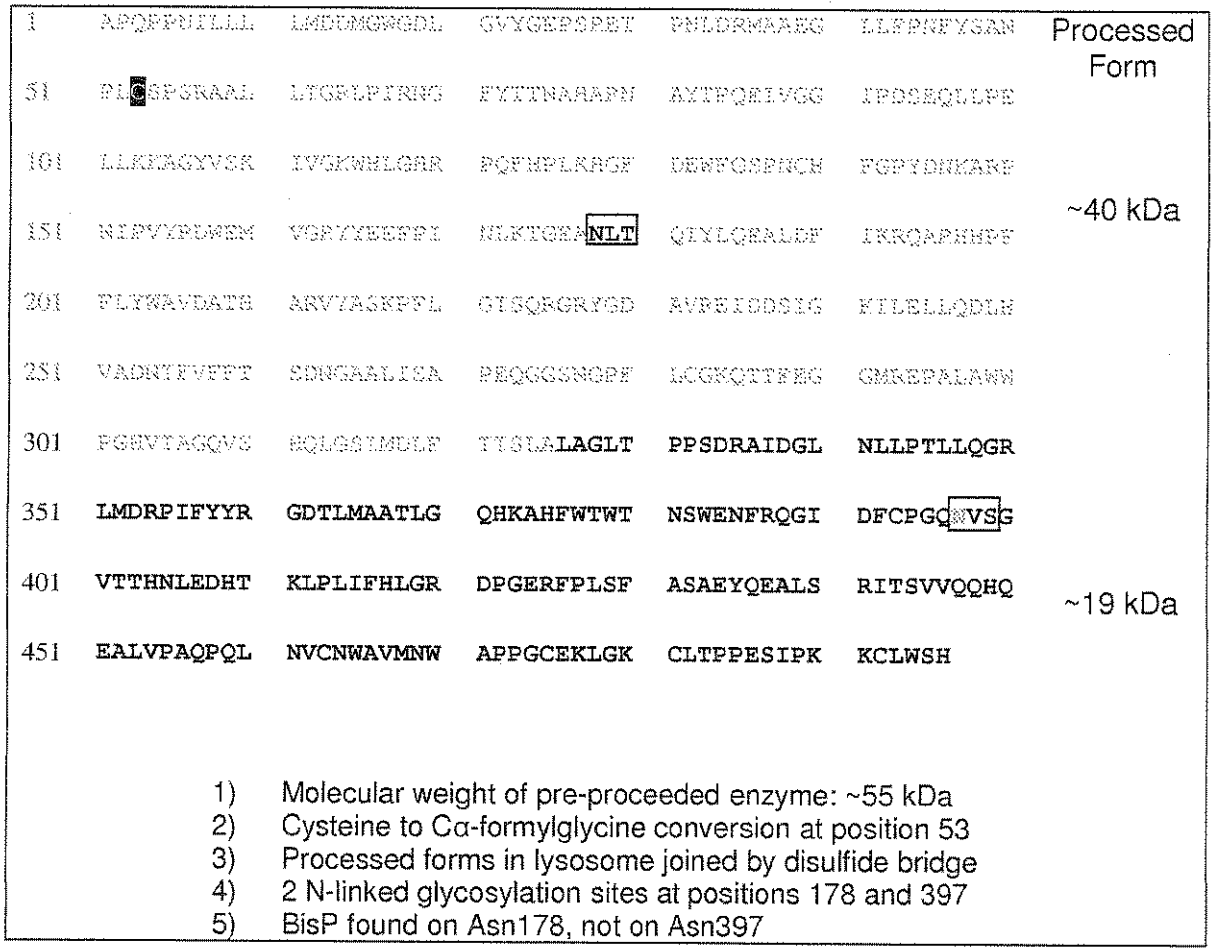
FIGURE 4

HUMAN N-ACETYLGALACTOSAMINE-6-SULFATASE (GALNS) POLYPEPTIDE SEQUENCE (SEQ ID NO:4)

MAAVVAATRWWQLLLVLSAAGMGASGAPQPPNILLLLMDDMGWGDLGVYGEPSRETPNLDRMAAEGLLFPNFYSA
NPLCSPSRAALLTGRLPIRNGFYTTNAHARNAYTPQEIVGGIPDSEQLLPELLKKAGYVSKIVGKWHLGHRPQFH
PLKHGFDEWFGSPNCHFGPYDNKARPNIPVYRDWEMVGRYYEEFPINLKTGEANLTQIYLQEALDFIKRQARHHP
FFLYWAVDATHAPVYASKPFLGTSQRGRYGDAVREIDDSIGKILELLQDLHVADNTFVFFTSDNGAALISAPEQG
GSNGPFLCGKQTTFEGGMREPALAWWPGHVTAGQVSHQLGSIMDLFTTSLALAGLTPPSDRAIDGLNLLPTLLQG
RLMDRPIFYYRGDTLMAATLGQHKAHFWTWTNSWENFRQGIDFCPGQNVSGVTTHNLEDHTKLPLIFHLGRDPGE
RFPLSFASAEYQEALSRITSVVQQHQEALVPAQPQLNVCNWAVMNWAPPGCEKLGKCLTPPESIPKKCLWSH

FIGURE 5

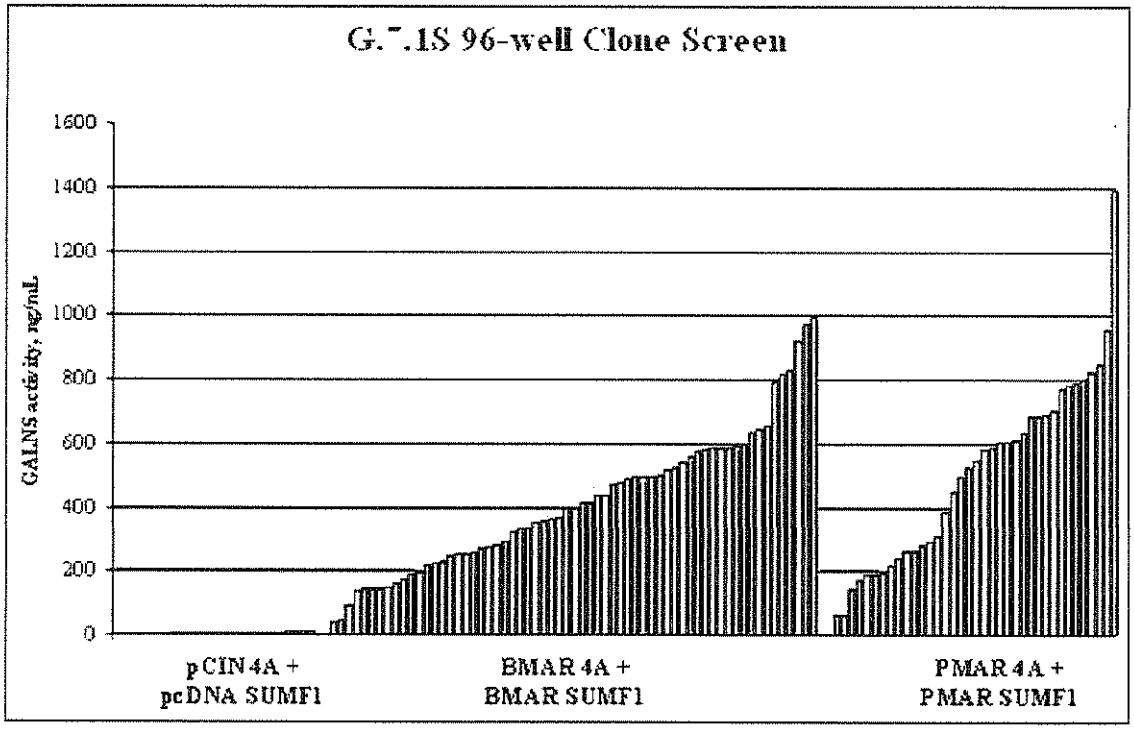
STRUCTURE AND CHARACTERISTICS OF SECRETED HUMAN N-ACETYLGALACTOSAMINE-6-SULFATASE (GALNS) (SEQ ID NO:5)



1) Molecular weight of pre-proceeded enzyme: ~55 kDa
2) Cysteine to Cα-formylglycine conversion at position 53
3) Processed forms in lysosome joined by disulfide bridge
4) 2 N-linked glycosylation sites at positions 178 and 397
5) BisP found on Asn178, not on Asn397

FIGURE 6A-B

A.



B.

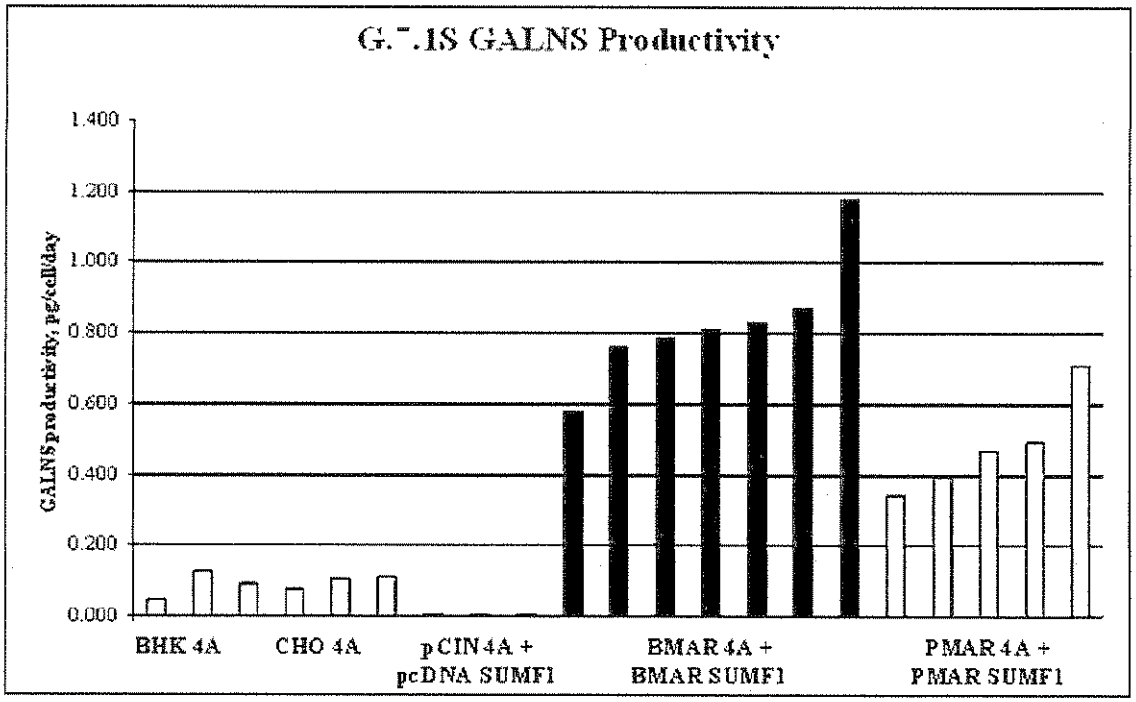


FIGURE 7

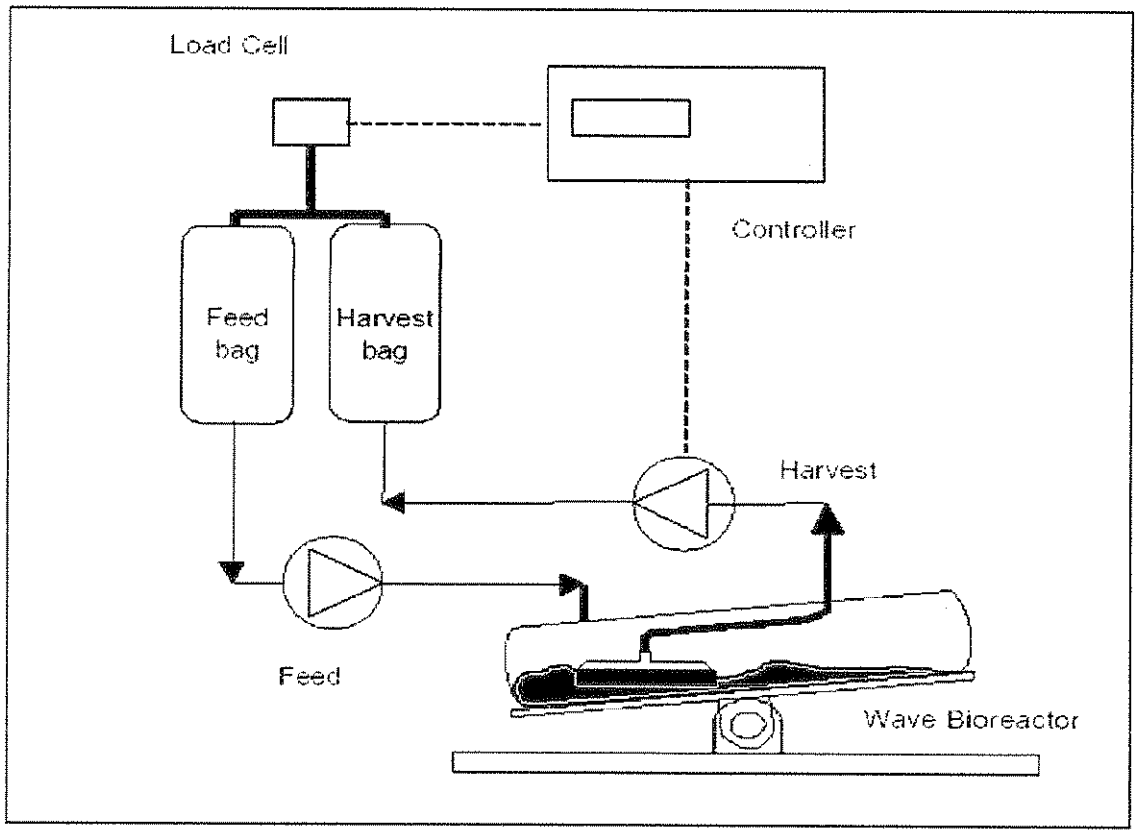

Load Cell
Controller
Feed bag
Harvest bag
Harvest
Feed
Wave Bioreactor

FIGURE 8

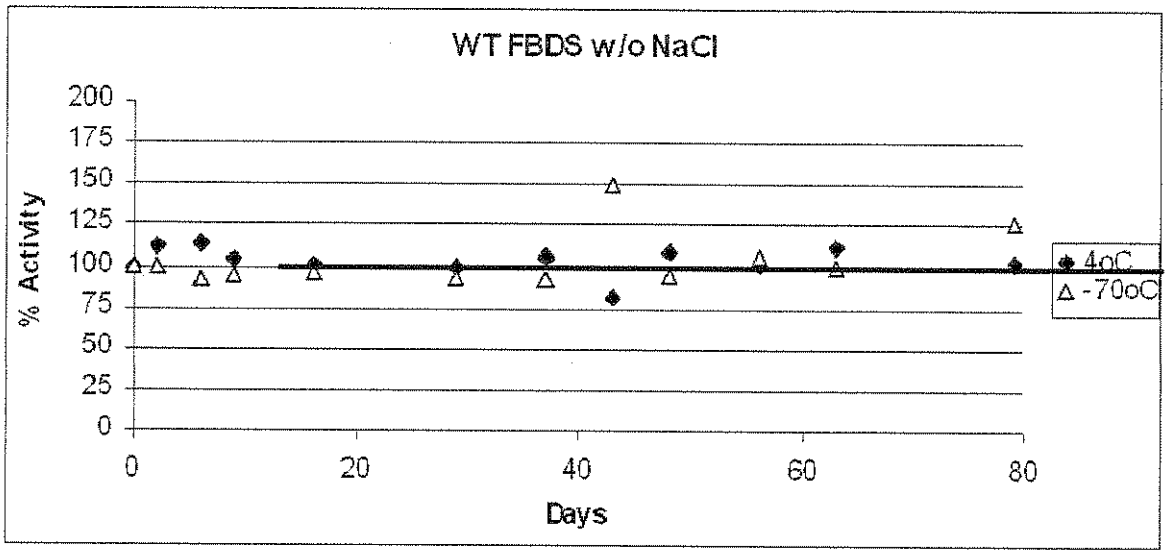
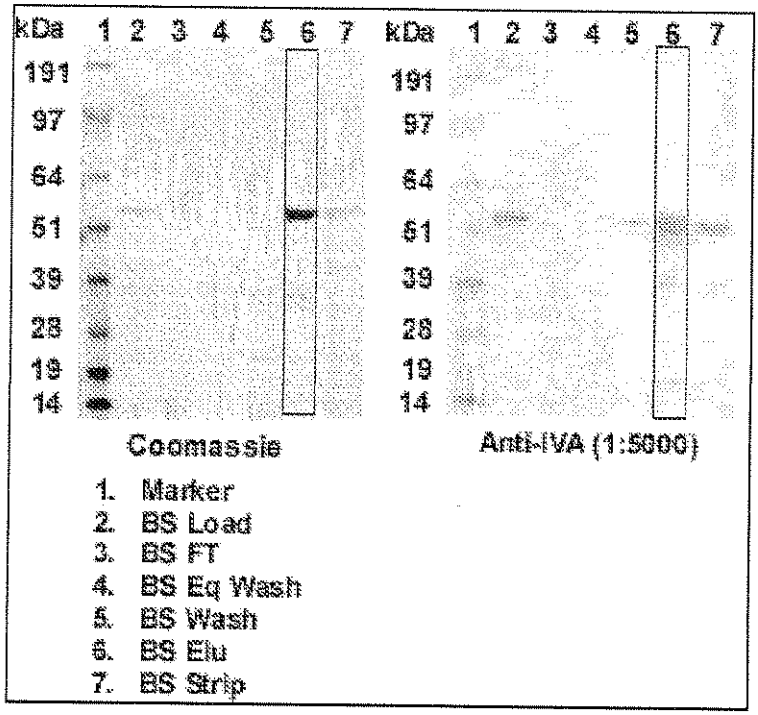

WT FBDS w/o NaCl
% Activity
200
175
150
125
100
75
50
25
0
0
20
40
60
80
Days
4oC
-70oC

FIGURE 9A-B

A. Blue Sepharose 6 FF Chromatography



B. Fractogel SE Hi-Cap Chromatography

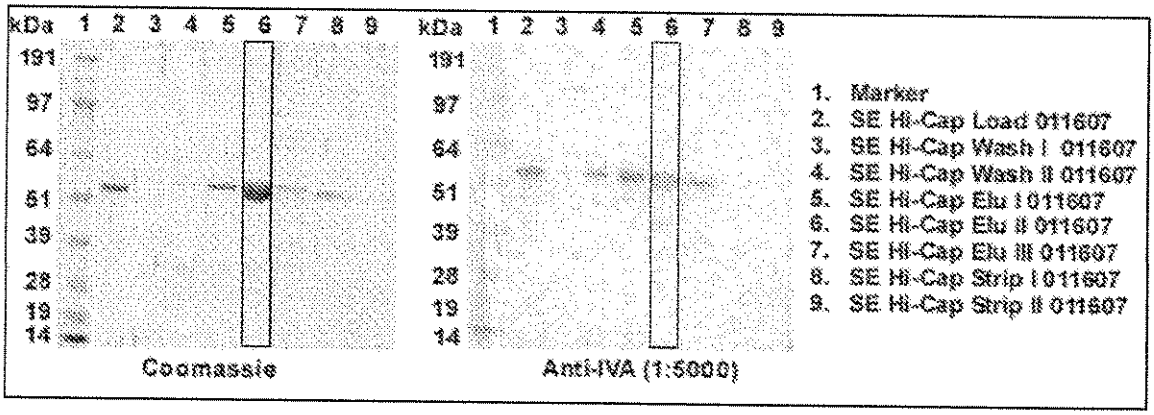
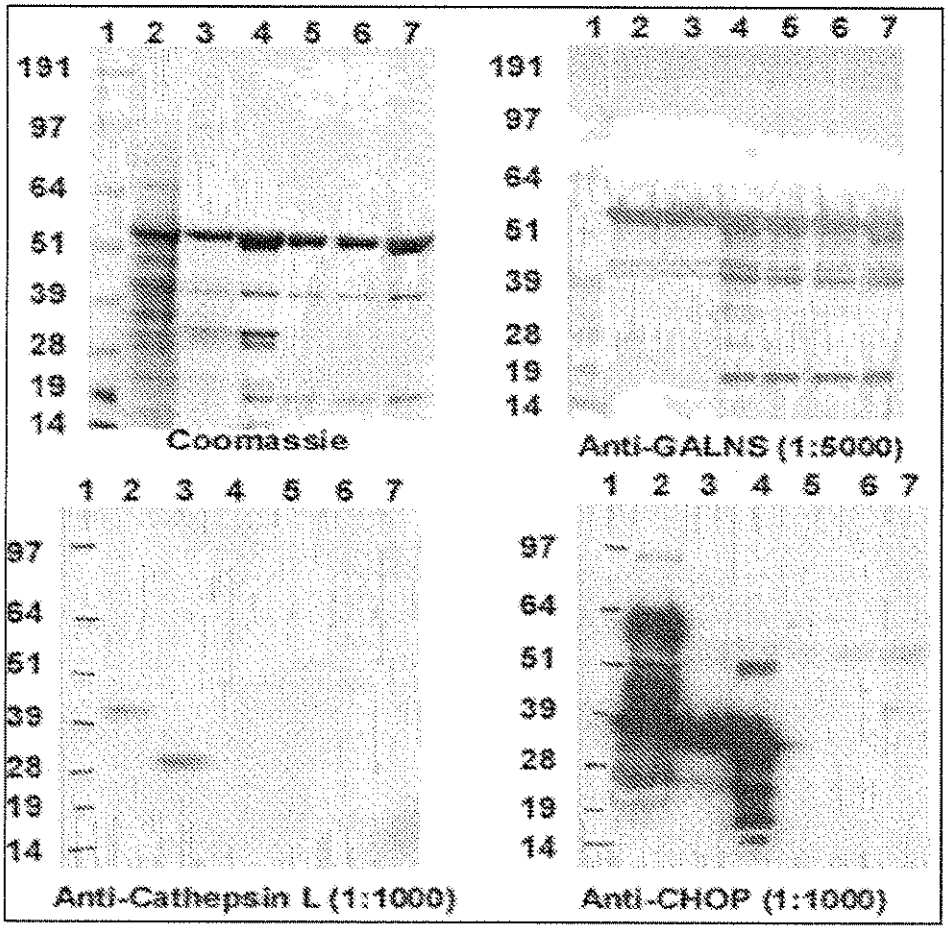


FIGURE 10



1. Marker
2. UF/DF
3. SE Hi-Cap load 042108
4. SE Hi-Cap Eluate 042108
5. Zn Eluate 042208
6. TPB Eluate 042408
7. FBDS 042508

FIGURE 11

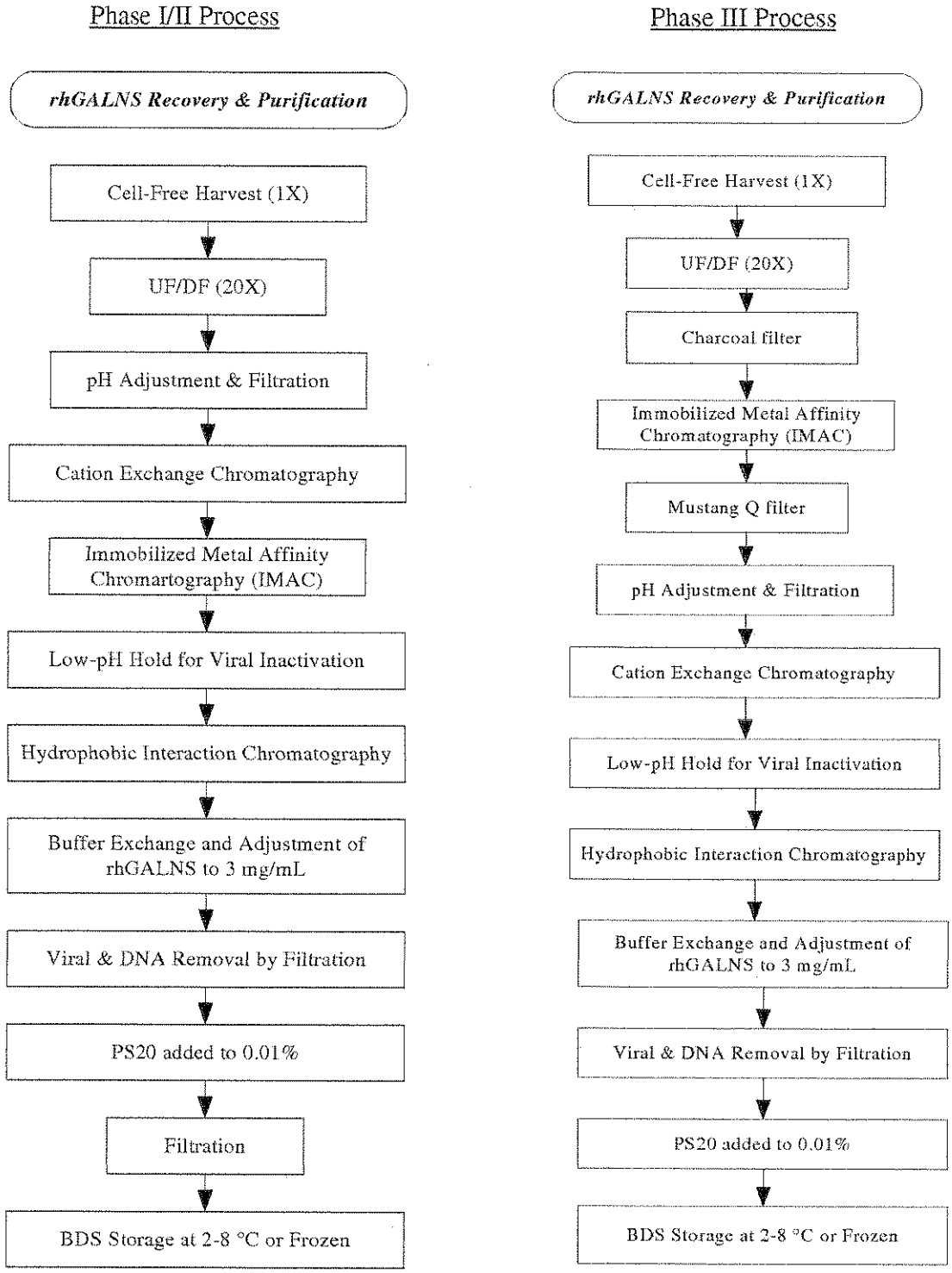

Phase I/II Process
rhGALNS Recovery & Purification
Cell-Free Harvest (1X)
UF/DF (20X)
pH Adjustment & Filtration
Cation Exchange Chromatography
Immobilized Metal Affinity Chromartography (IMAC)
Low-pH Hold for Viral Inactivation
Hydrophobic Interaction Chromatography
Buffer Exchange and Adjustment of rhGALNS to 3 mg/mL
Viral & DNA Removal by Filtration
PS20 added to 0.01%
Filtration
BDS Storage at 2-8 °C or Frozen
Phase III Process
rhGALNS Recovery & Purification
Cell-Free Harvest (1X)
UF/DF (20X)
Charcoal filter
Immobilized Metal Affinity Chromatography (IMAC)
Mustang Q filter
pH Adjustment & Filtration
Cation Exchange Chromatography
Low-pH Hold for Viral Inactivation
Hydrophobic Interaction Chromatography
Buffer Exchange and Adjustment of rhGALNS to 3 mg/mL
Viral & DNA Removal by Filtration
PS20 added to 0.01%
BDS Storage at 2-8 °C or Frozen

FIGURE 12

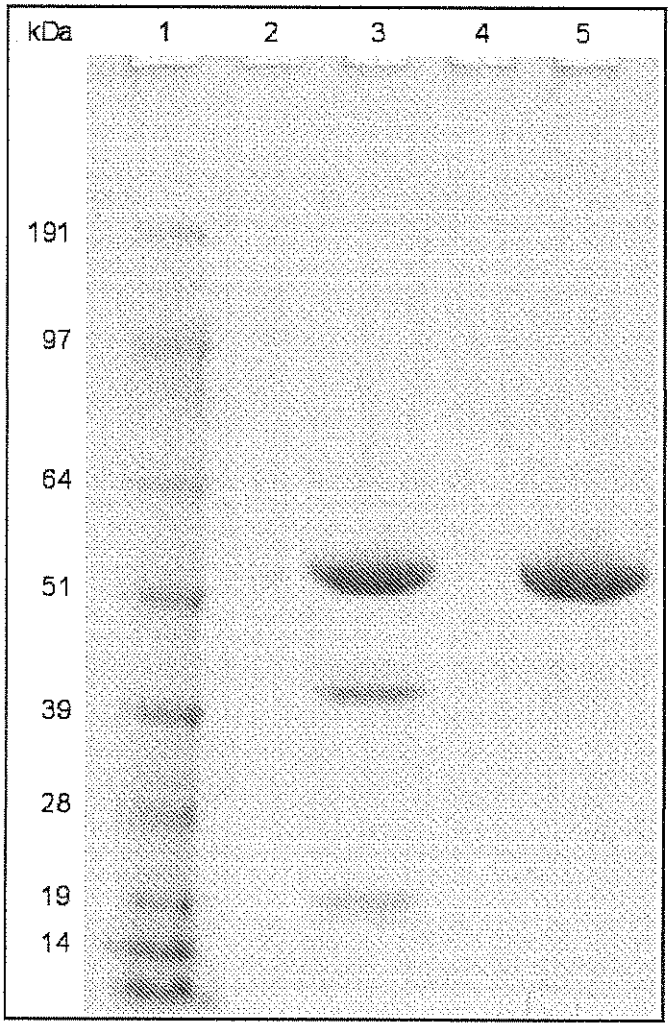


FIGURE 13

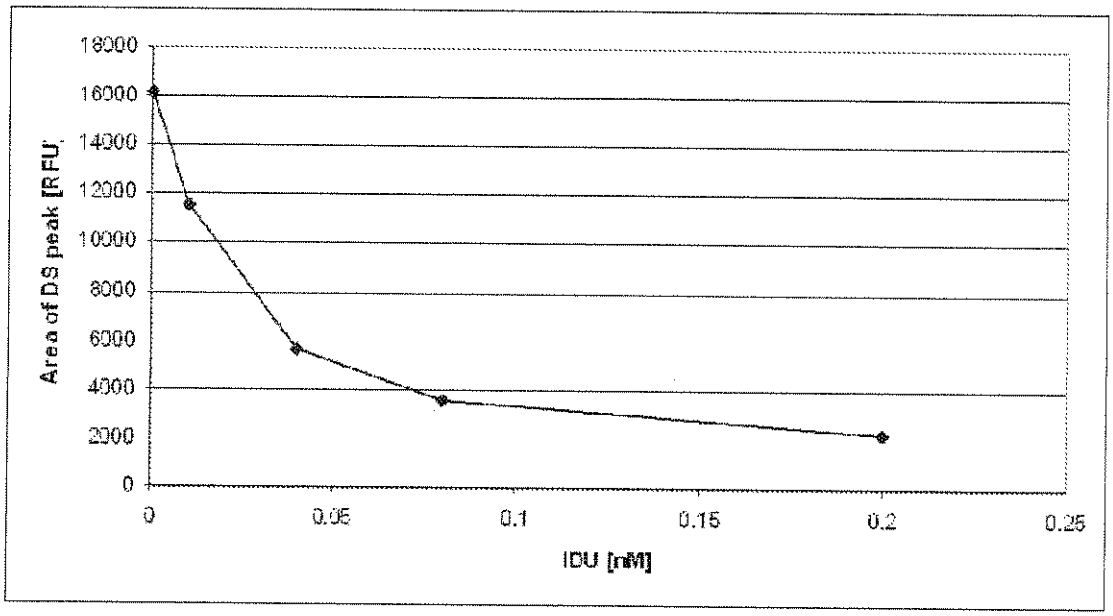

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Area of DS peak [RFU]
0
0.05
0.1
0.15
0.2
0.25
IDU [nM]

FIGURE 14

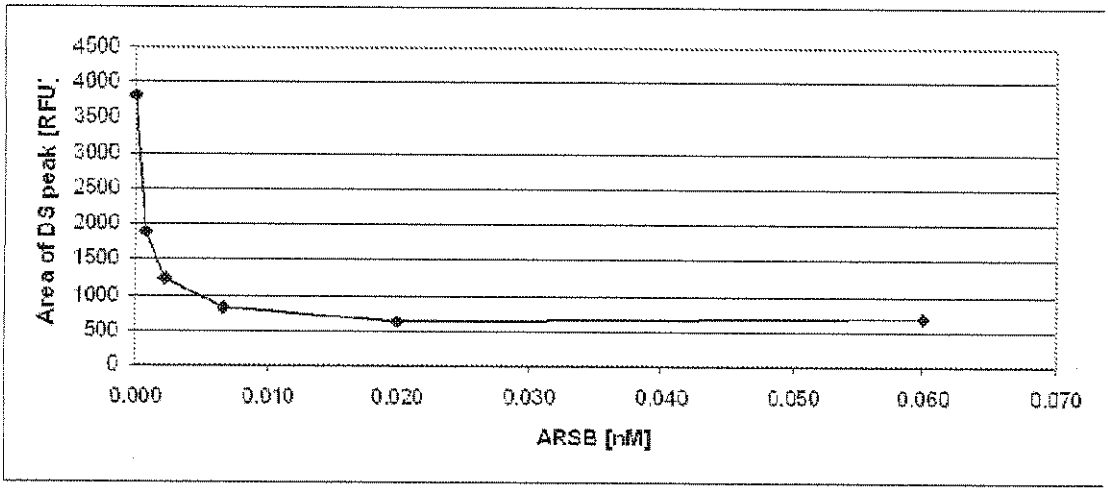

Area of DS peak [RFU]
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.000
0.010
0.020
0.030
0.040
0.050
0.060
0.070
ARSB [nM]

FIGURE 15

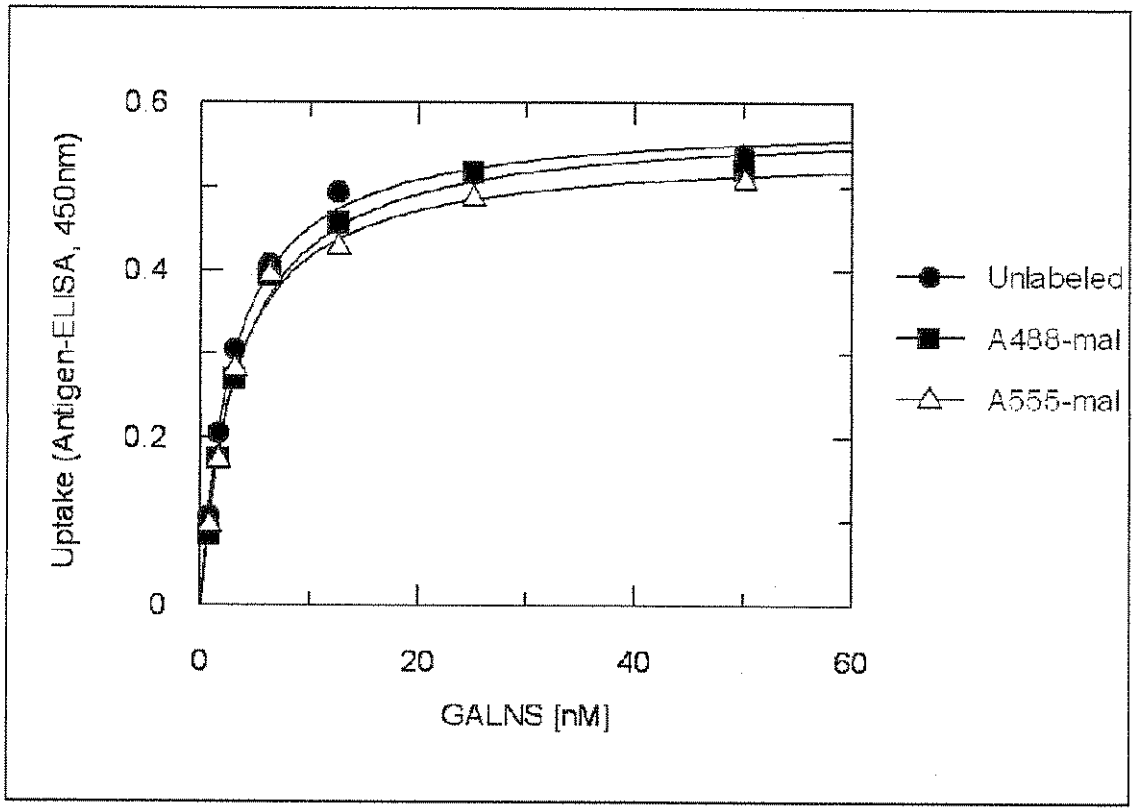

Uptake (Antigen-ELISA, 450nm)
0.6
0.4
0.2
0
0
20
40
60
GALNS [nM]
Unlabeled
A488-mal
A555-mal

FIGURE 16

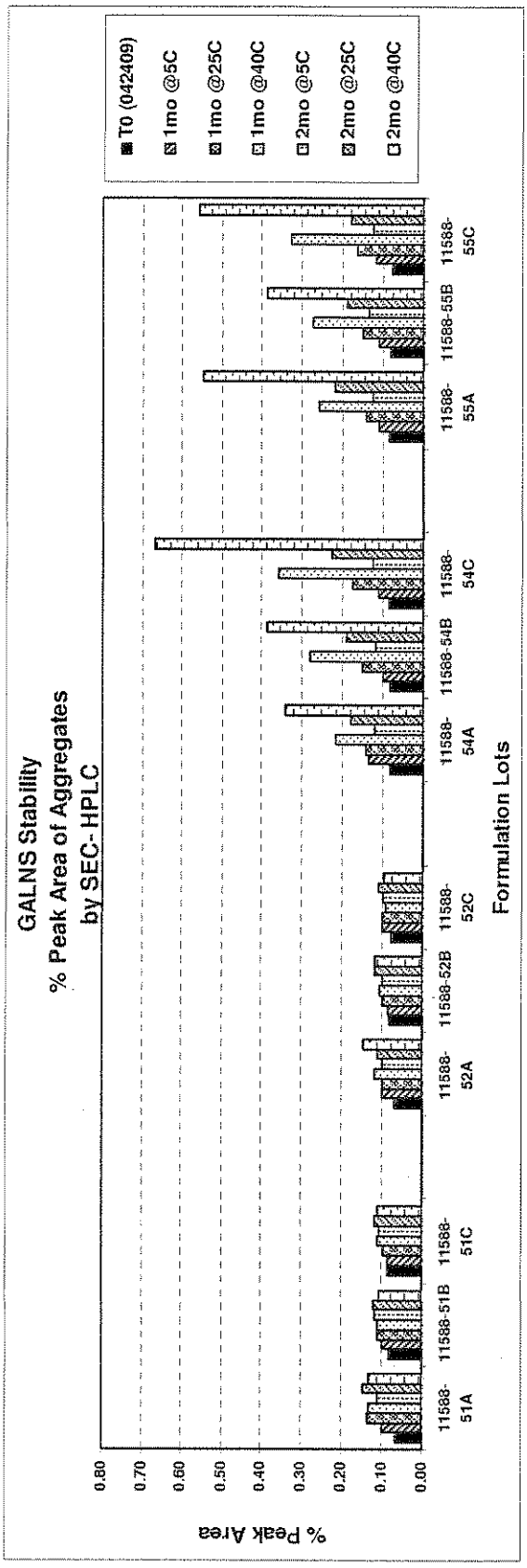

GALNS Stability
% Peak Area of Aggregates
by SEC-HPLC
T0 (042409)
1mo @5C
1mo @25C
1mo @40C
2mo @5C
2mo @25C
2mo @40C
% Peak Area
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
11588-51A
11588-51B
11588-51C
11588-52A
11588-52B
11588-52C
11588-54A
11588-54B
11588-54C
11588-55A
11588-55B
11588-55C
Formulation Lots

FIGURE 17

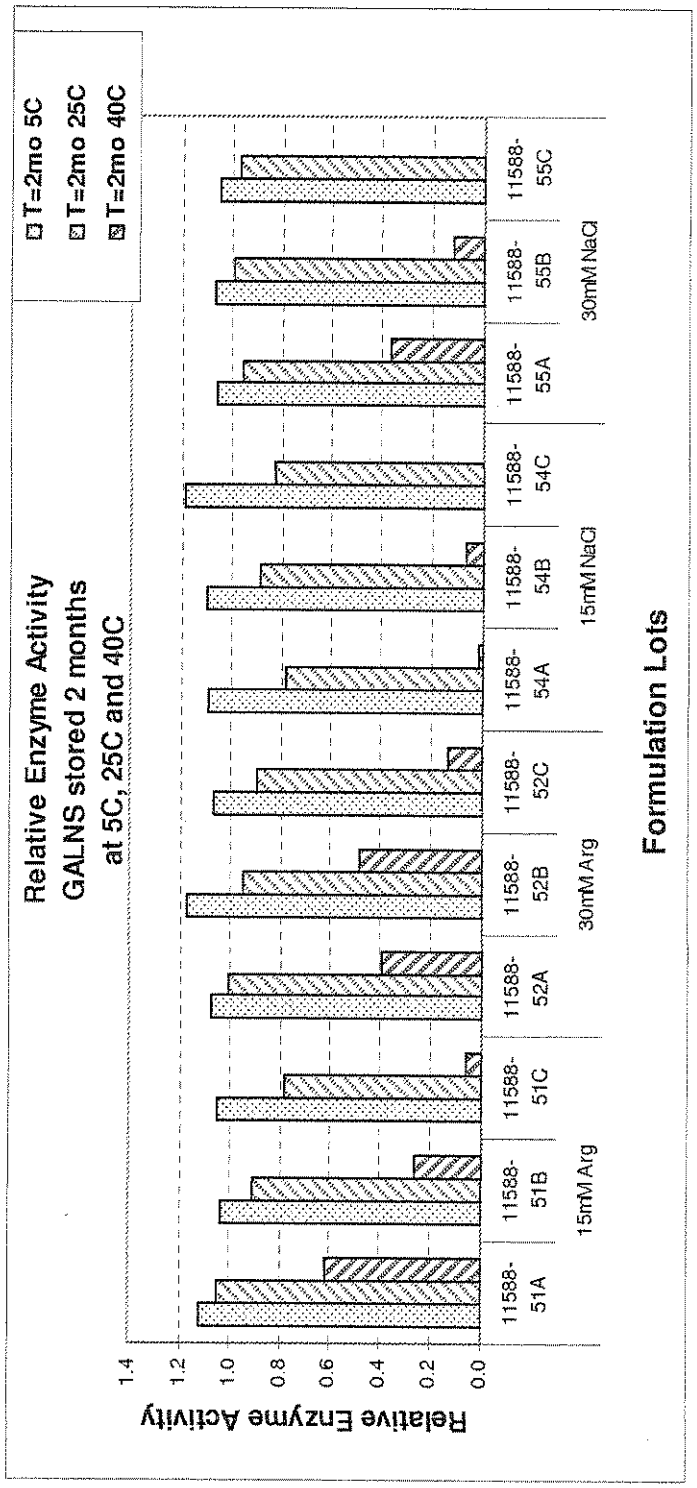

Relative Enzyme Activity
GALNS stored 2 months
at 5C, 25C and 40C
T=2mo 5C
T=2mo 25C
T=2mo 40C
Relative Enzyme Activity
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
11588-51A
11588-51B
11588-51C
11588-52A
11588-52B
11588-52C
11588-54A
11588-54B
11588-54C
11588-55A
11588-55B
11588-55C
15mM Arg
30mM Arg
15mM NaCl
30mM NaCl
Formulation Lots

FIGURE 18

Time 2 month- 5°C

	15mM L-Arginine-HCl			30mM L-Arginine-HCl			15mM NaCl			30mM NaCl			
	pH5.0	pH5.4	pH 5.8	pH5.0	pH5.4	pH 5.8	pH5.0	pH5.4	pH 5.8	pH5.0	pH5.4	pH 5.8	Reference
BPM7	34.3	32.3	34.9	33.9	33.2	34.3	34.0	33.3	34.3	34.1	33.4	31.7	32.1

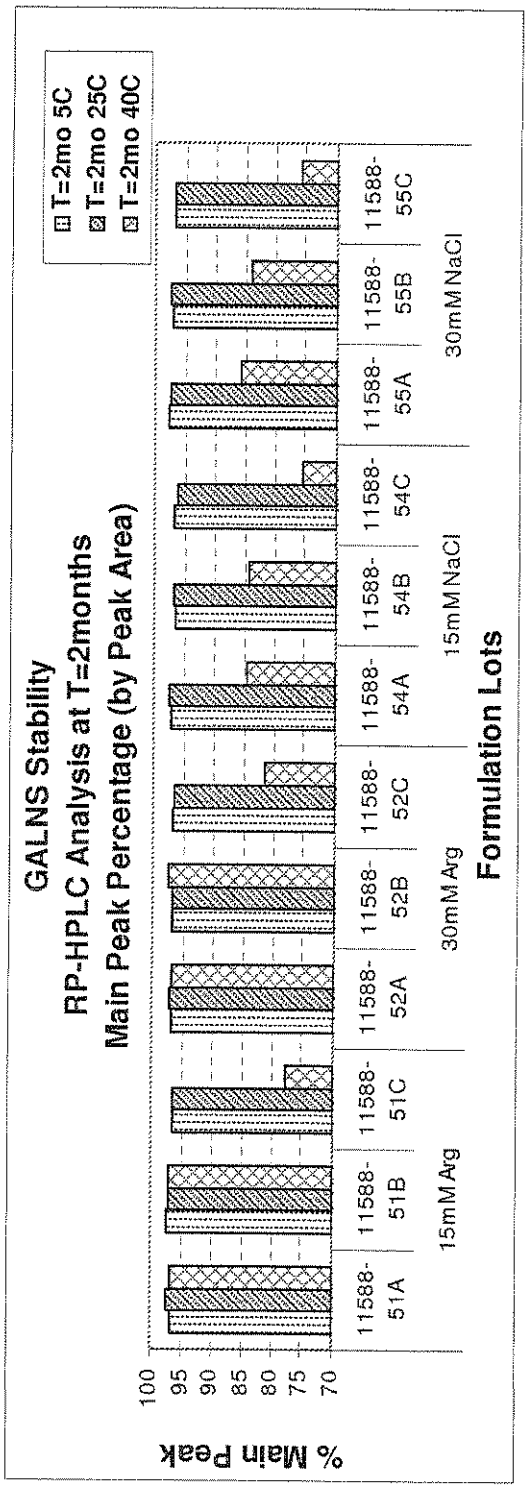
Time 2 month- 25°C

	15mM L-Arginine-HCl			30mM L-Arginine-HCl			15mM NaCl			30mM NaCl			
	pH5.0	pH5.4	pH 5.9	pH5.0	pH5.4	pH 5.9	pH5.0	pH5.4	pH 5.9	pH5.0	pH5.4	pH 5.8	Reference
BPM7	34.4	34.6	35.2	34.6	33.6	35.1	34.1	34.0	35.0	33.7	33.8	34.9	34.4

Time 2 month- 40°C

	15mM L-Arginine-HCl			30mM L-Arginine-HCl			15mM NaCl			30mM NaCl			
	pH5.0	pH5.4	pH 5.10	pH5.0	pH5.4	pH 5.10	pH5.0	pH5.4	pH 5.10	pH5.0	pH5.4	pH 5.8	Reference
BPM7	30.2	29.7	32.5	30.0	30.0	31.2	33.3	30.7	33.9	30.0	30.3	31.1	34.4

FIGURE 19


GALNS Stability
RP-HPLC Analysis at T=2months
Main Peak Percentage (by Peak Area)
T=2mo 5C
T=2mo 25C
T=2mo 40C
% Main Peak
100
95
90
85
80
75
70
11588-51A
11588-51B
11588-51C
11588-52A
11588-52B
11588-52C
11588-54A
11588-54B
11588-54C
11588-55A
11588-55B
11588-55C
15mM Arg
30mM Arg
15mM NaCl
30mM NaCl
Formulation Lots