# 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

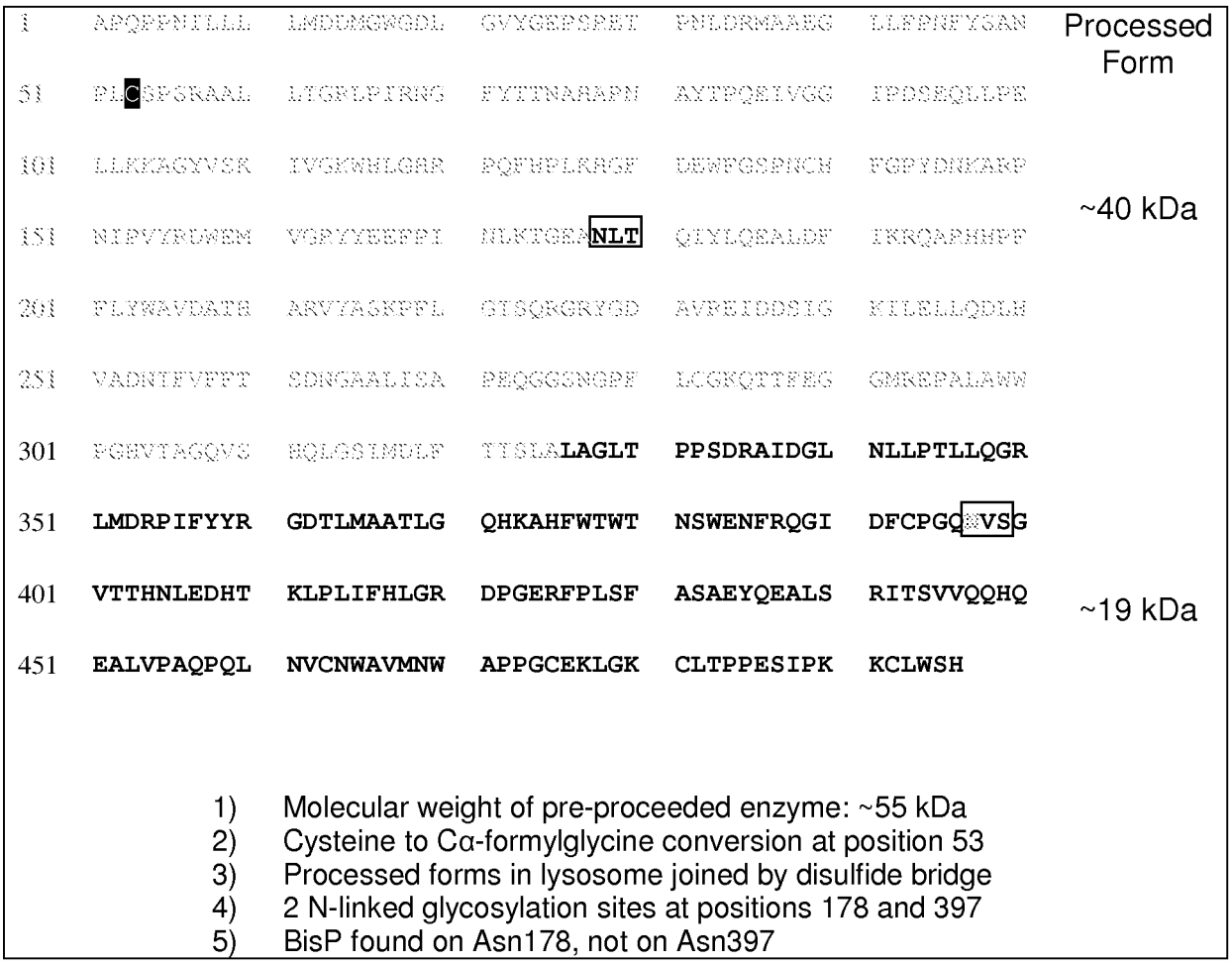
# FIGURE 4

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

MAAVVAATRWWQLLLVLSAAGMGASGAPQPPNILLLLMDDMGWGDLGVYGEPSRETPNLDRMAAEGLLFPNFYSA
NPLCSPSRAALLTGRLPIRNGFYTTNAHARNAYTPQEIVGGIPDSEQLLPELLKKAGYVSKIVGKWHLGHRPQFH
PLKHGFDEWFGSPNCHFGPYDNKARPNIPVYRDWEMVGRYYEEFPINLKTGEANLTQIYLQEALDFIKRQARHHP
FFLYWAVDATHAPVYASKPFLGTSQRGRYGDAVREIDDSIGKILELLQDLHVADNTFVFFTSDNGAALISAPEQG
GSNGPFLCGKQTTFEGGMREPALAWWPGHVTAGQVSHQLGSIMDLFTTSLALAGLTPPSDRAIDGLNLLPTLLQG
RLMDRPIFYYRGDTLMAATLGQHKAHFWTWTNSWENFRQGIDFCPGQNVSGVTTHNLEDHTKLPLIFHLGRDPGE
RFPLSFASAEYQEALSRITSVVQQHQEALVPAQPQLNVCNWAVMNWAPPGCEKLGKCLTPPESIPKKCLWSH

# FIGURE 5

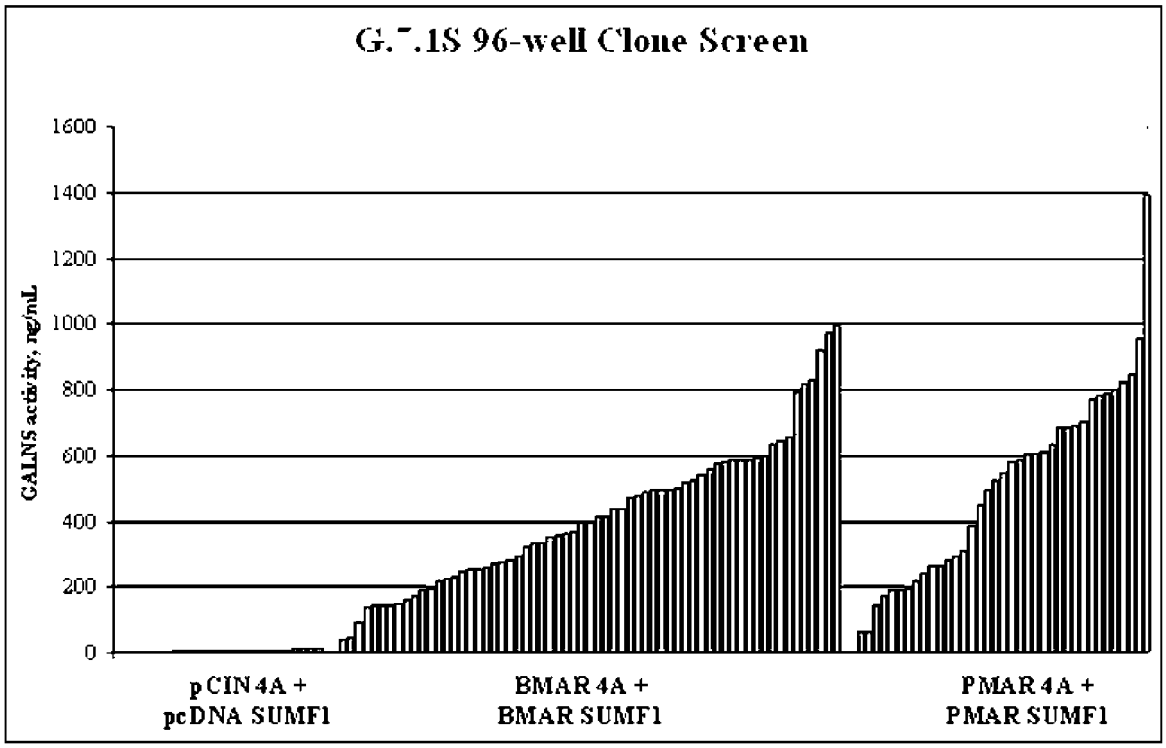
## STRUCTURE AND CHARACTERISTICS OF SECRETED HUMAN N-ACETYLGALACTOSAMINE-6-SULFATASE (GALNS) (SEQ ID NO:5)




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.

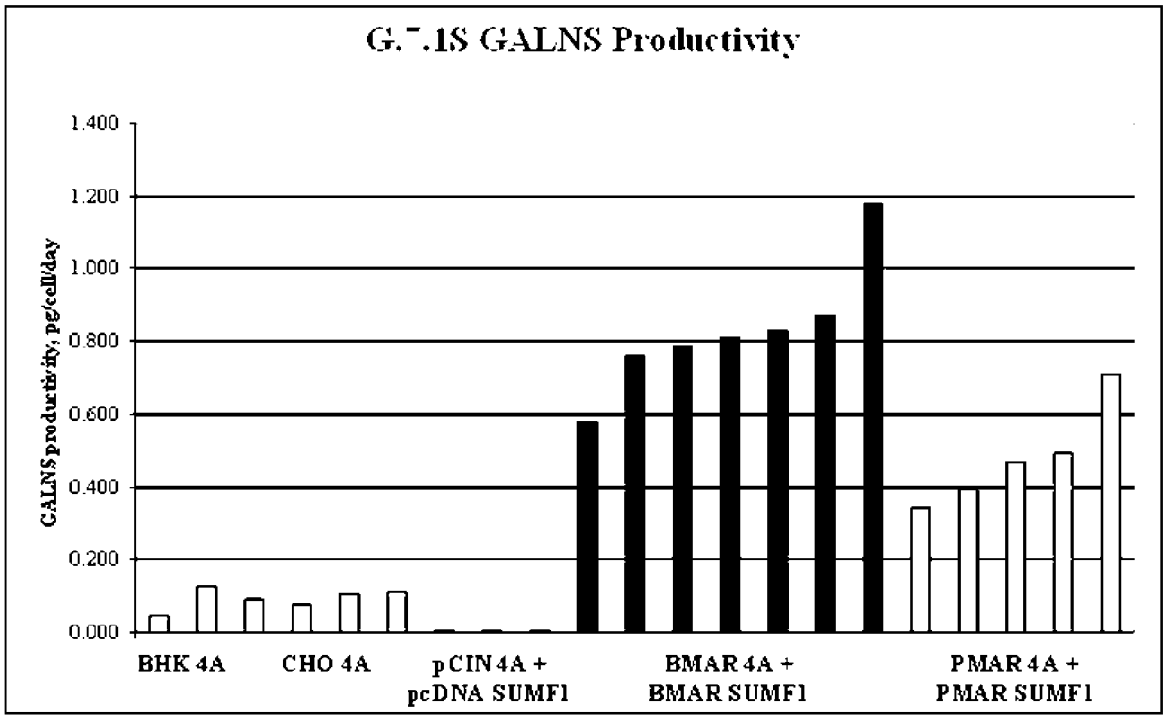


**FIGURE 7**

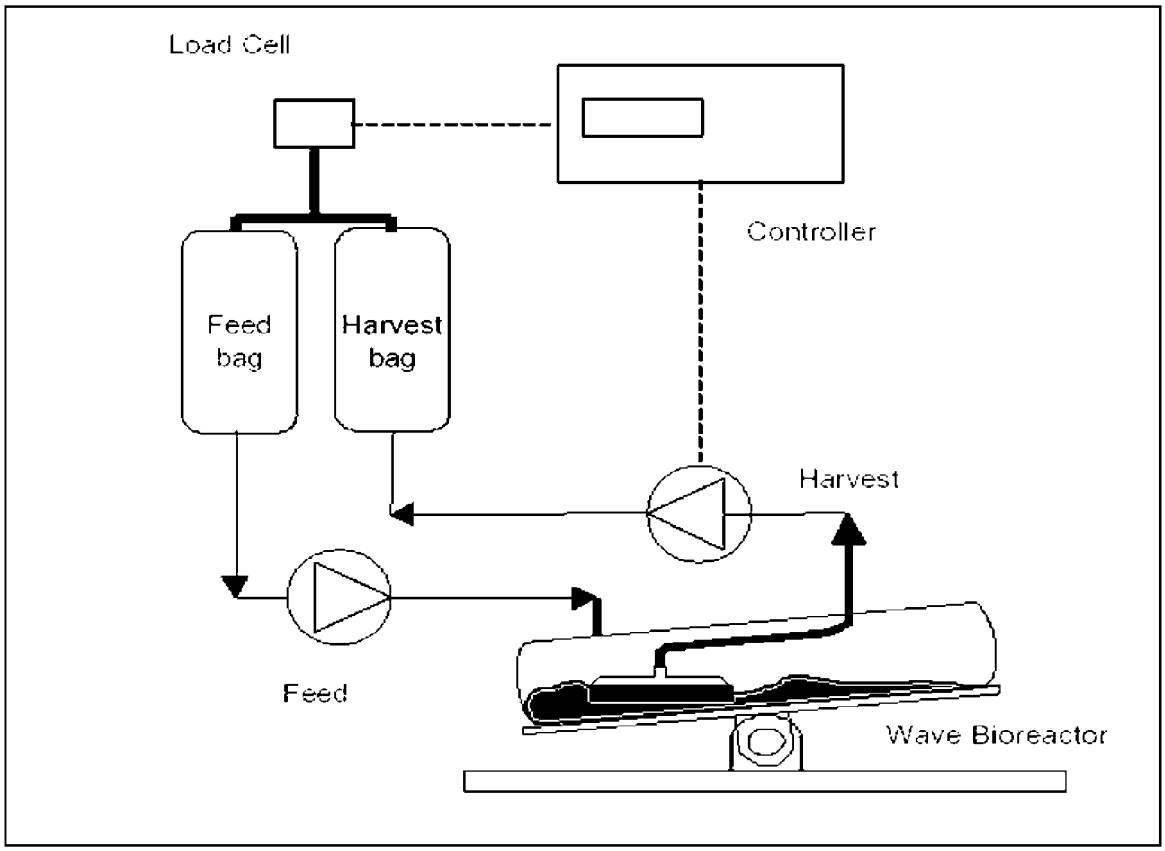
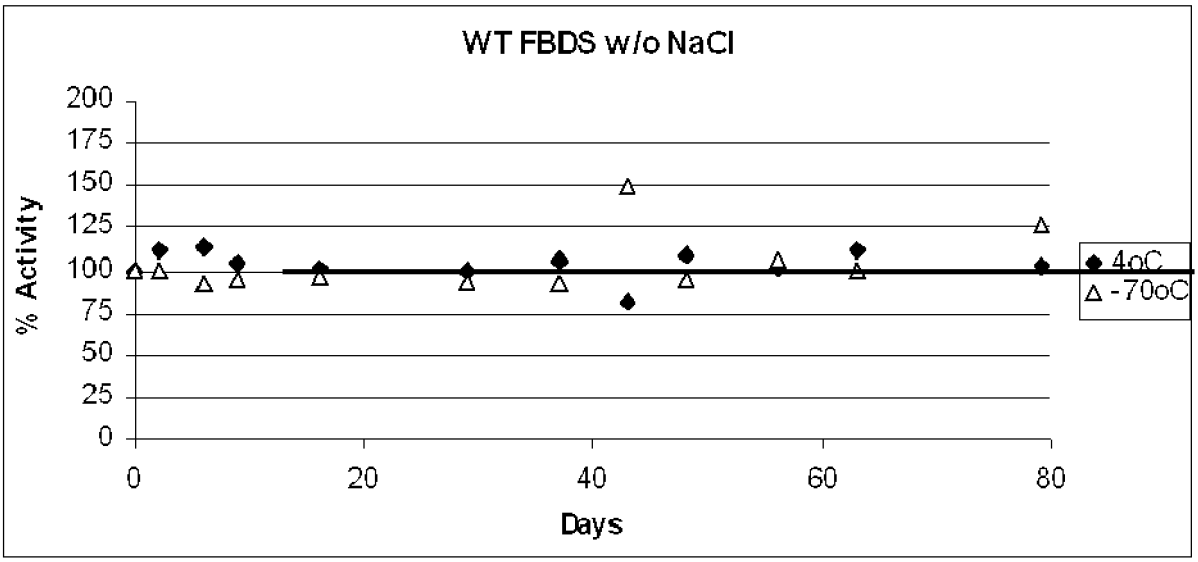
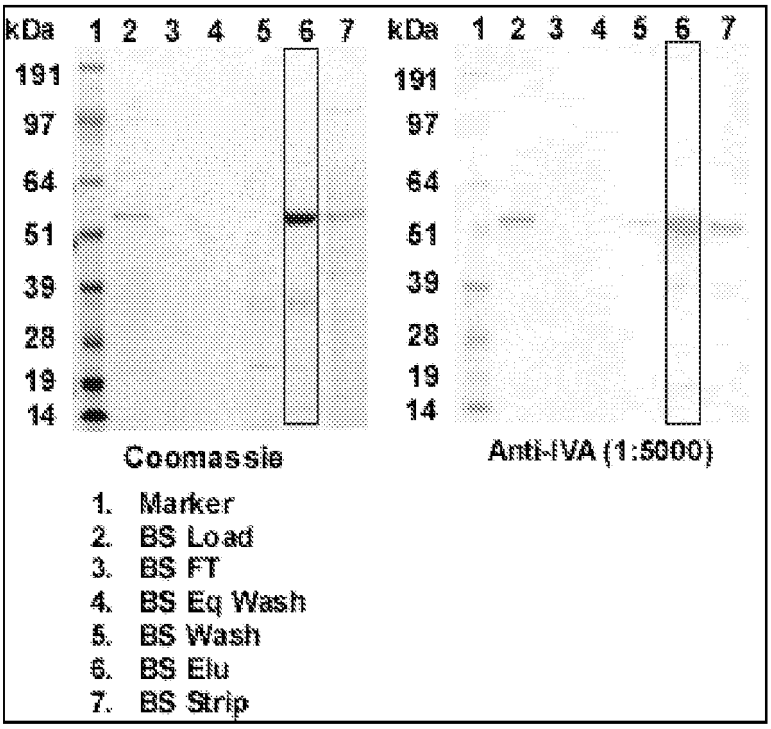

Load Cell
Controller
Feed bag
Harvest bag
Harvest
Feed
Wave Bioreactor

**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

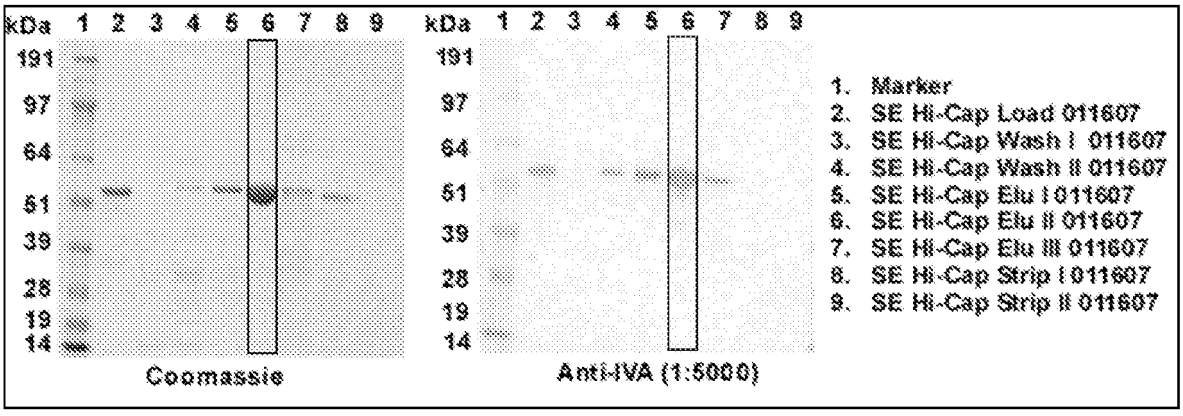
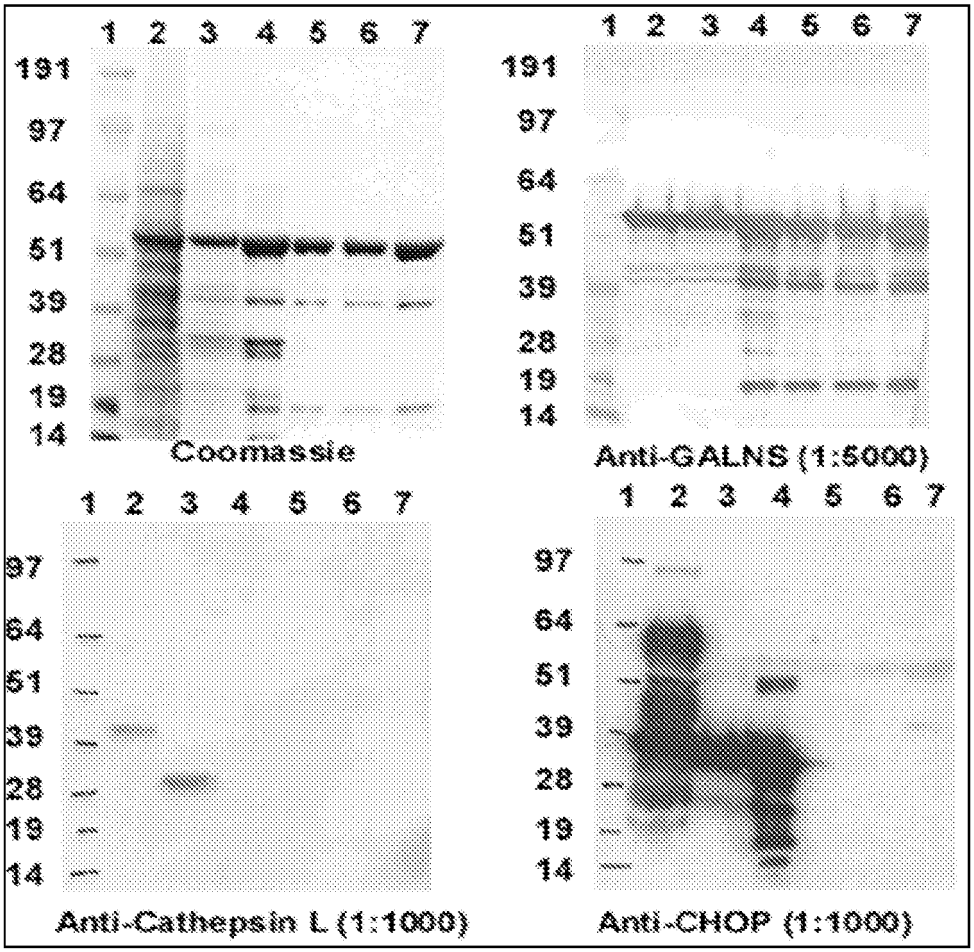


FIGURE 10




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**

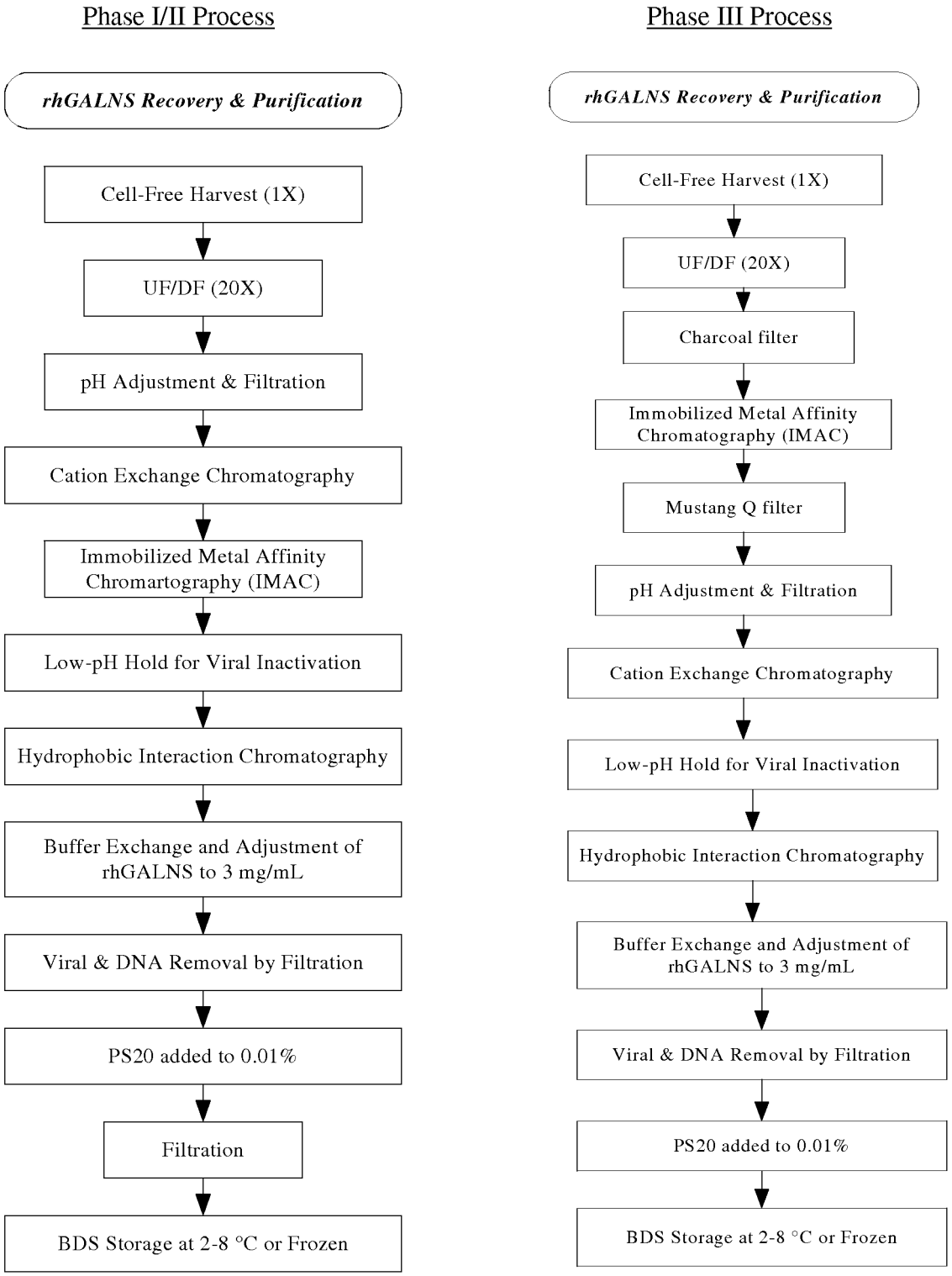

Phase I/II Process
rhGALNS Recovery & Purification
Cell-Free Harvest (1X)
UF/DF (20X)
pH Adjustment & Filtration
Cation Exchange Chromatography
Immobilized Metal Affinity Chromartography (IMAC)
Low-pH Hold for Viral Inactivation
Hydrophobic Interaction Chromatography
Buffer Exchange and Adjustment of 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**

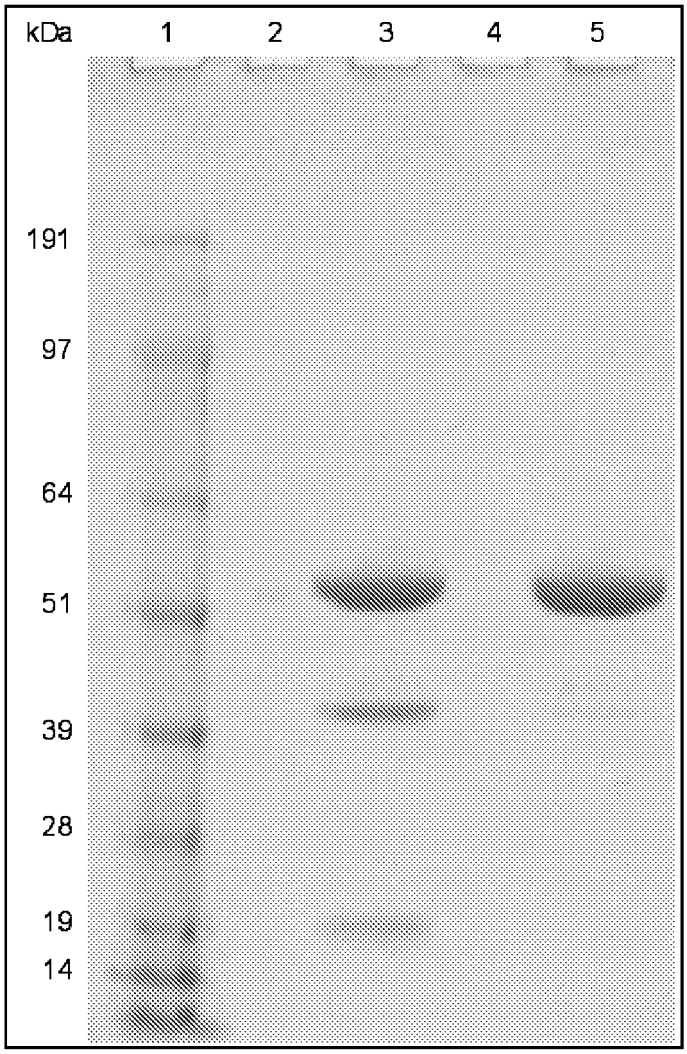


**FIGURE 13**

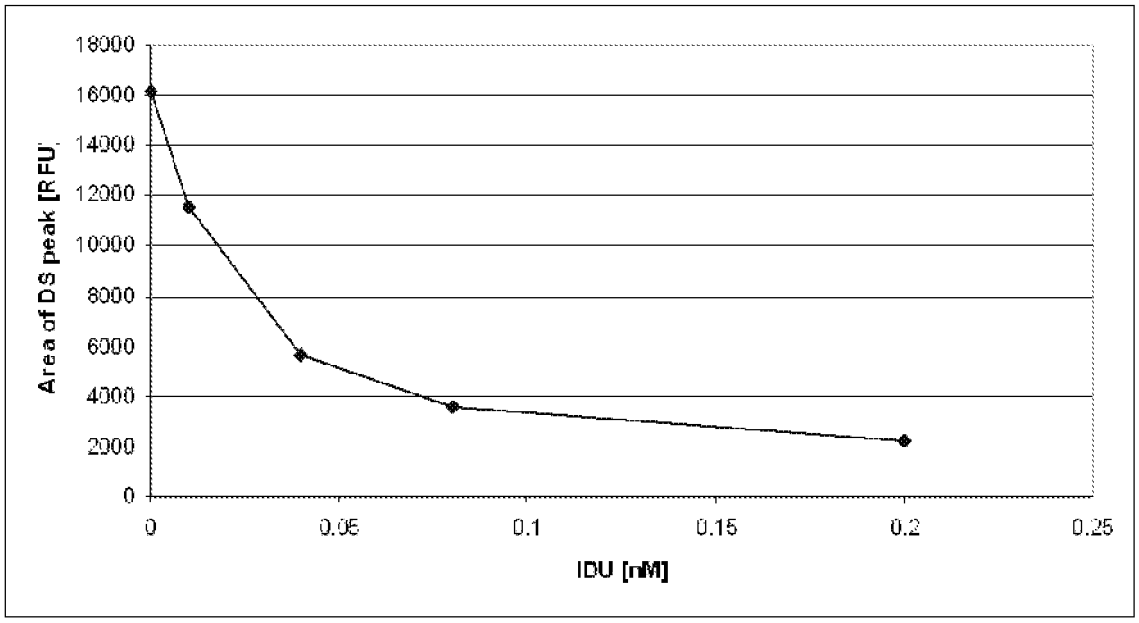

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Area of DS peak [RFU]
0
0.05
0.1
0.15
0.2
0.25
IDU [nM]

**FIGURE 14**

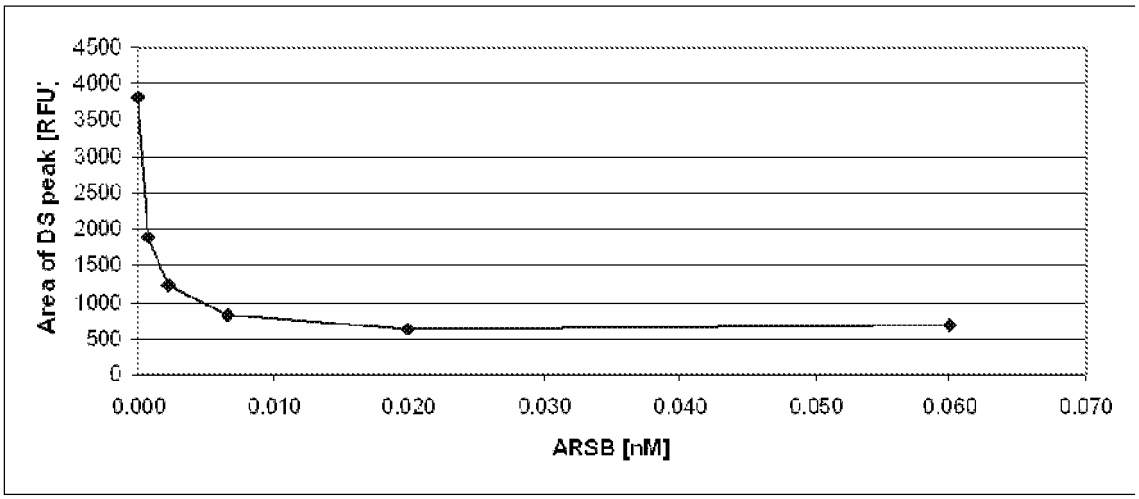

Area of DS peak [RFU]
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.000
0.010
0.020
0.030
0.040
0.050
0.060
0.070
ARSB [nM]

**FIGURE 15**

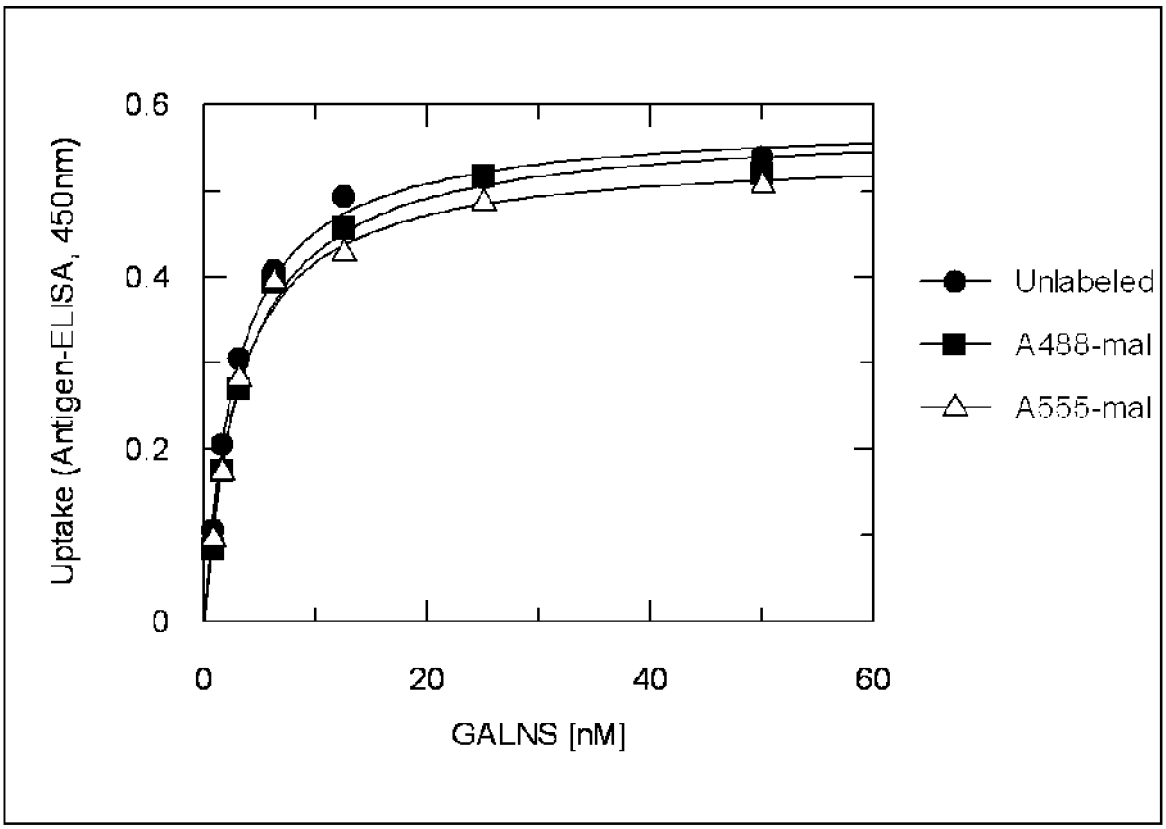

Uptake (Antigen-ELISA, 450nm)
0.6
0.4
0.2
0
0
20
40
60
GALNS [nM]
Unlabeled
A488-mal
A555-mal

FIGURE 16

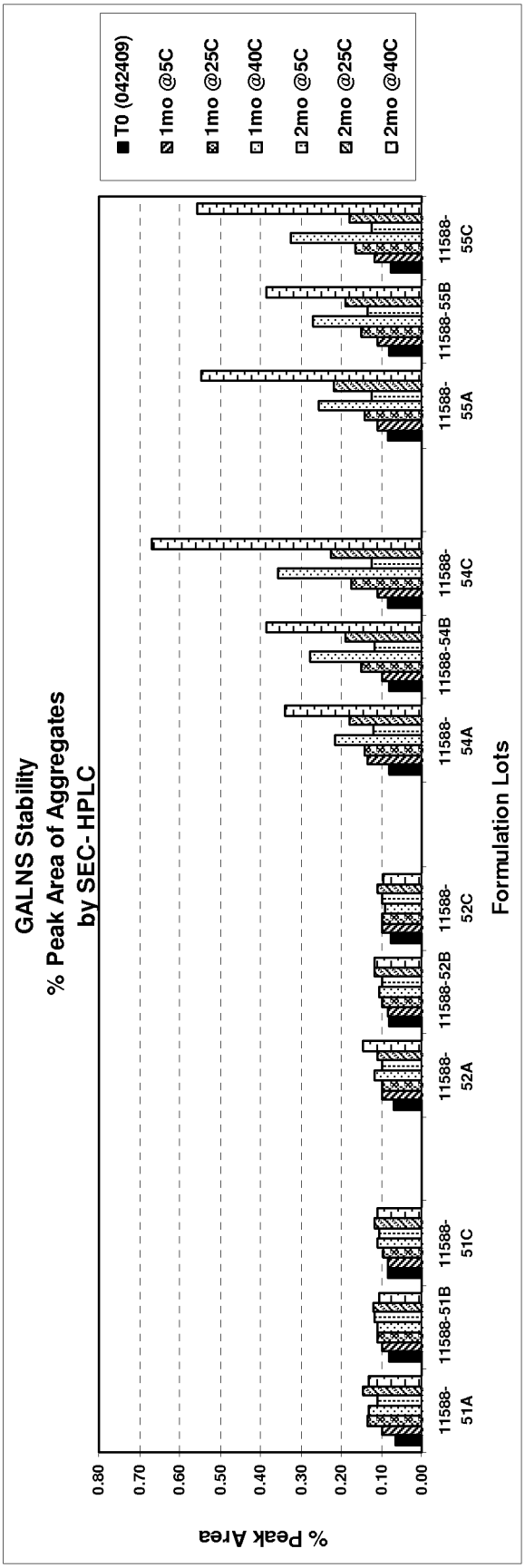

GALNS Stability
% Peak Area of Aggregates
by SEC- HPLC
T0 (042409)
1mo @5C
1mo @25C
1mo @40C
2mo @5C
2mo @25C
2mo @40C
% Peak Area
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
11588-51A
11588-51B
11588-51C
11588-52A
11588-52B
11588-52C
11588-54A
11588-54B
11588-54C
11588-55A
11588-55B
11588-55C
Formulation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

FIGURE 17

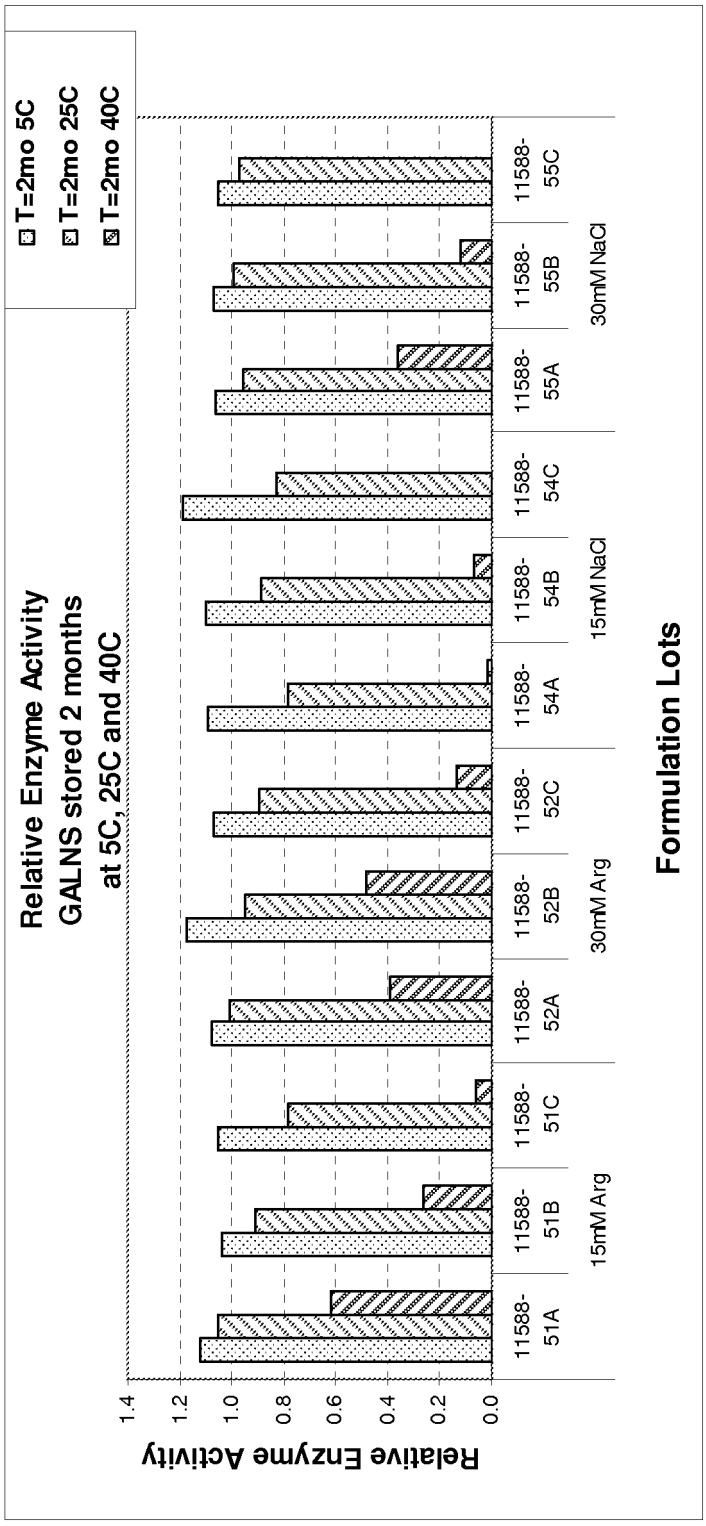
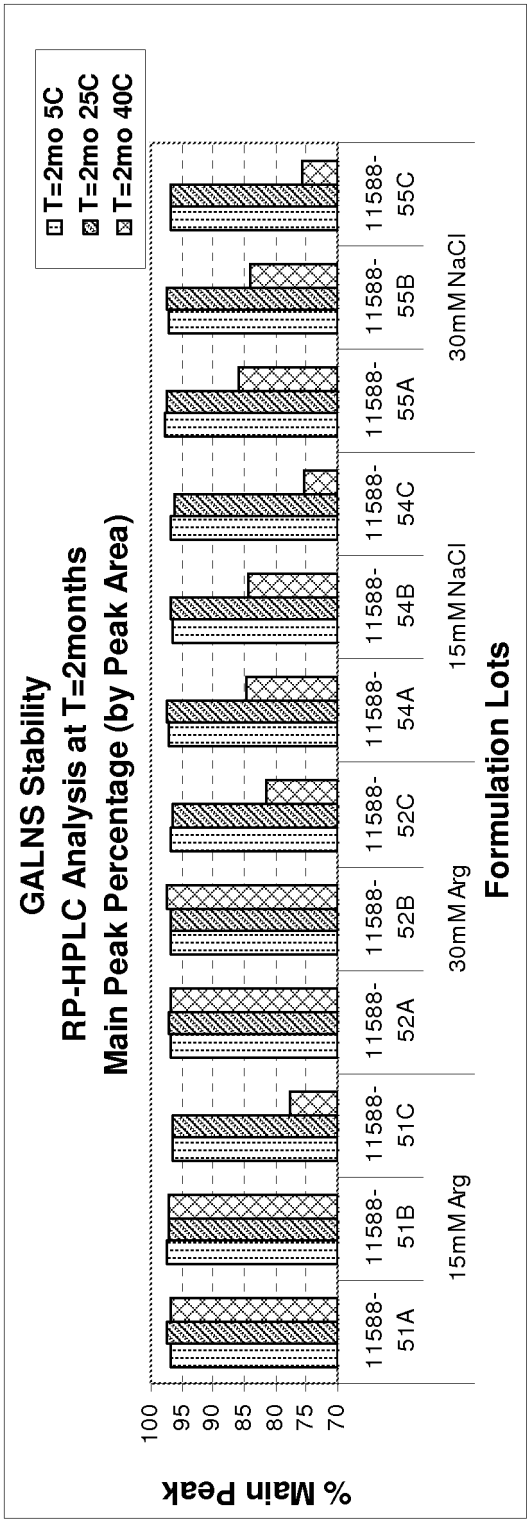

Relative Enzyme Activity
GALNS stored 2 months
at 5C, 25C and 40C
T=2mo 5C
T=2mo 25C
T=2mo 40C
Relative Enzyme Activity
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
11588-51A
11588-51B
11588-51C
11588-52A
11588-52B
11588-52C
11588-54A
11588-54B
11588-54C
11588-55A
11588-55B
11588-55C
15mM Arg
30mM Arg
15mM NaCl
30mM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