

Title:	MODIFIED HUMAN PLASMA POLYPEPTIDES OR FC SCAFFOLDS AND THEIR USES		
Applicant:	AMBRX, INC.		
Appl. No.:	PCT/US2007/019528		
Atty:	John W. Wallen, III	Telephone:	(858) 875-2403
Docket No.:	AMBX-0122.00PCT		

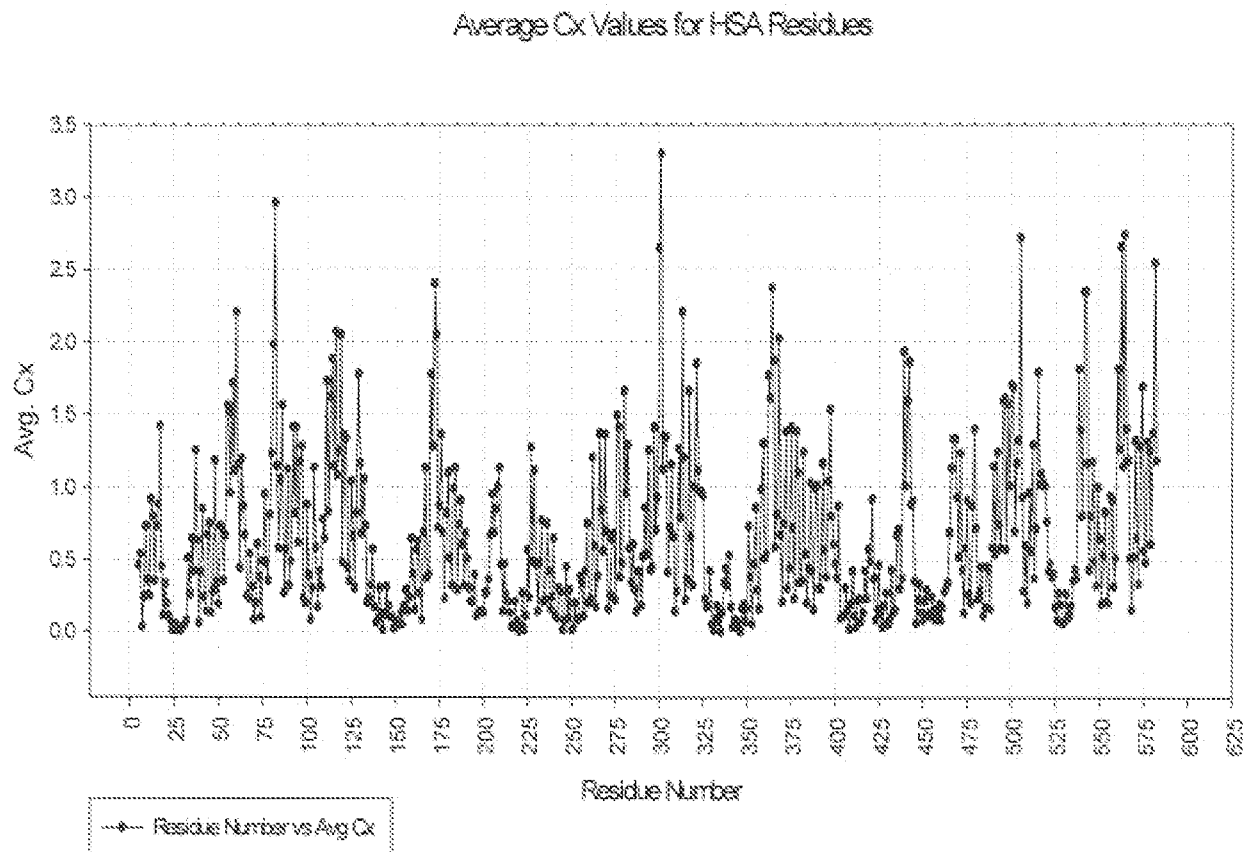
Figure 1A

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CCAKQEPERNECFLOHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARR
HPYFYAPELLFFAKRYKAAFTCCQAADKAAACLLPKLDEL RDEGKASSAKQRLKCA
SLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDR
ADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPADLPSLAADFVESKDV
CKNYAEAKDVFLGMFLY EYARRHPDYSVVL LRLAKTYETTLEKCCAAADPHECY
AKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLVRYTKKVPQVSTPTLVEVS
RNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVN
RRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATK
EQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL

(SEQ ID NO:1)

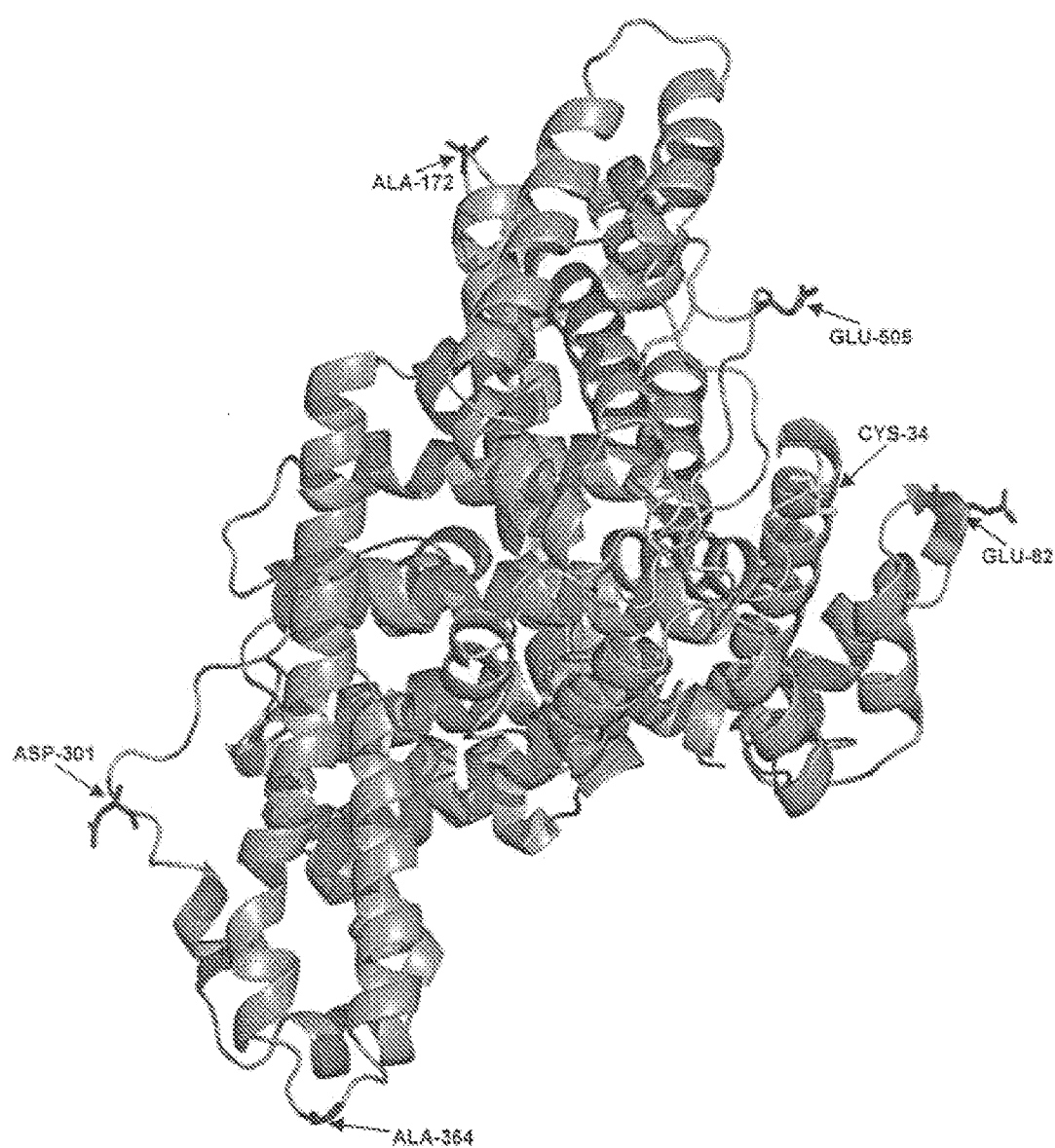
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Figure 2



Title:	MODIFIED HUMAN PLASMA POLYPEPTIDES OR FC SCAFFOLDS AND THEIR USES		
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Figure 3



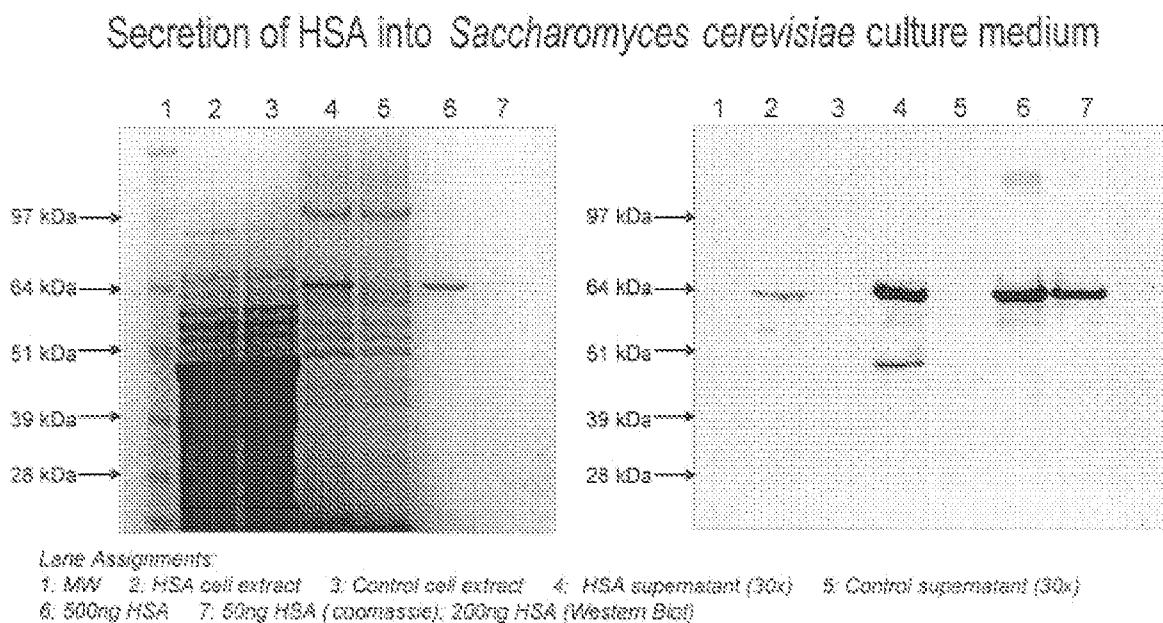
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Figure 4

Group I	Residue #	Cx value	Group II	Residue #	Cx value	Group III	Residue #	Cx value
ASP	301	3.3	ARG	81	1.98	GLU	280	1.56
GLU	82	2.96	LYS	439	1.93	LYS	317	1.66
LYS	564	2.73	ARG	114	1.88	ALA	363	1.61
GLU	505	2.71	ASP	365	1.87	THR	496	1.61
ASP	562	2.65	GLU	442	1.86	GLU	495	1.59
ALA	300	2.64	GLU	321	1.85	VAL	498	1.57
ALA	581	2.54	LYS	538	1.81	ALA	55	1.56
ALA	172	2.4	LYS	560	1.81	GLU	65	1.56
ALA	364	2.37	THR	515	1.79	GLN	397	1.53
LYS	541	2.34	ASP	129	1.78	ASP	56	1.51
GLU	542	2.34	GLN	170	1.78	LYS	276	1.48
GLU	60	2.21	ALA	362	1.77	GLU	17	1.42
LYS	313	2.21	ASN	111	1.73	ALA	92	1.41
VAL	116	2.07	SER	58	1.72	GLN	94	1.41
GLU	119	2.05	LYS	500	1.7	GLU	277	1.41
ASP	173	2.05	LYS	574	1.69	GLU	297	1.41
GLU	368	2.02	GLU	501	1.68	ASP	375	1.41

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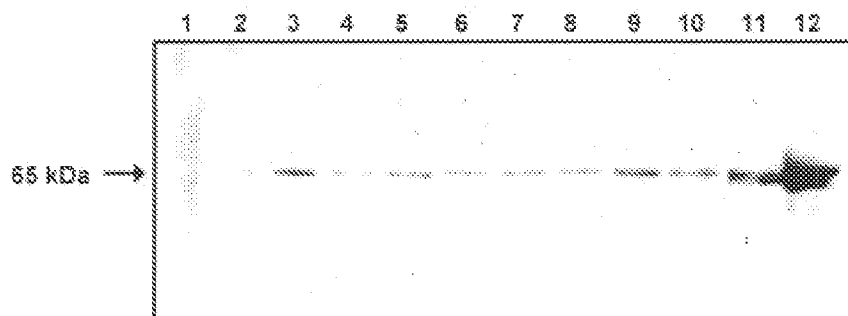
Figure 5



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Figure 6

Detection of suppressed HSA by Western Blot

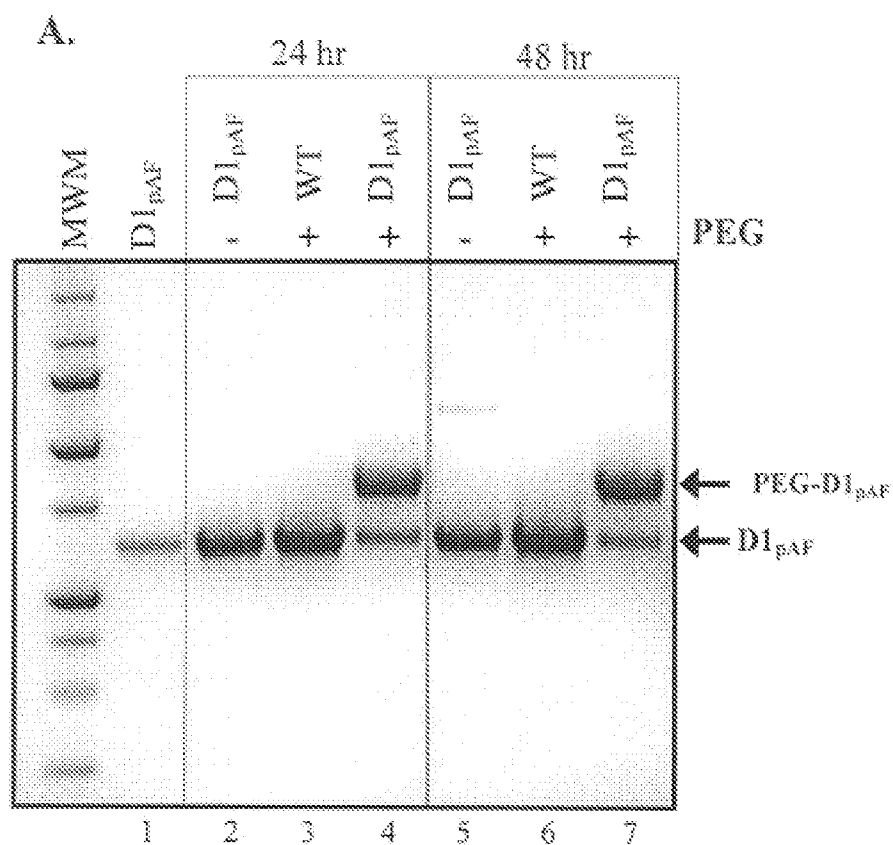


Lane Assignments:

- 1: MW
- 2: pGADHSA(C34Amb)
- 3: pGADHSA(C34Amb) + *E. coli* TyrRS/tRNA_{CUA}
- 4: pGADHSA(C34Amb) + *E. coli* pAFRS/tRNA_{CUA}
- 5: pGADHSA(C34Amb) + *E. coli* pAFRS/tRNA_{CUA} + 1mM pAF
- 6: pGADHSA(C34Amb) + *E. coli* pAzRS/tRNA_{CUA}
- 7: pGADHSA(C34Amb) + *E. coli* pAzRS/tRNA_{CUA} + 1mM AzF
- 8: pGADHSA(C34Amb) + *E. coli* pBzRS/tRNA_{CUA}
- 9: pGADHSA(C34Amb) + *E. coli* pBzRS/tRNA_{CUA} + 1mM BzF
- 10: pGADHSA(C34Amb) + *E. coli* pOMeRS/tRNA_{CUA}
- 11: pGADHSA(C34Amb) + *E. coli* pOMeRS/tRNA_{CUA} + 1mM OMeF
- 12: 500 ng HSA control

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Figure 7



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Figure 8A

WT Fc Nucleotide sequence:

ATGTACAGGATGCAACTCCTGTCTTGCAATTGCACTAAGTCTTGCACTTGTACCGAATTCGGA
CAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTG
GTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGA
GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA
GCGTCCTCACCGTCCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA
ACCACAGGTGTACACCCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGA
CCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGCAGAGCAATGGGCAG
CCGAGAAACAACACTACAAGACCACCCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGAGGGGAACGTCTTCTCATGCTCCGTGA
TGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

(SEQ ID NO: 20)

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Figure 8B

Fc Protein Sequence:

Signal Sequence - completely absent in purified protein
(underlined)

Mature Protein: wt has D at position 1
Suppressed protein has pAF at position 1
(marked with an asterisk)

*

```

MYRMQLLSCT ALSLALVTNS DKTHTCPPCP APELLGGPSV FLFPPKPKDT
LMISRTPEVT CVVDVSHED FEVKFNWYVD GVEVHNAKTK FREEQINSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPFVLD
DGSFFLYSKL TVDKSRWQQG NVFSCSVMH EALHNHYTQKS LSLSPGK-

```

(SEQ ID NO: 21)

11/22

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Figure 8C

Mature Fc Protein Sequence:

Mature Protein: wt has D at position 1
Suppressed protein has pAF at position 1
(marked with an asterisk)

*

DKTHTCPPCP AFELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKENWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVME ALHNHYTQKS LSLSPGK

(SEQ ID NO: 22)

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Figure 8D

Fc Nucleotide sequence without signal sequence:

GACAAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGSGGACCGTCAGTCTT
CCTCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG
TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGT
CAGCGTCCTCACCGTCCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCT
CCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCCAGCCCCGA
GAACCAAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCT
GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC
TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
GATGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GA

(SEQ ID NO: 23)

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Figure 9A

(RNA:

GGTGGGGTAGCGAAGTGGCTAAACGCGGCGGACTCTAAATCCGCTCCCTTTGGG
TTCGCGGGTTCGAATCCGTCCCCACCA

(SEQ ID NO: 24)

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Figure 9B

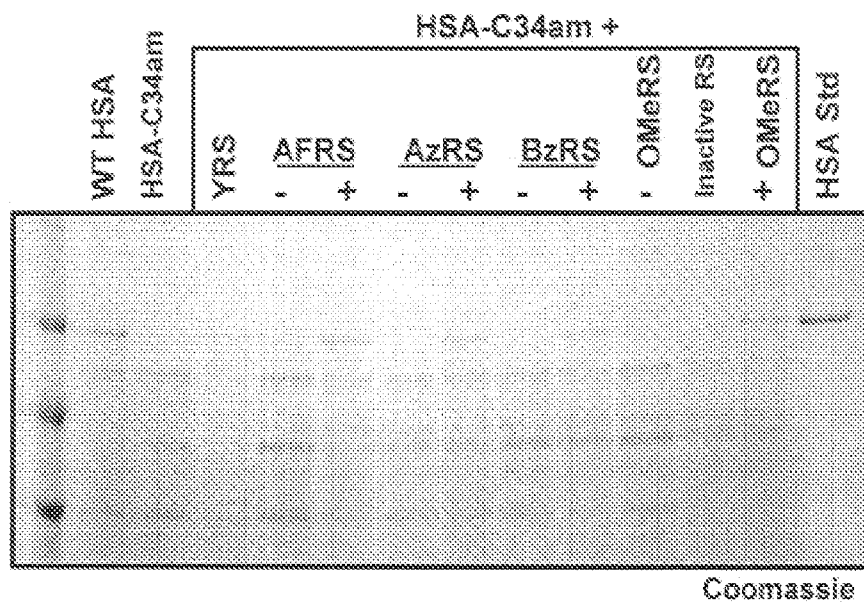
RS:

ATGGCAAGCAGTAACTTGATTAAACAATTGCAAGAGCGGGGGCTGGTAGCCCAAG
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ATTTGCGGCTTCGATCCTACCGCTGACAGCTTGCAATTGGGGCATCTTGTTCCATT
GTTATGCCTGAAACGCTTCCAGCAGGCGGGGCCACAAGCCGGTTGCGCTGGTAGG
CGGCGCGACGGGTCTGATTGGCGACCCGAGCTTCAAAGCTGCCGAGCGTAAGCT
GAACACCGAAGAACTGTTCAAGGAGTGGGTGGACAAAATCCGTAAGCAGGTTGC
CCCGTTCCTCGATTTGACTGTGGAGAAAACCTCTGCTATCGCGGCGAACAACAT
GACTGGTTCGGCAATATGAATGTGCTGACCTTCCTGCGCGATATTGGCAAACACT
TCTCCGTTAACCAGATGATCAACAAAGAAGCGGTTAAGCAGCGTCTCAACCGTG
AAGGTCAGGGGATTTTCGTTCACTGAGTTTTCCTACAACCTGTTGCAGGGTTATGG
TATGGCCTGTGCTAACAAACAGTACGGTGTGGTGCTGCAAATTGGTGGTTCTGAC
CAGTGGGGTAACATCACTTCTGGTATCGACCTGACCCGTCGTCTGCATCAGAATC
AGGTGTTTGGCCTGACCGTTCGCTGATCACTAAAGCAGATGGCACCAAATTTGG
TAAAACTGAAGGCGGCGCAGTCTGGTTGGATCCGAAGAAAACCGCCCGTACAA
ATTCTACCAGTTCTGGATCAACACTGCGGATGCCGACGTTTACCGCTTCCTGAAG
TTCTTCACCTTTATGAGCATTGAAGAGATCAACGCCCTGGAAGAAGAAGATAAA
AACAGCGGTAAAGCACCGCGCGCCAGTATGTACTGGCGGAGCAGGTGACTCGT
CTGGTTCACGGTGAAGAAGGTTTACAGGCGGCAAAACGTATTACCGAATGCCTG
TTCAGCGGTTCTTTGAGTGCCTGAGTGAAGCGGACTTCGAACAGCTGGCGCAGG
ACGGCGTACCGATGOTTGAGATGGAAAAGGGCGCAGACCTGATGCAGGCACTGG
TCGATICTGAACTGCAACCTTCCCGTGGTCAGGCACGTAAAACTATCGCCTCCAA
TGCCATCACCATTAAACGGTGAAAAACAGTCCGATCCTGAATACTTCTTTAAAGAA
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(SEQ ID NO: 25)

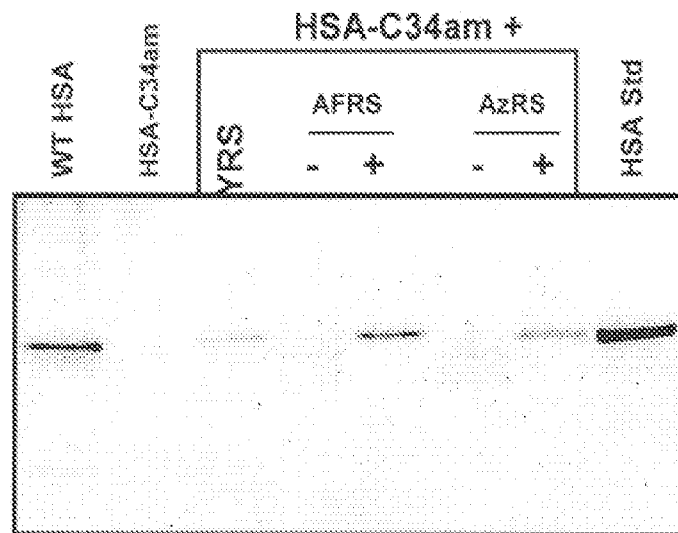
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Figure 10A



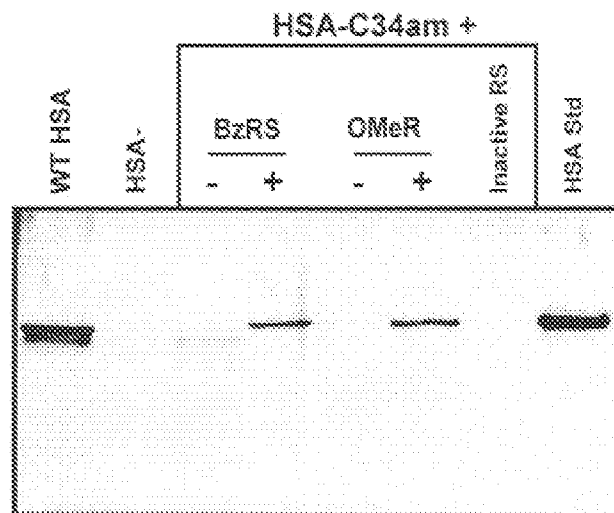
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Figure 10B

 α -HSA Western

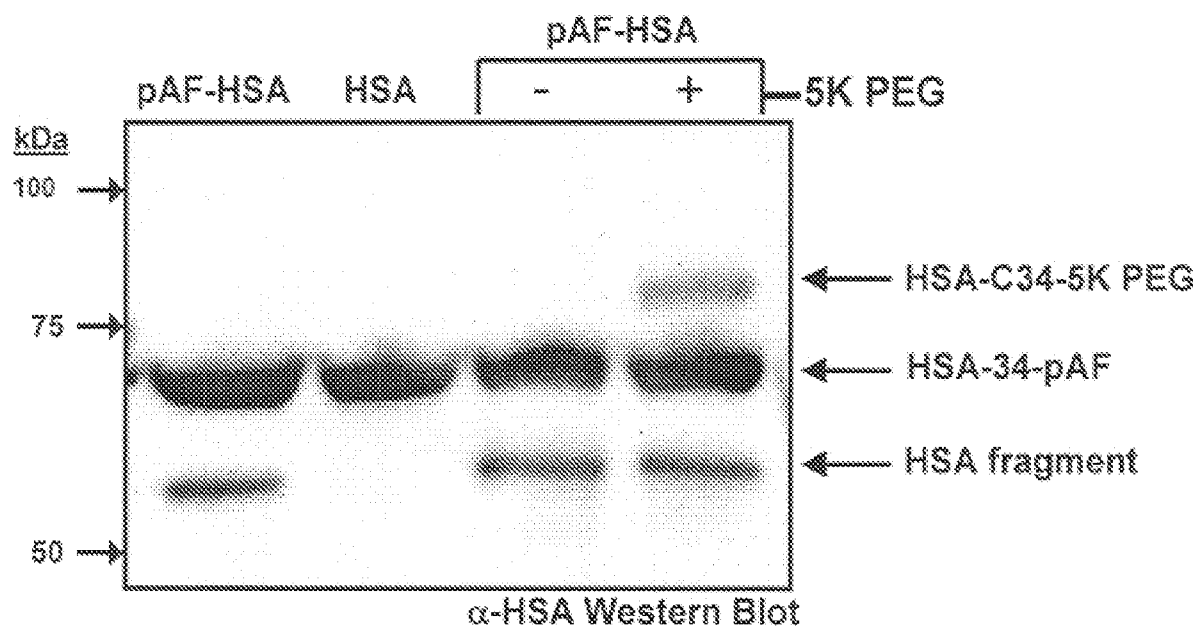
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Figure 10C

 α -HSA Western

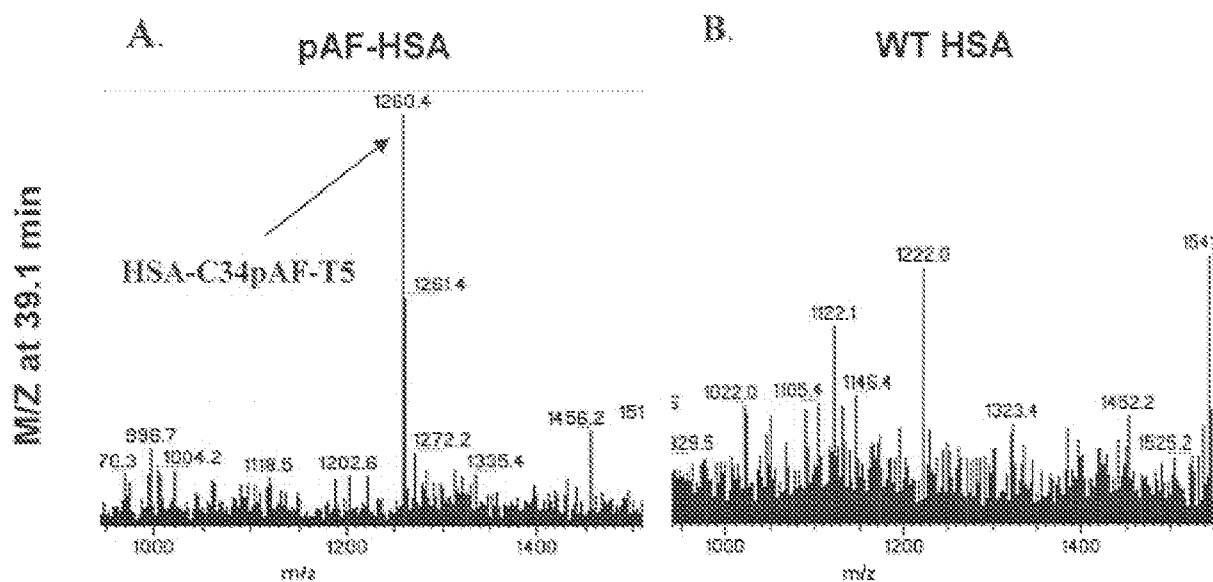
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Figure 11



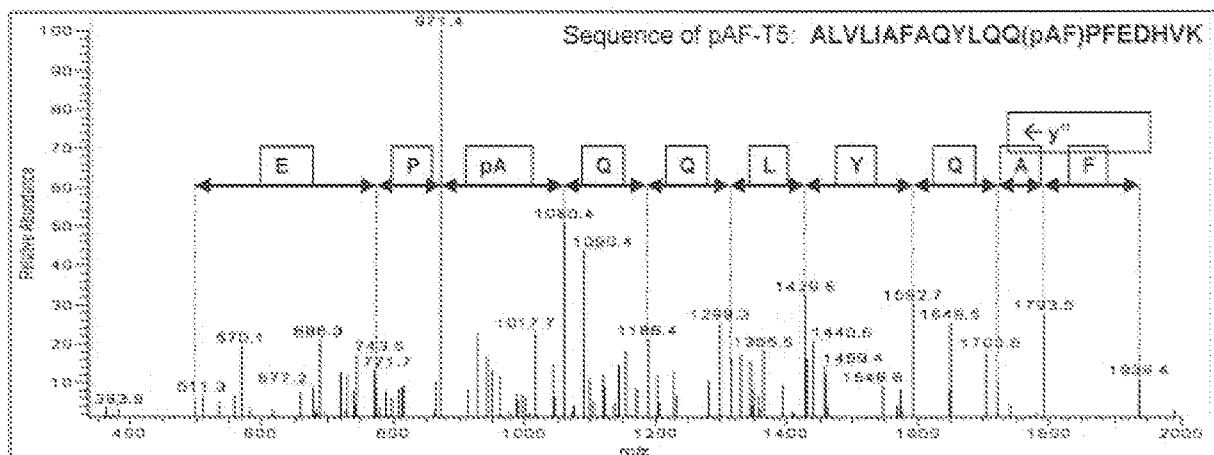
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Figure 12



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Figure 13A



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Figure 13B

b	Amino Acid	y ⁿ
72.05	Ala	x
186.13	Leu	2412.23
284.20	Val	2335.13
387.28	Leu	2236.05
510.37	Ile	2122.98
581.40	Ala	2009.90
722.47	Phe	1938.85
799.51	Ala	1791.80
927.57	Gln	1720.75
1090.53	Tyr	1592.70
1203.71	Leu	1429.63
1321.77	Gln	1216.55
1459.83	Gln	1188.49
1648.83	pAF	1060.43
1845.88	Pro	871.43
1932.95	Phe	774.38
2022.00	Glu	627.31
2137.02	As	498.27
2274.06	His	363.24
2373.15	Val	246.19
x	Lys	147.11

Predicted ion masses

Identified ions in Figure 13A

Identified ions (not shown)