

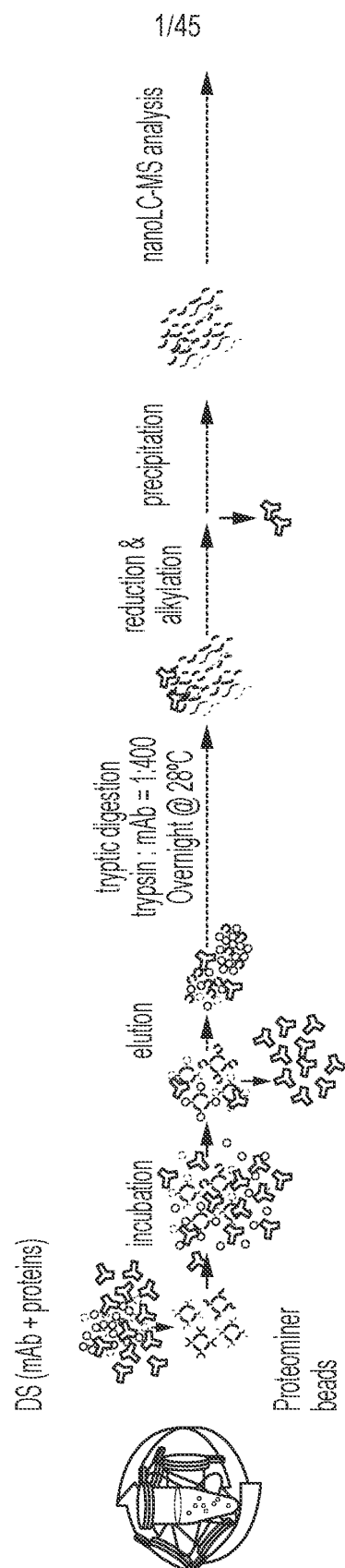


FIG. 1

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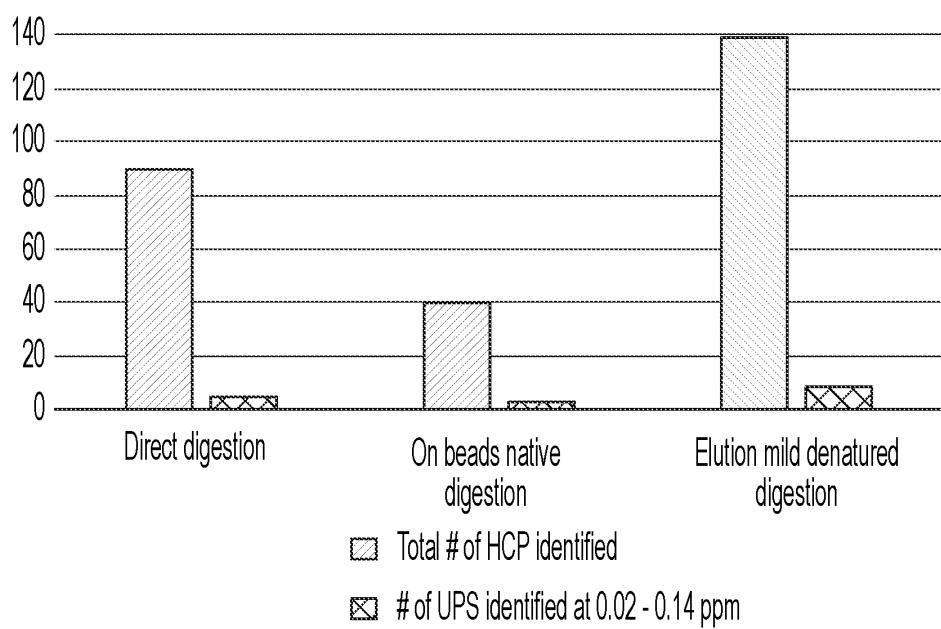


FIG. 2

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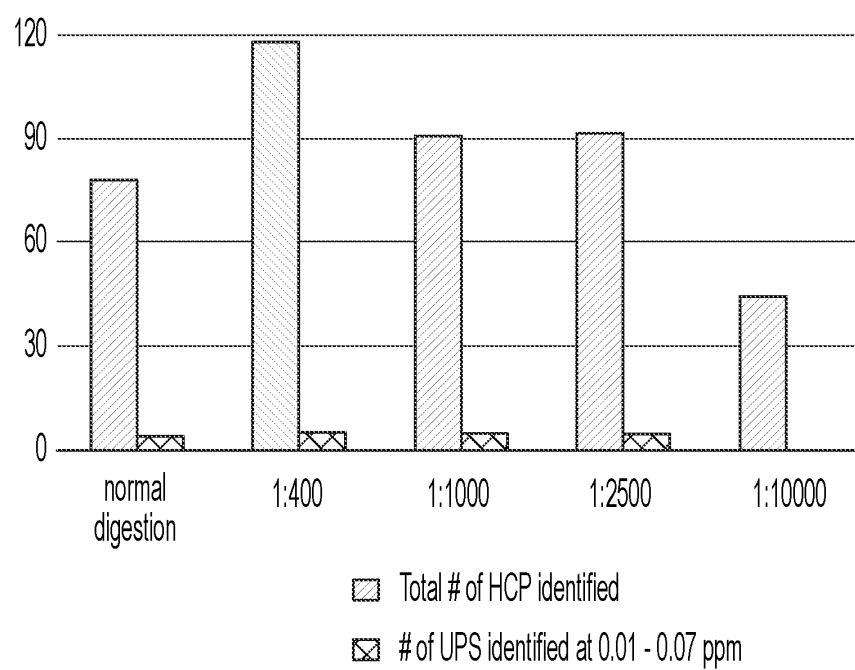


FIG. 3

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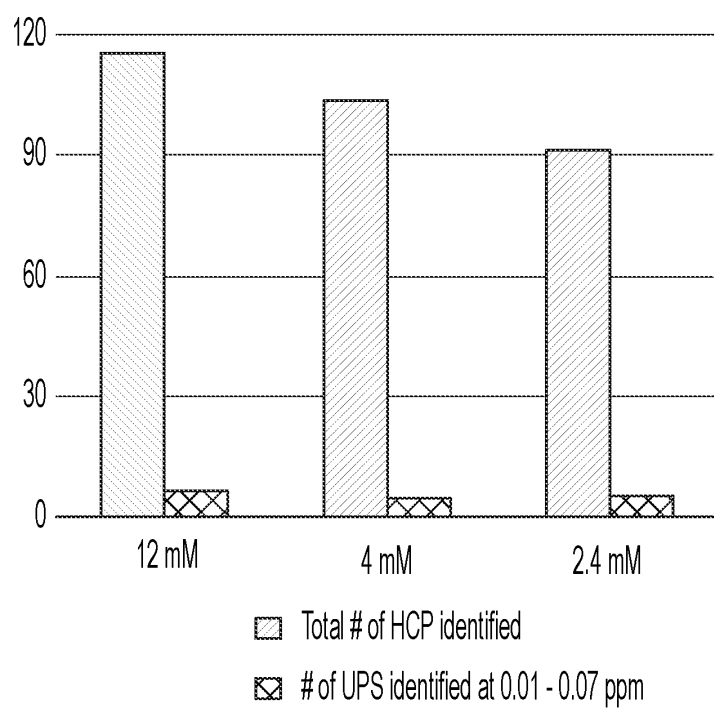


FIG. 4

Accession Number	UPS2 Amount (fmol)	UniProt Protein Name [Synonym]	MW (Da)	UPS2 ppm (w/w)	Optimized Limited Digestion	ProteoMiner + Limited Digestion	ProteoMiner
		liver carboxylesterase 1 like protein			22	36	20
		liver carboxylesterase B-1 like protein			4	4	3
P00915	50000	Carbonic anhydrase 1	28738	3.59	16	23	13
P00918	50000	Carbonic anhydrase 2	29115	3.64	18	27	20
P01031	50000	Complement C5 [Complement C5a]	8536	1.07	4	10	5
P69905	50000	Hemoglobin alpha chain	15126	1.89	7	13	11
P68871	50000	Hemoglobin beta chain	15867	1.98	11	17	14
P41159	50000	Leptin	16158	2.02	6	9	8
P02768	50000	Serum Albumin	66357	8.29	37	71	69
P62988	50000	Ubiquitin	10597	1.32		8	7
P04040	5000	Catalase	59625	0.75	21	30	18
P00167	5000	Cytochrome b5	16022	0.20	4	8	6
P01133	5000	Epidermal Growth Factor	6353	0.08			5
P02144	5000	Myoglobin C	17053	0.21	5	18	12
P15559	5000	NAD(P)H dehydrogenase [quinone] 1 [DT Diaphorase] C	30736	0.38	5	8	5
P62937	5000	Peptidyl-prolyl cis-trans isomerase A [Cyclophilin A]	20176	0.25		19	7
Q06830	5000	Peroxioredoxin 1	21979	0.27	3	6	6
P63165	5000	Small ubiquitin-related modifier 1 [SUMO-1]	38815	0.49	11	31	12

FIG. 5A

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Accession Number	UPS2 Amount (fmol)	UniProt Protein Name [Synonym]	MW(Da)	UPS2 ppm (w/w)	Optimized Limited Digestion	ProteoMiner + Limited Digestion	ProteoMiner
P00709	500	Alpha-lactalbumin	14078	0.02		4	5
P06732	500	Creatine kinase M-type [CK-MM]	43101	0.05		9	5
P12081	500	Histidyl-tRNA synthetase [Jo-1]	58233	0.07		12	
P61626	500	Lysozyme C	14701	0.02		2	2
Q15843	500	Neddylin [Nedd8]	9072	0.01		1	
P02753	500	Retinol-binding protein	21071	0.03		2	
P16083	500	Ribosylidihydronicotinamide dehydrogenase [quinone] [Quinone oxidoreductase 2] [NQO2]	25821	0.03		5	
P63279	500	Ubiquitin-conjugating enzyme E2 [UbcH9]	18007	0.02		3	
P01008	50	Antithrombin-III	49039	0.006		3	
P61769	50	Beta-2-microglobulin	11731	0.001			
P55957	50	BH3 Interacting domain death agonist [BID]	21995	0.003		2	
O76070	50	Gamma-synuclein	15363	0.002			
P08263	50	Glutathione S-transferase A1 [GST A1-1]	25500	0.003			
P01344	50	Insulin-like growth factor II	7475	0.001			
P01127	50	Platelet-derived growth factor B chain	12294	0.002			
P10599	50	Thioredoxin	12429	0.002		2	
				total	67	181	97
				total UPS	14	27	19

FIG. 5B

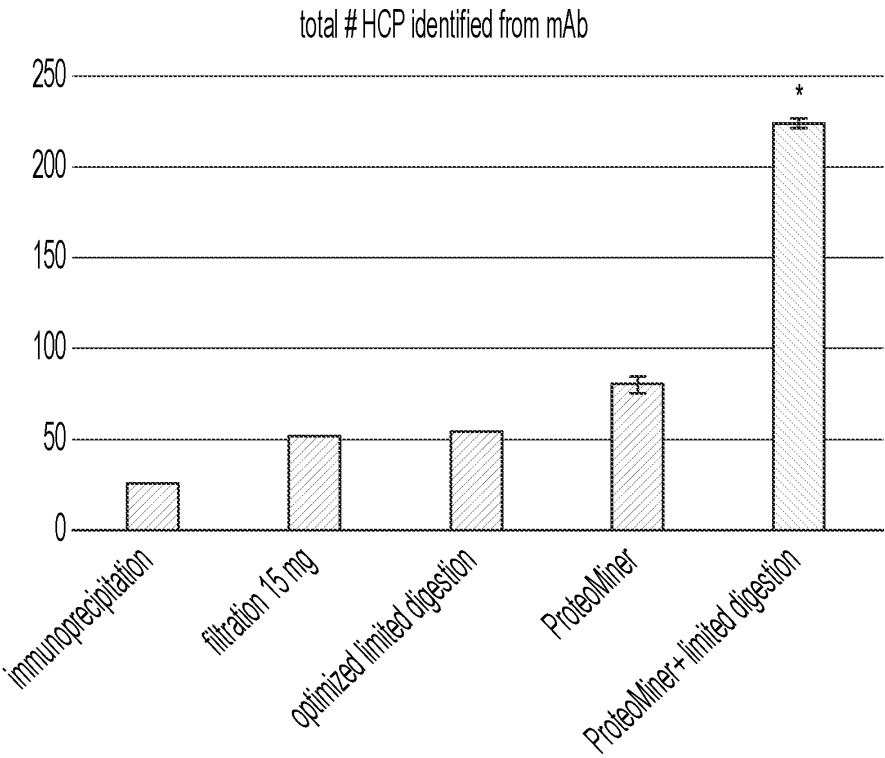


FIG. 6

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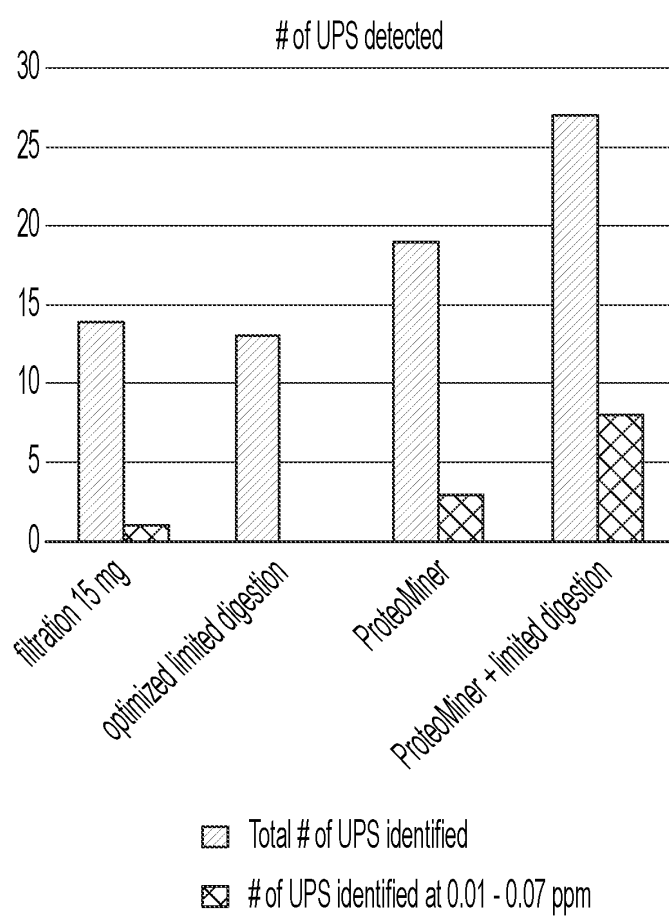


FIG. 7

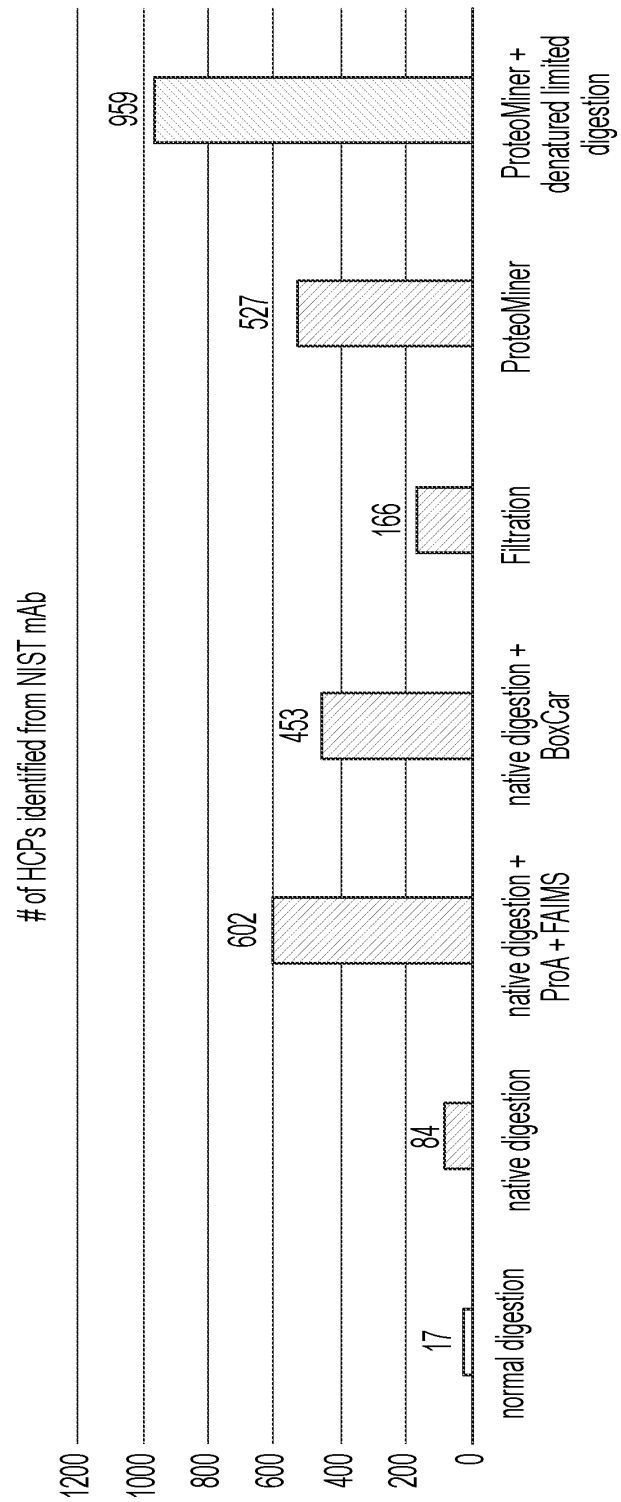


FIG. 8

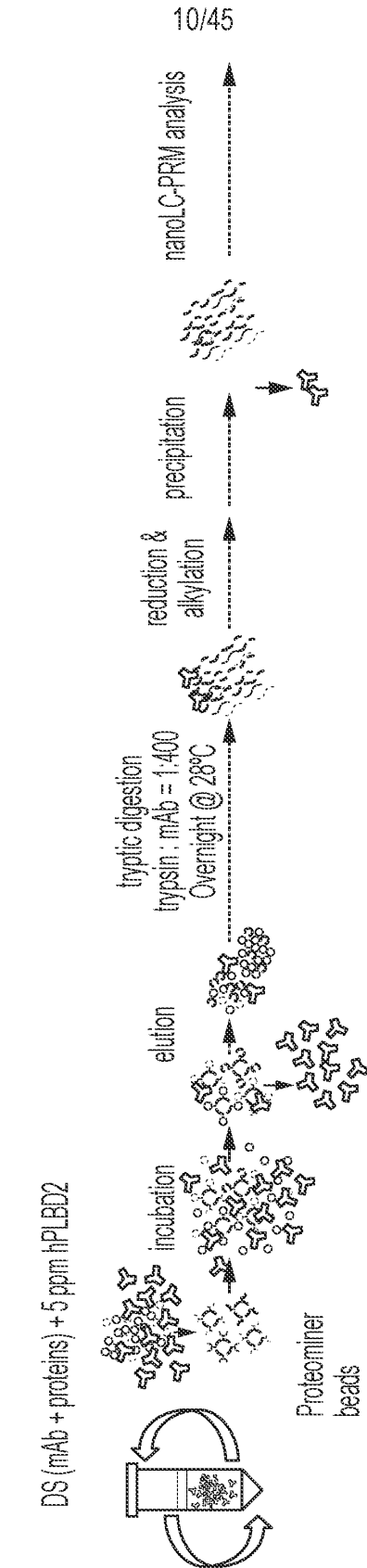


FIG. 9

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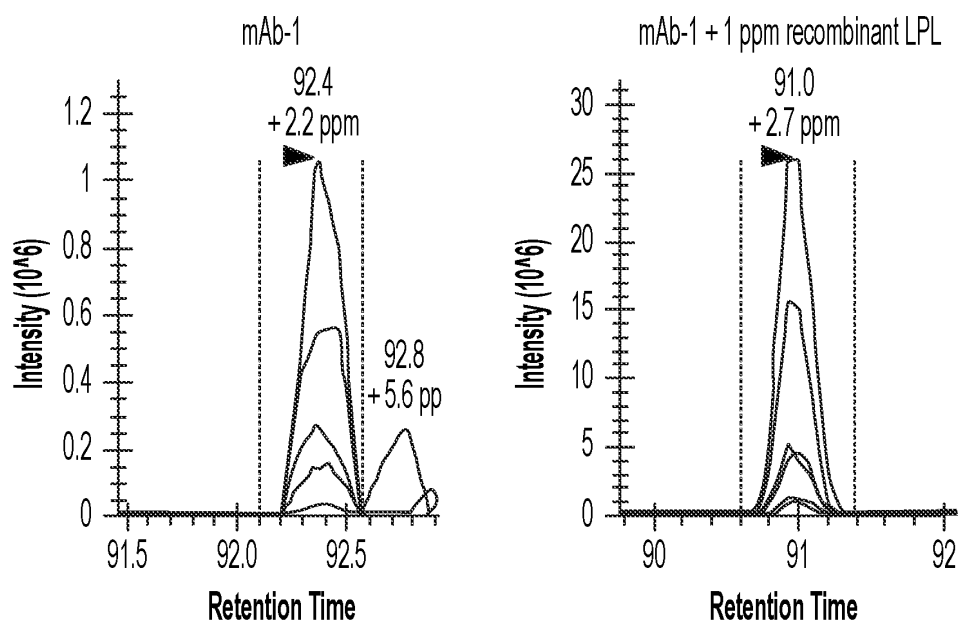


FIG. 10A

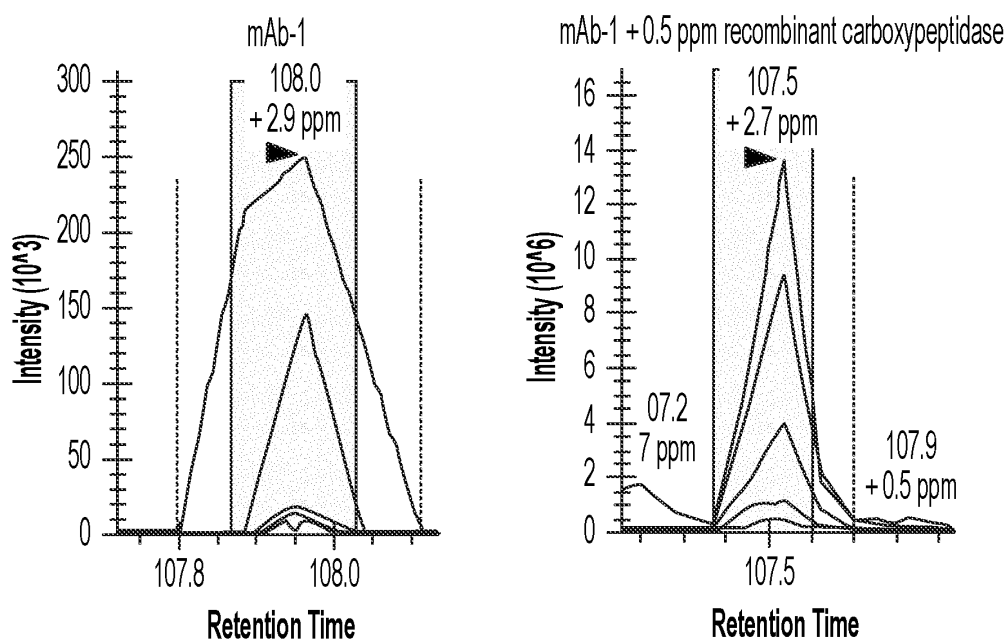


FIG. 10B

SUBSTITUTE SHEET (RULE 26)

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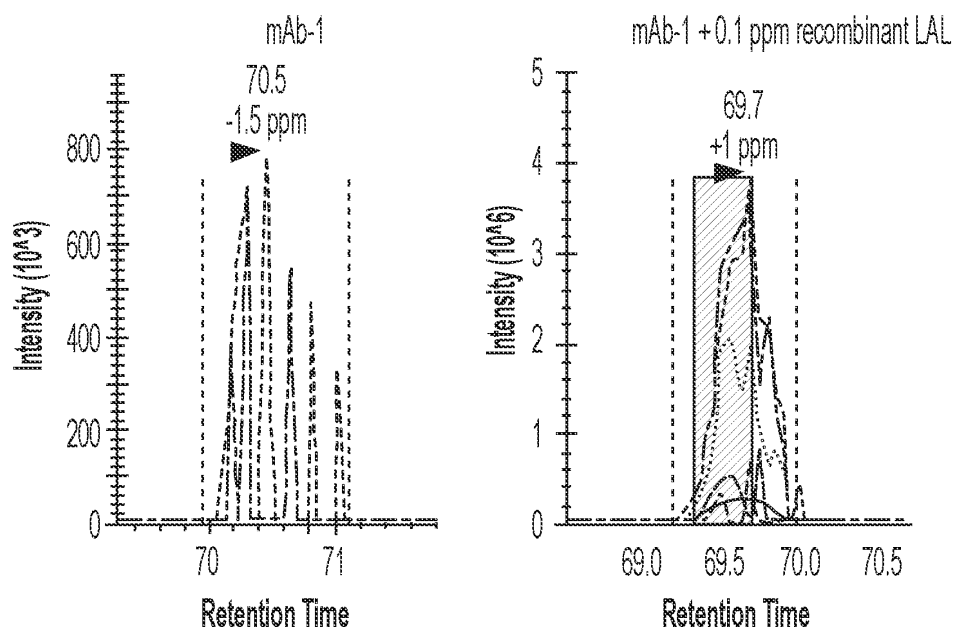


FIG. 10C

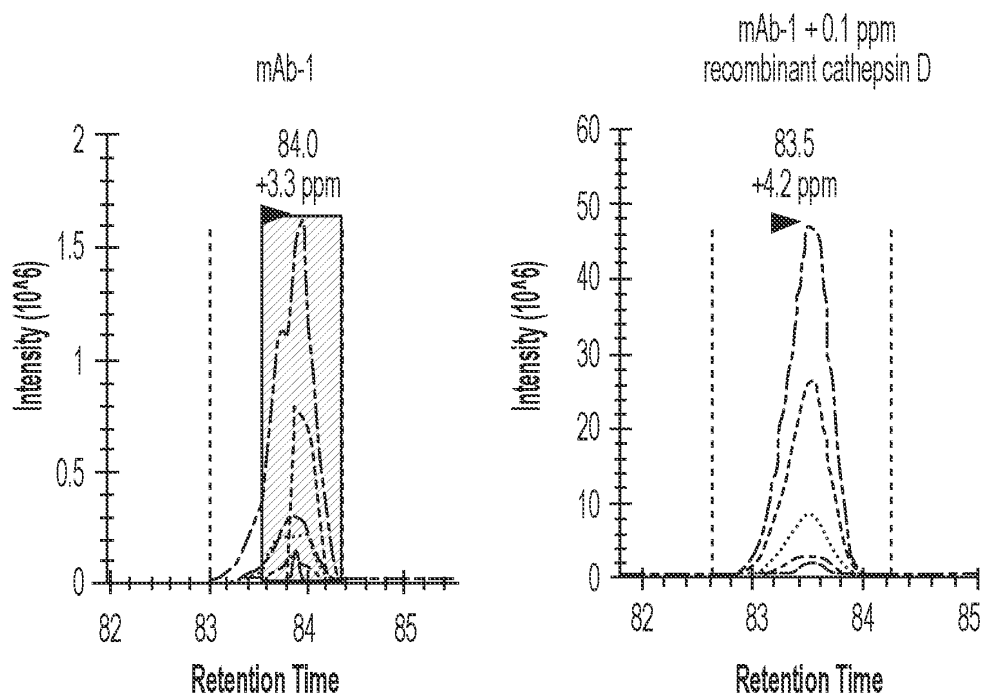


FIG. 10D

SUBSTITUTE SHEET (RULE 26)

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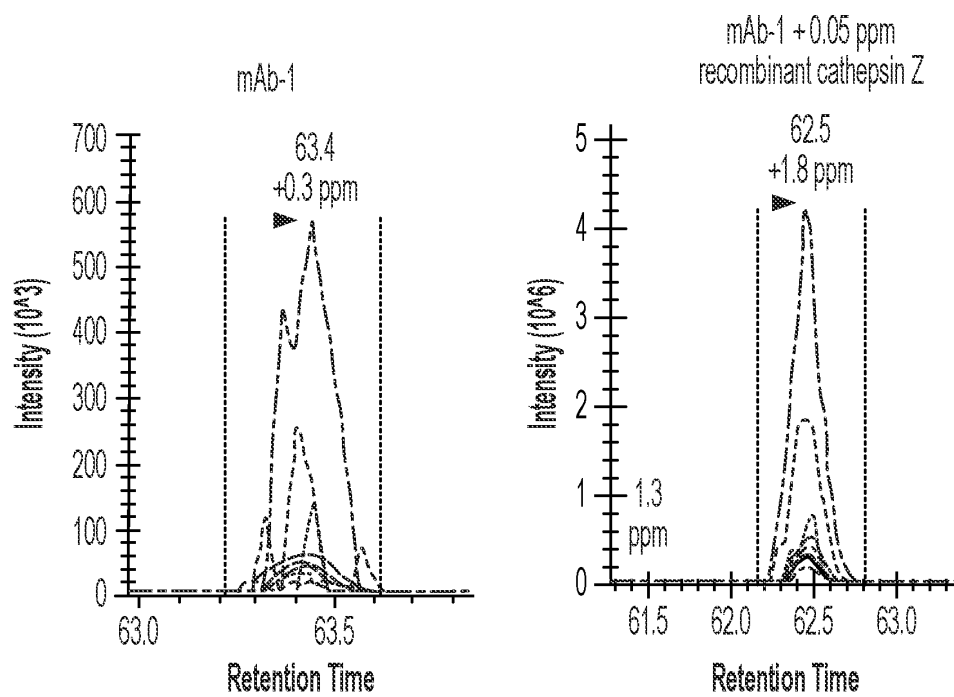


FIG. 10E

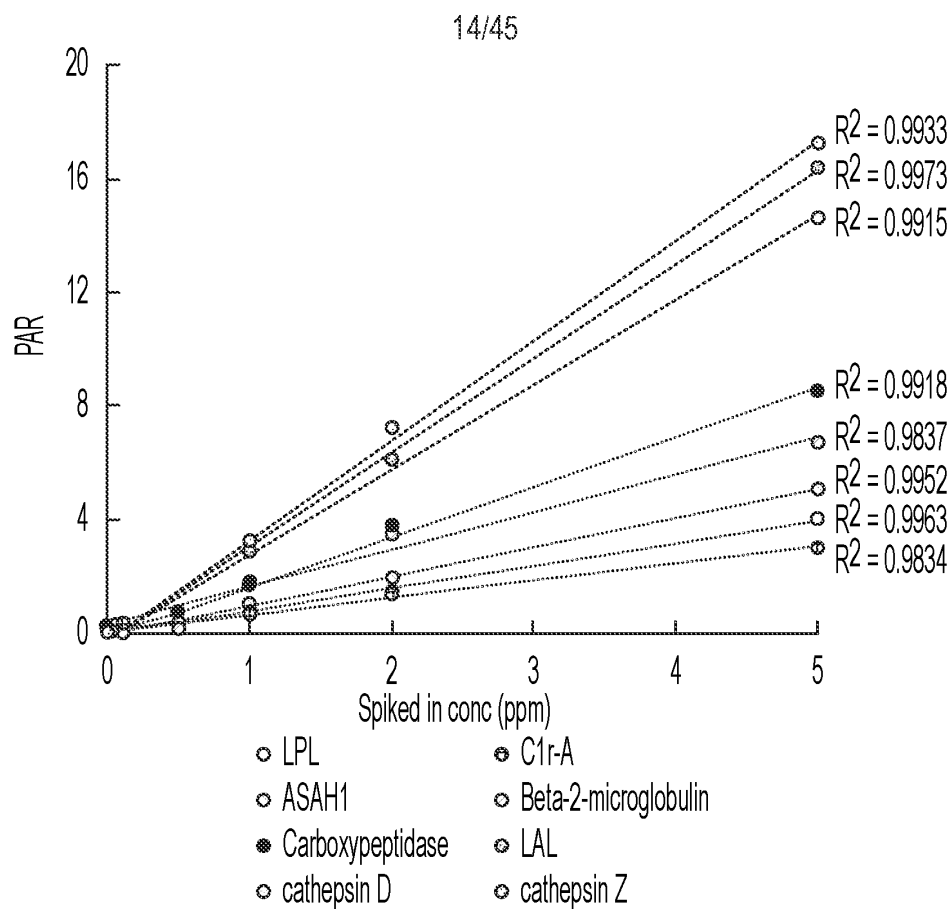


FIG. 11A

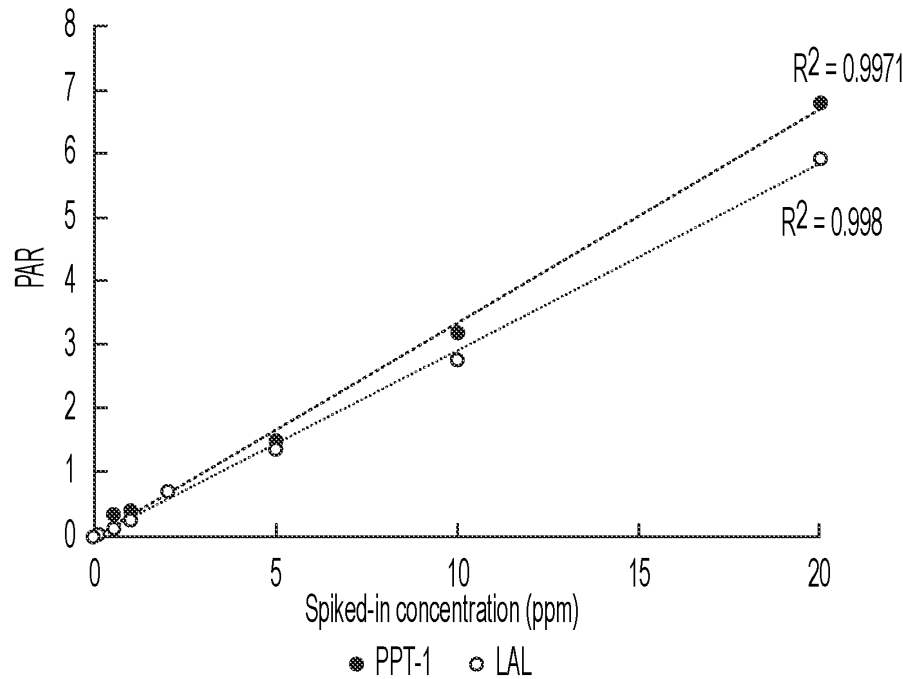


FIG. 11B

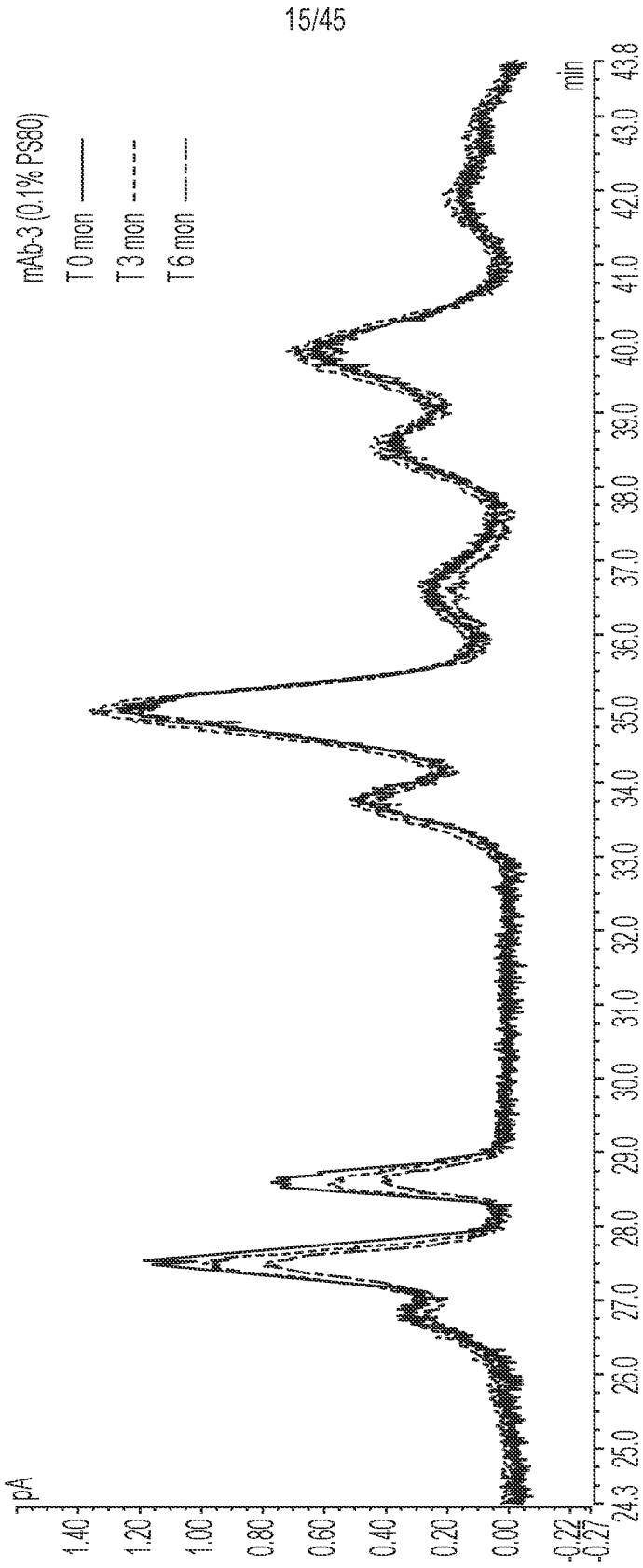


FIG. 12A

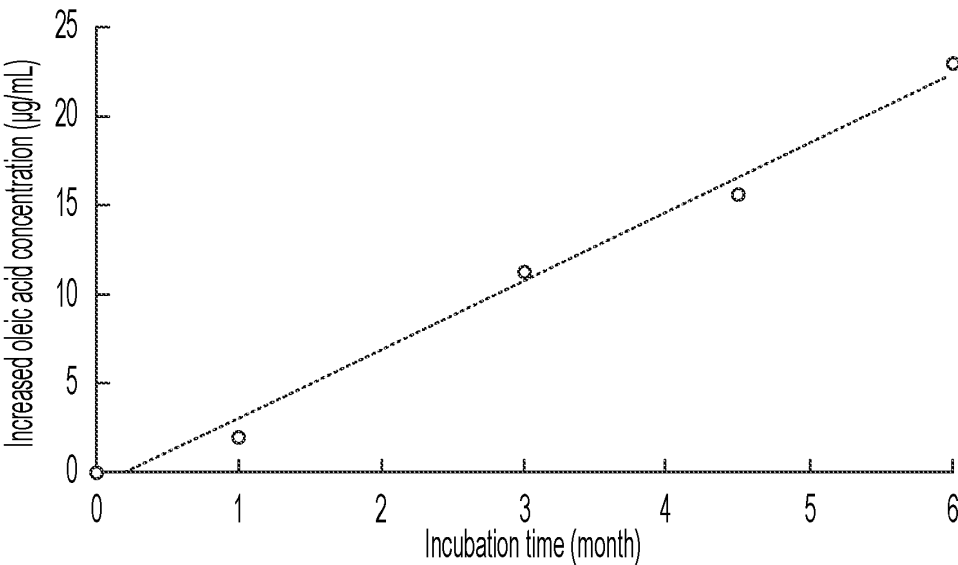


FIG. 12B

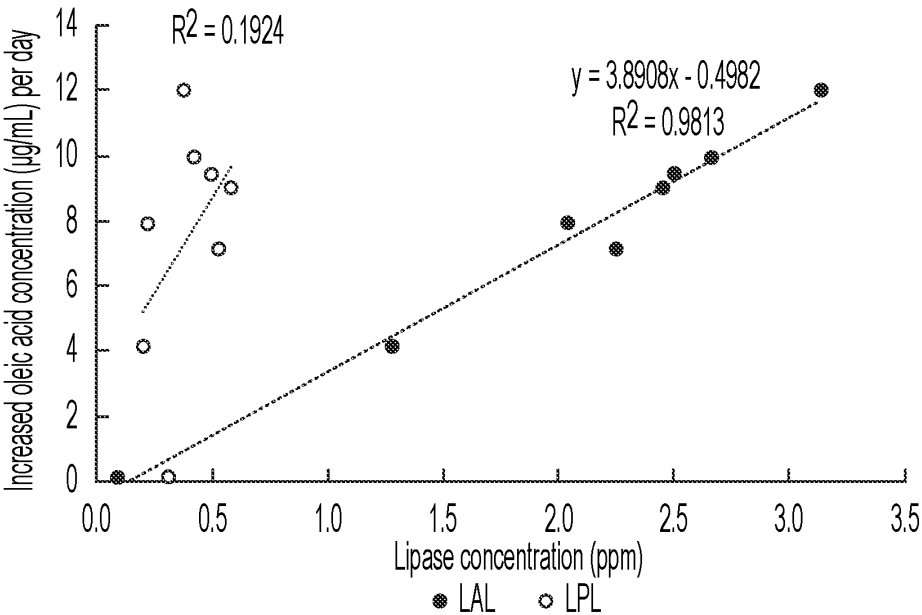


FIG. 12C

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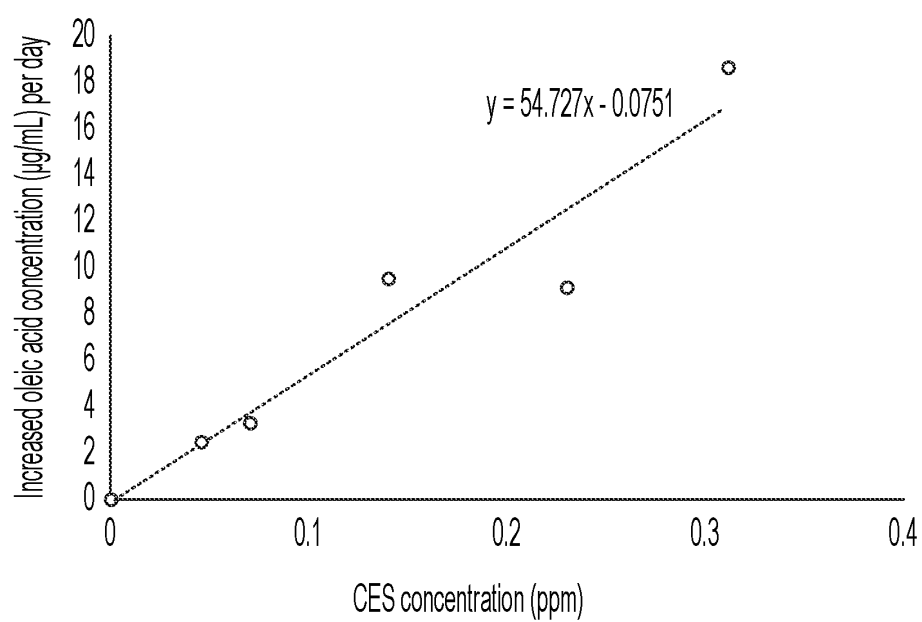


FIG. 13

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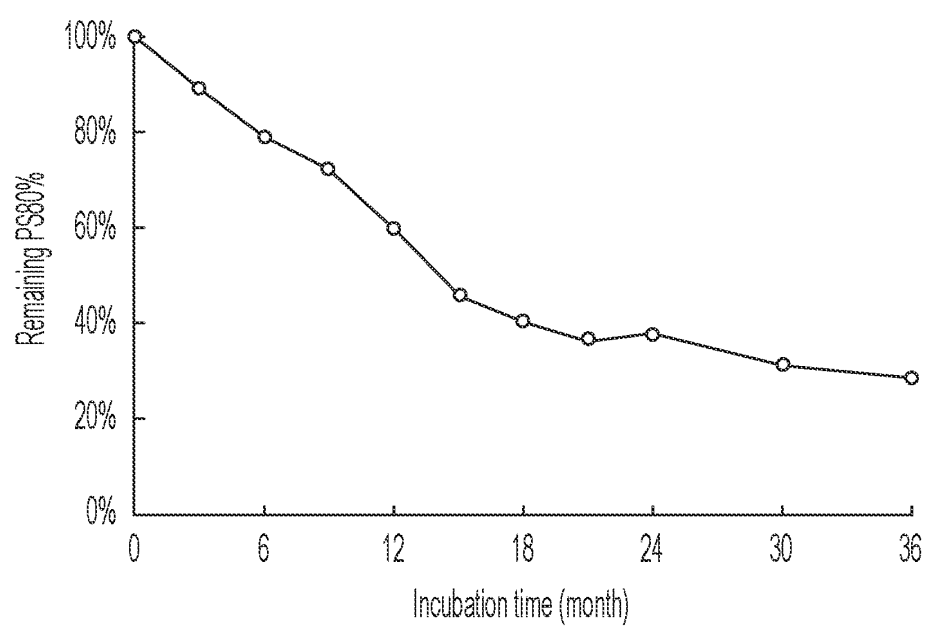


FIG. 14

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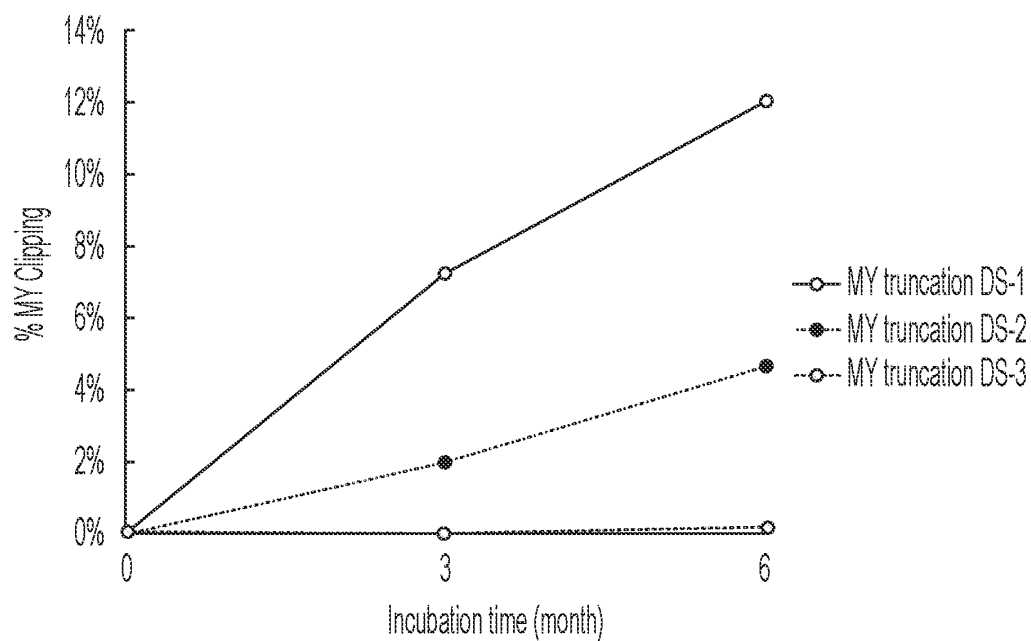


FIG. 15A

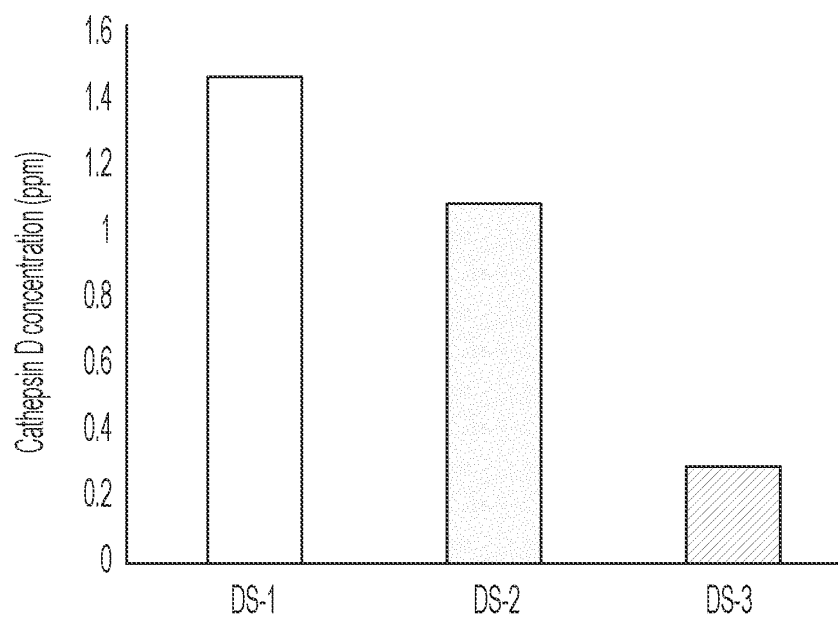


FIG. 15B

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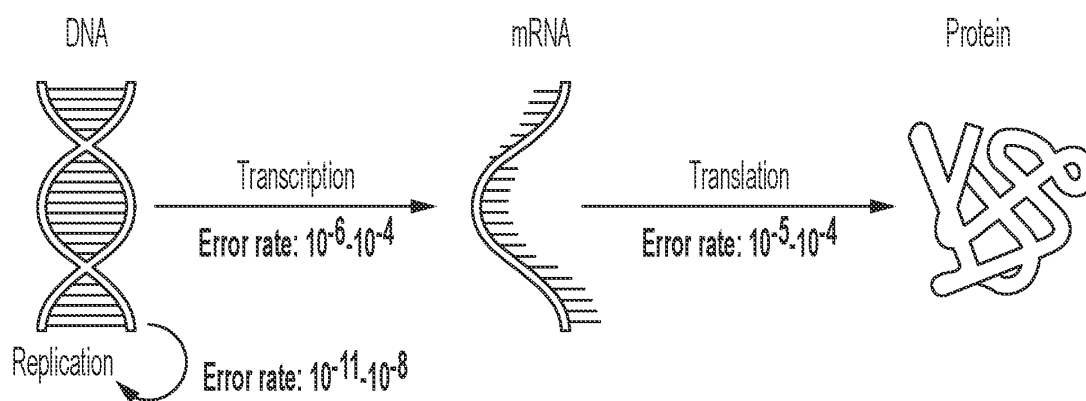


FIG. 16

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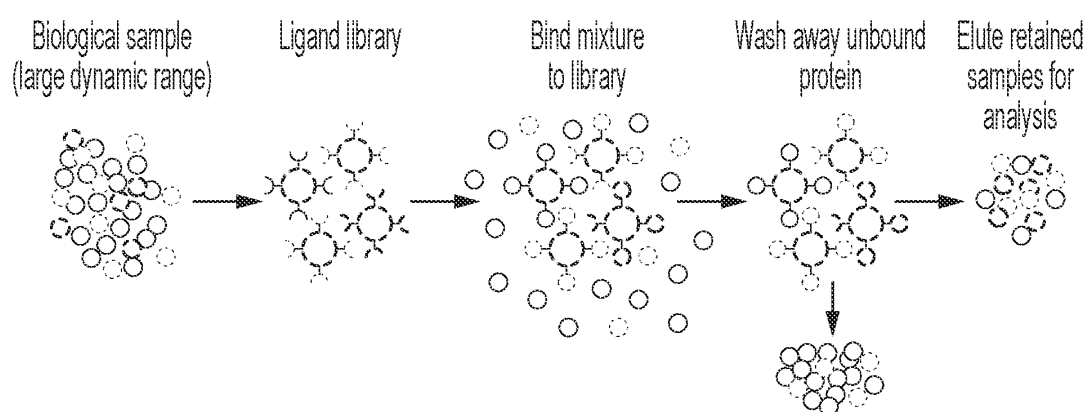


FIG. 17

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC
Ala → Glu	HC	90		0.37	0.68	0.30
	LC	110	0.08	0.09	0.07	0.09
	LC	111				0.11
	LC	192	0.01		0.01	0.002
Ala → Pro	HC	381		0.14	0.15	0.14
	LC	54		0.03	0.04	0.03
	LC	129	0.07	0.13	0.14	0.07
	LC	152	0.02	0.04	0.02	0.04
Ala → Thr	LC	192	0.02	0.05	0.05	0.03
	HC	330	0.00	0.00	0.00	0.00
	LC	25	0.02	0.01	0.01	0.01
	HC	132	0.01	0.01		0.01
Ala → Val	HC	143			0.00	
	HC	144				0.01
	LC	192	0.01	0.02	0.01	0.01
	HC	333			0.00	
Arg → Lys	LC	54	0.00	0.01		0.00
	HC	132		0.00		0.00
	HC	143				0.00
	HC	144			0.00	
Arg → Lys	HC	330	0.00			
	LC	60	0.02	0.02	0.02	0.01

FIG. 18A

Asp→Glu	HC	273					0.01
Cys→Gly	LC	193	0.00			0.00	0.00
Cys→Ser	LC	23	0.04			0.07	0.02
	HC	97			0.02	0.03	
	LC	133	0.06		0.16	0.13	0.03
	HC	264	0.03		0.04	0.05	0.05
Cys→Tyr	HC	370	0.02		0.03	0.03	0.00
	LC	23	0.01				
	HC	147	0.05		0.04		0.00
	HC	370	0.03		0.02	0.03	
Glu→Asp	HC	385	0.12		0.14	0.12	
Glu→Val	LC	104	35.10		42.10	30.80	37.00
Gly→Asp	HC	125	0.004		0.003	0.005	0.004
	LC	127	0.05		0.07		0.03
	HC	140	0.01		0.01		0.003
	HC	141	0.03		0.03	0.03	0.03
	HC	146	0.07				
	LC	156			0.19	0.21	
	LC	199	0.07		0.09	0.14	0.09
	HC	284	0.02		0.03	0.02	0.02
	HC	405	0.03		0.03	0.03	0.02
	HC	423			0.11		

Gly → Glu	LC	16	0.01	0.02	0.01	0.01
	LC	127		0.03	0.04	0.01
	HC	140		0.01	0.01	0.01
	HC	146				0.00
Gly → Ser	HC	146			0.01	0.00
	LC	156		0.02	0.01	
	LC	199			0.02	
	HC	374		0.09		0.04
His → Asn	HC	227	0.29	0.15	0.20	
	HC	271	0.63	0.61	0.53	
	HC	288	0.02	0.02	0.02	0.00
	HC	313	0.21	0.23	0.17	
His → Asp	HC	227	0.19	0.13	0.21	
	HC	271	1.05	1.14	0.93	
	HC	288	0.03	0.06	0.03	
	HC	313	0.52	0.49	0.36	
His → Tyr	LC	197	0.01	0.01	0.01	0.01
Ile → Arg	LC	2			0.03	0.05
Leu → Arg	HC	435		0.05		
Leu → Gln	HC	317	0.06	0.03	0.04	
Leu → Phe	LC	45	0.00	0.00	0.00	0.00
Leu → Pro	HC	371		0.00	0.00	0.00

FIG. 18A
CONTINUED

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC
Lys → Arg	HC	150	0.05	0.05	0.05	0.05
	HC	412	0.02			0.01
Met → Ile or Leu	HC	255				0.02
Met → Thr	LC	4	0.21	0.50	0.51	0.44
	LC	32	0.05	0.08	0.05	0.04
	HC	87	0.02		0.02	0.01
Phe → Ile or Leu	HC	255	0.07	0.08	0.09	0.08
	HC	408	0.04	0.04	0.04	0.02
Phe → Ser	HC	408		0.01	0.01	0.01
Phe → Tyr	HC	129	0.03	0.02	0.01	0.01
	HC	407	0.03	0.04	0.03	
	HC	408	0.02	0.01		
Pro → Ala	HC	260				0.03
Pro → His	HC	133			0.00	
Pro → Leu	LC	58		0.00	0.00	0.00
	LC	118	0.00	0.08	0.01	0.01
	LC	119			0.00	0.01
	HC	126	0.00	0.00	0.00	0.00
	HC	130		0.00	0.00	0.00
	HC	133				0.00
	LC	140	0.01		0.01	0.01
	HC	356		0.01		

FIG. 18B

	HC	448	0.07		0.05			0.06
Pro → Ser	HC	133			0.00			
	LC	140	0.02		0.03	0.01		
	LC	203				0.00		0.00
	HC	233			0.02			0.02
Ser → Asn	HC	241	0.02		0.02	0.02		
	LC	26				0.01		0.01
	LC	51	0.002		0.001	0.003		
	HC	137	0.04		0.02	0.04		0.01
	LC	167	0.03		0.04	0.03		0.05
	LC	170	0.02		0.03	0.02		
	LC	173	0.07		0.08	0.08		0.06
	LC	176	0.03		0.07	0.03		0.02
	LC	181				0.03		0.02
	LC	201	0.03		0.05	0.04		0.04
	HC	270	0.04		0.02	0.02		0.03
	HC	307	0.04		0.04	0.04		0.04
	HC	411	0.19		0.28	0.24		0.22
	HC	443	0.08			0.10		0.08
Ser → Ile or Leu	LC	26			0.03	0.01		0.00
	LC	27			0.01			
	LC	59			0.00			0.00
	LC	155	0.00		0.01	0.01		0.01

FIG. 18B
CONTINUED

	HC	242				0.01
Ser → Phe	LC	12			0.00	
	LC	120				0.02
	HC	135	0.00			0.00
	LC	158				0.00
	HC	357				0.00
Ser → Pro	HC	403		0.01	0.00	0.00
	HC	406		0.01		0.00
	LC	27	0.00			0.00
	LC	161				0.01
Thr → Ala	LC	50		0.00	0.00	
Thr → Asn	HC	228	0.04		0.04	
Thr → Ile	LC	10	0.00		0.00	
	LC	50			0.00	0.00
	HC	138			0.02	0.00
	LC	163	0.00		0.00	0.00
	LC	177			0.00	
	HC	259			0.00	0.00
	HC	369	0.01		0.01	
Thr → Ser	HC	85	0.01		0.01	0.01
Trp → Ser	HC	280	0.01		0.01	
	HC	316		0.01	0.01	

FIG. 18B
CONTINUED

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC
Tyr → Asp	LC	172	0.06	0.09	0.04	
	LC	191		0.00	0.00	
Tyr → Cys	LC	48	0.01	0.00	0.01	0.00
	LC	172	0.02			
	LC	191	0.09	0.01	0.01	0.04
Tyr → Phe	HC	281		0.02	0.01	
	HC	299				0.21
Val → Ala	LC	15	1.14	1.75	0.98	
	LC	19	0.001	0.001	0.001	
	HC	149	0.01	0.00	0.00	
	LC	190	0.01	0.01	0.00	0.01
Val → Glu	HC	372				0.01
	LC	190			0.02	0.04
	HC	282	0.04	0.07	0.06	0.05
	HC	351		0.02	0.03	0.04
	HC	382	0.12	0.09	0.04	
	HC	400		0.03		0.03
	LC	19	0.01	0.01	0.02	0.01
	LC	57	0.02	0.02	0.02	0.02
	LC	114			0.02	
	HC	128	0.10	0.06	0.07	0.04
	LC	131	0.02	0.03	0.03	0.02

FIG. 18C

Val → Ile or Leu	LC	132			0.03			
	HC	149	0.01		0.01		0.01	0.01
	LC	162	0.01		0.02		0.01	
	LC	190	0.04		0.04		0.03	0.02
	LC	195	0.02				0.02	0.01
	LC	204	0.01		0.01		0.01	0.01
	HC	262	0.02		0.01			0.01
	HC	276	0.02		0.02		0.02	0.01
	HC	308					0.08	0.05
	HC	311			0.06		0.04	0.02
	HC	366	0.07		0.06		0.07	0.01
	HC	372			0.01		0.01	0.00
	LC	132						0.003
Val → Met	HC	149			0.004			0.004
	LC	149	0.006		0.003		0.003	0.007
	LC	204			0.002		0.004	0.002
	HC	265	0.03		0.02		0.03	0.02
	HC	266	0.02		0.02		0.02	0.03
	HC	267	0.04		0.04		0.03	0.02
	HC	269	0.04		0.04		0.04	0.02
	HC	282	0.07		0.06		0.03	0.01
	HC	285					0.01	0.01
	Total SVs Detected		76		90		87	64

FIG. 18C
CONTINUED

AA Substitution	Chain	Position	ProteoMiner™ Enriched Peptides	Color
Cys → Ser	LC	133	SVVCLNNF	Pink
Glu → Asp	HC	385	SDIAVEWESNGQP	Orange (loop)
Gly → Asp	LC	156	VDNALQSGN	Light Pink (loop)
His → Asn or Asp	HC	227	THTCPPEAPE	Red (loop)
	HC	271	VVDVSHEDPEVK	
	HC	313	SVLTVLHQDNL	
Val → Ala	LC	15	-	-
Val → Glu	HC	382	SDIAVEWESNGQP	Yellow

FIG. 19A

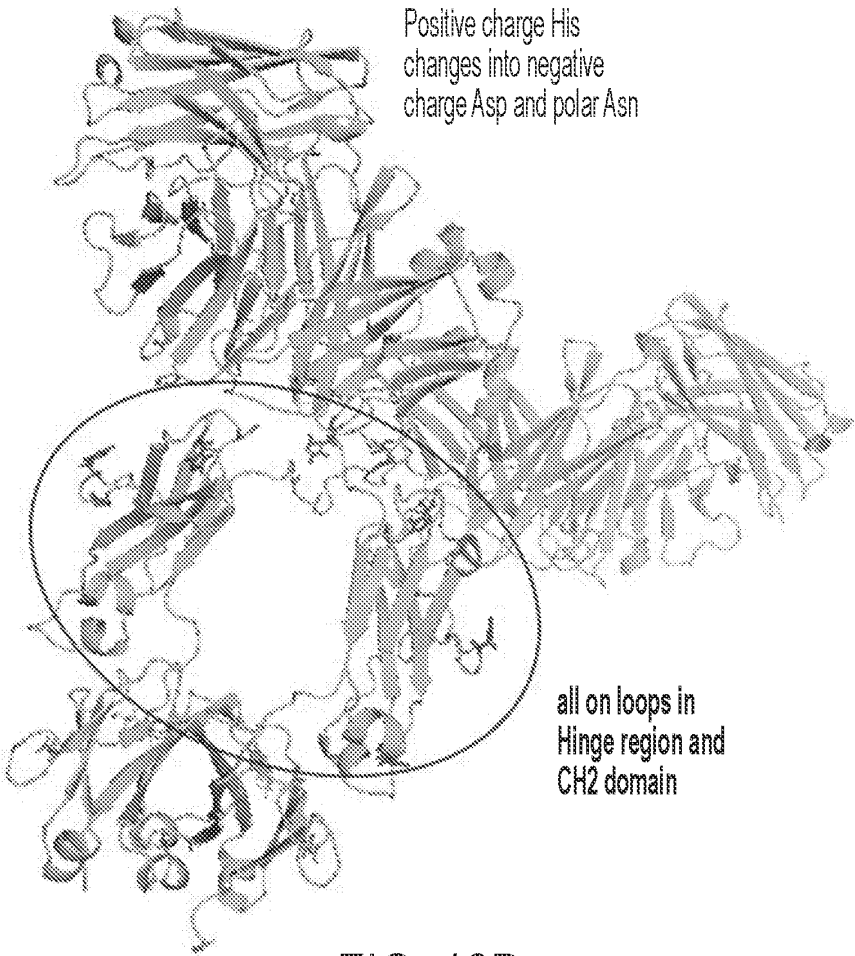


FIG. 19B

SUBSTITUTE SHEET (RULE 26)

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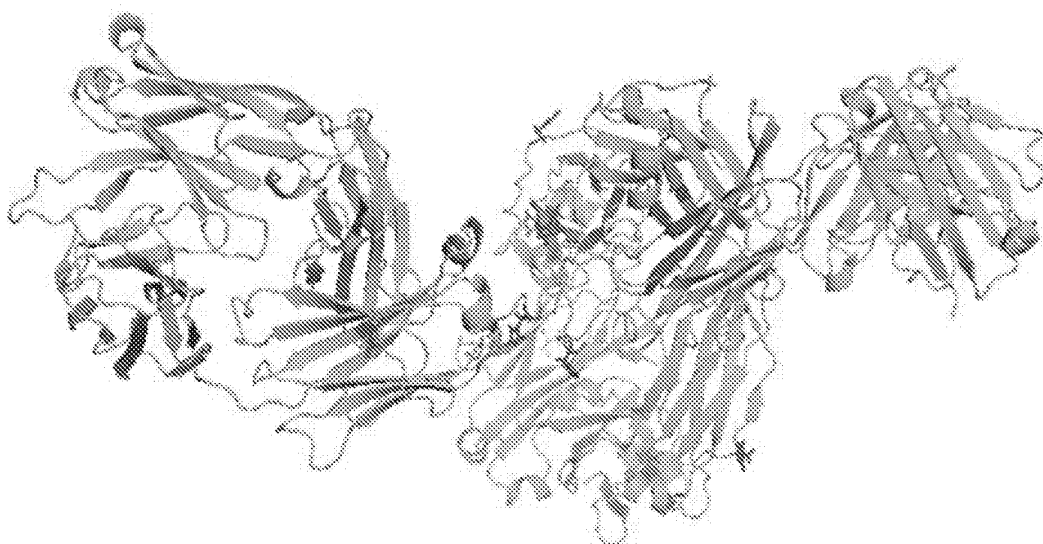


FIG. 19C

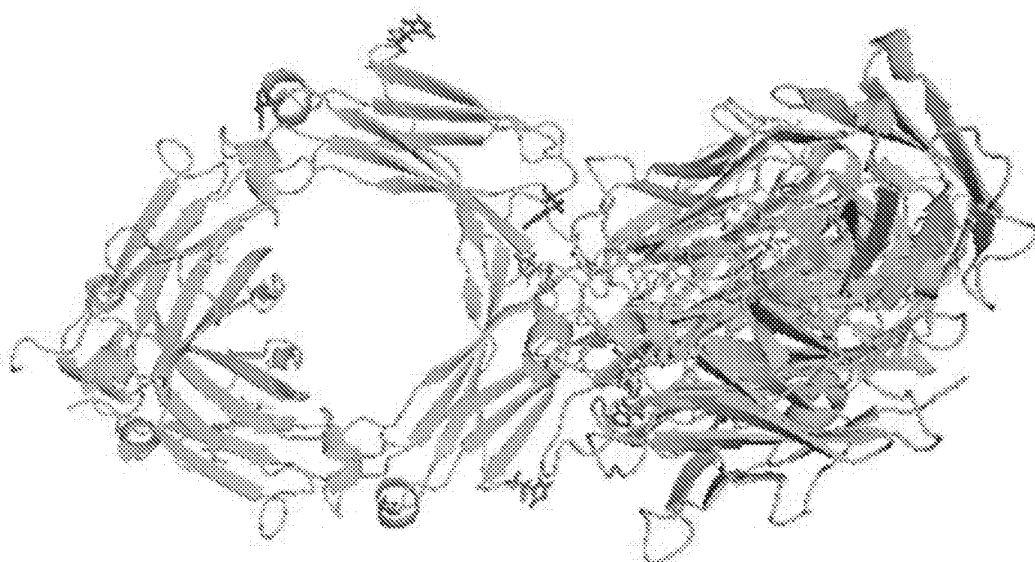


FIG. 19D

SUBSTITUTE SHEET (RULE 26)

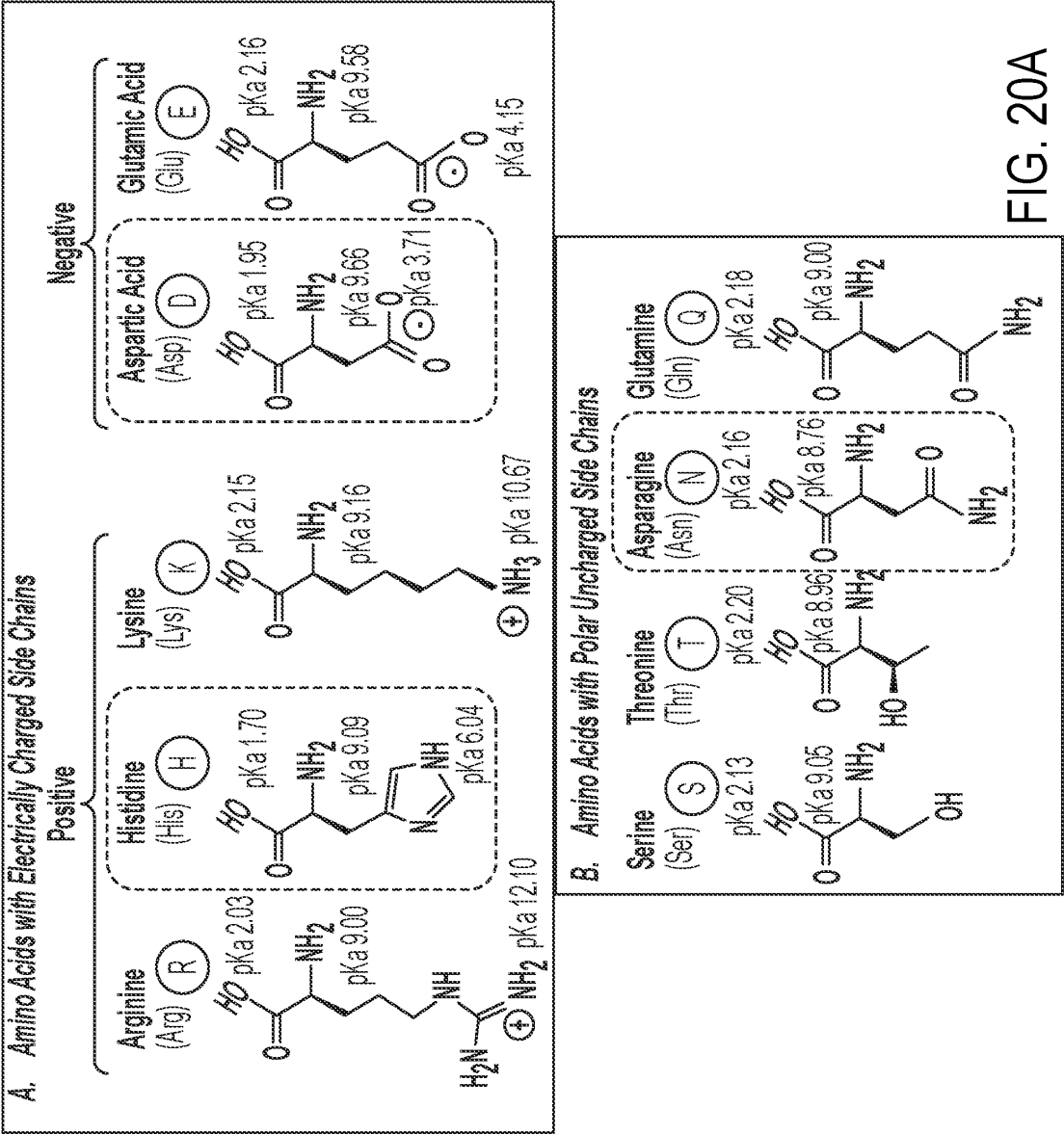


FIG. 20A

One letter code	Three letter code	Amino acid	Possible codons
D	Asp	Aspartic acid	GAC, GAT
H	His	Histidine	CAC, CAT
N	Asn	Asparagine	AAC, AAT

FIG. 20B

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC	Direct Digest CSH LC Lab-1	Direct Digest CSH LC Lab-2	Direct Digest CSH LC Lab-3
His → Asn	HC	227	0.29	0.21	0.20				
	HC	271	0.64	0.61	0.53				
	HC	288	0.02	0.02	0.02	0.004			
	HC	313	0.21	0.23	0.17				
His → Asp	HC	227	0.30	0.18	0.21				
	HC	271	1.05	1.14	0.93				
	HC	288	0.03	0.06	0.03				
	HC	313	1.10	0.49	0.36				

FIG. 20C

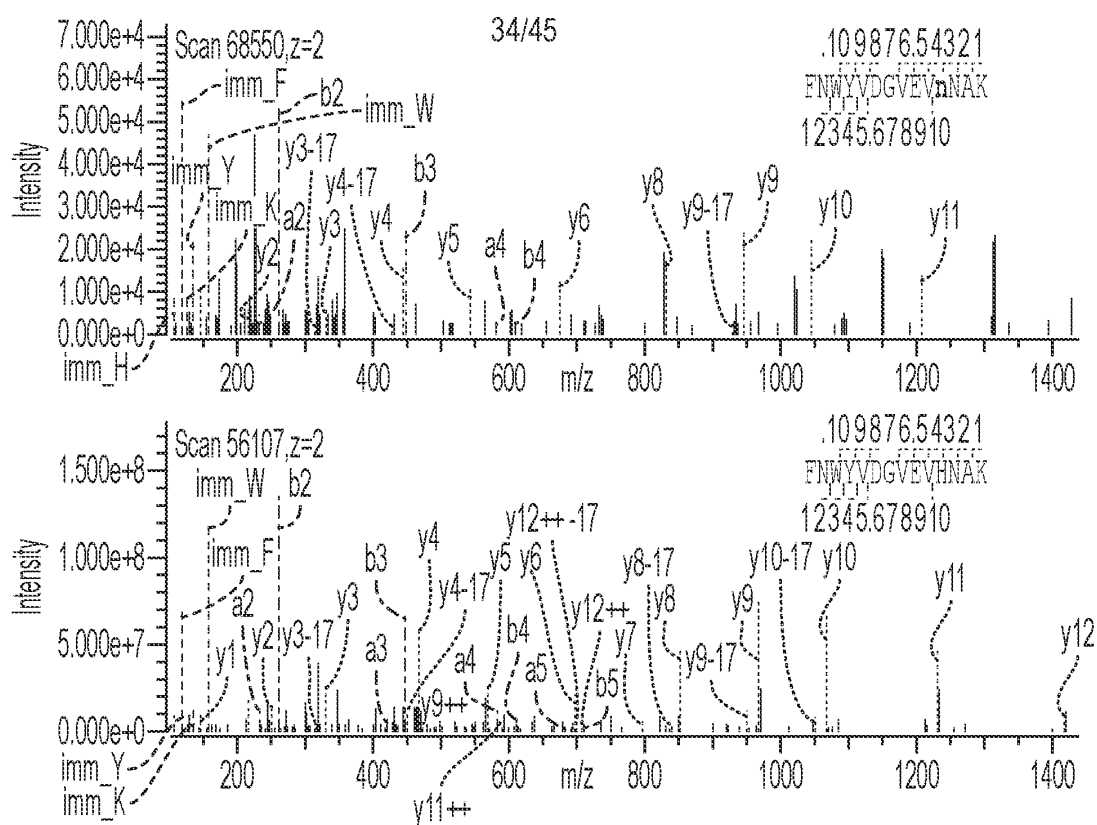


FIG. 20D

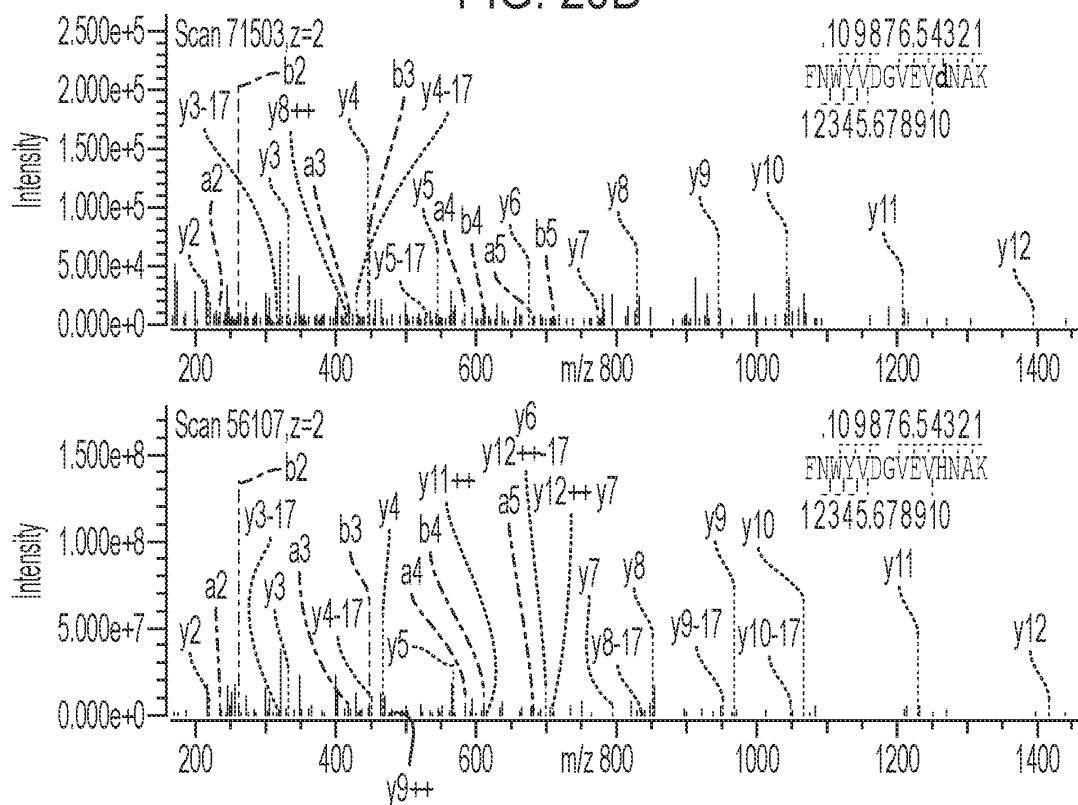


FIG. 20E

AA Substitution	Position	Process 1	Process 2	Process 3	FDS
His ➡ Asn	271	0.06	0.09	0.10	0.01
	288	0.01		0.01	
	313			0.01	
	432	0.47	0.38		
	436	0.33	0.27	0.20	0.09
	438	0.05	0.05		
His ➡ Asp	227	0.22	0.18	0.15	0.17
	271	0.09	0.09	0.10	0.05
	288	0.26	0.23	0.18	0.32
	313	0.04	0.03	0.02	0.04
	432	0.16	0.14	0.10	
	436	0.12	0.05	0.11	0.05

FIG. 20F

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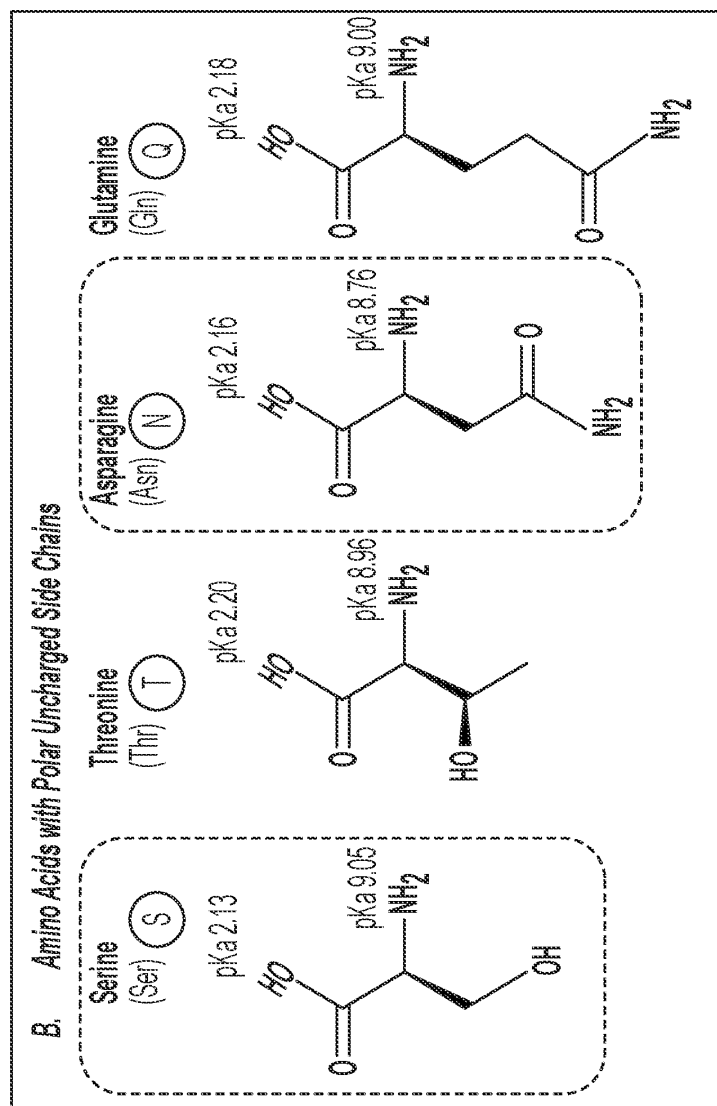


FIG. 21A

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC	Direct Digest CSH LC Lab-1	Direct Digest CSH LC Lab-2	Direct Digest CSH LC Lab-3
Ser→Asn	LC	26			0.01	0.01			
	LC	51	0.002	0.001	0.003				
	HC	137	0.04	0.02	0.04	0.01			
	LC	167	0.03	0.04	0.03	0.05			
	LC	170	0.02	0.03	0.02				
	LC	173	0.07	0.08	0.08	0.06	0.07	0.09	0.05
	LC	176	0.03	0.07	0.03	0.02			
	LC	181			0.03	0.02	0.07	0.03	0.04
	LC	201	0.03	0.05	0.04	0.04	0.01		
	HC	270	0.04	0.02	0.02	0.03	0.01	0.02	0.1
	HC	307	0.04	0.04	0.04	0.04	0.02	0.04	
	HC	411	0.19	0.28	0.24	0.22	0.08	0.06	0.02
	HC	443	0.08		0.10	0.08	0.08		

FIG. 21B

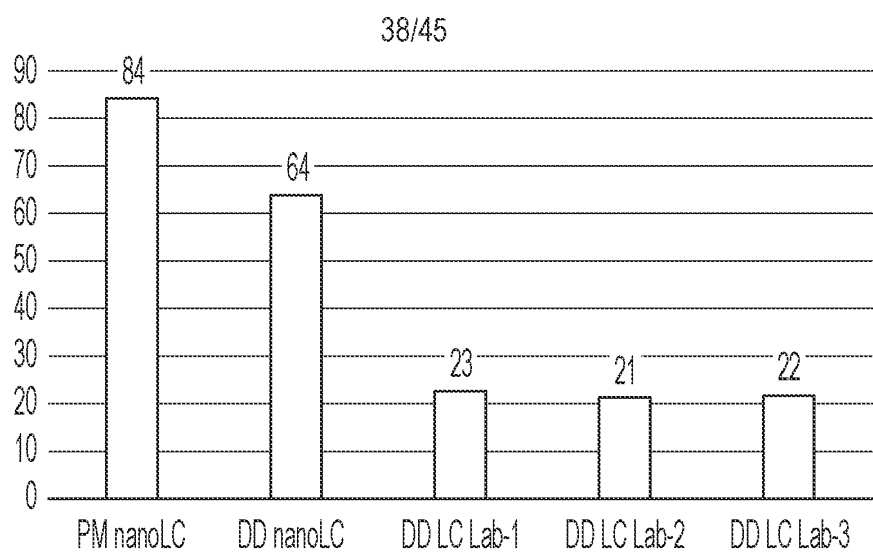


FIG. 22A

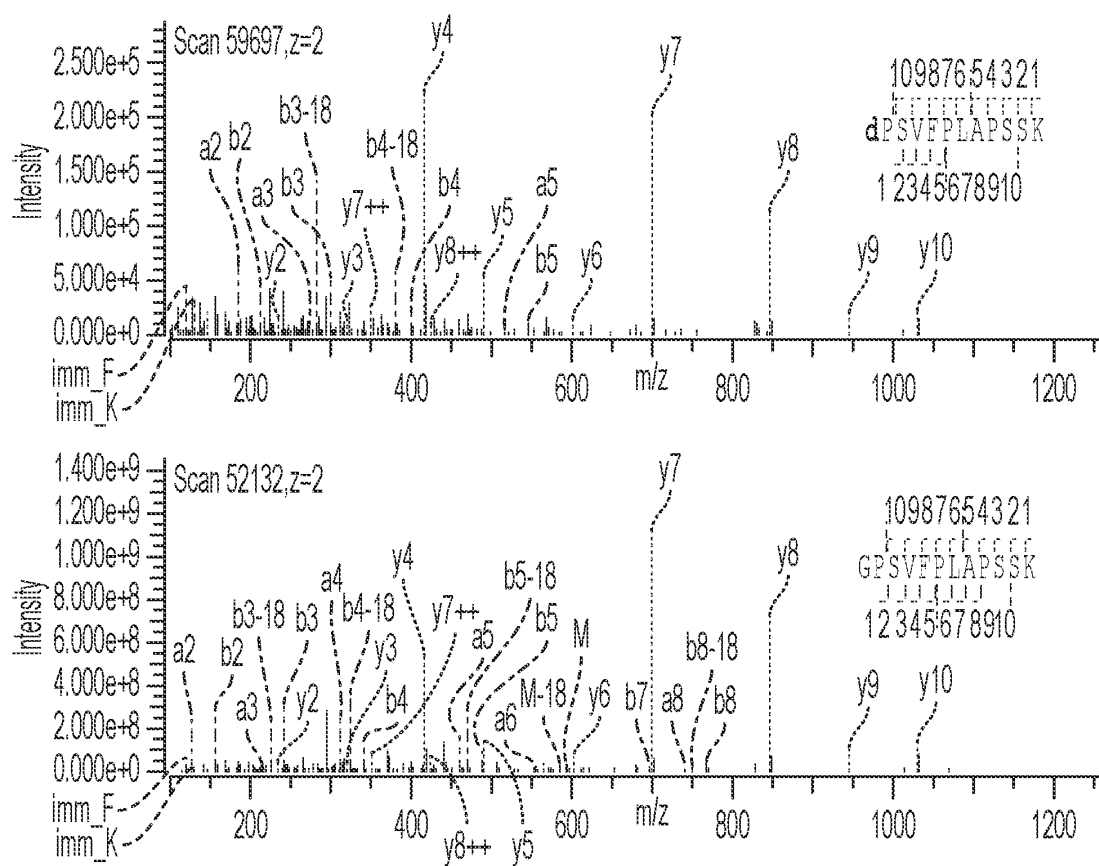


FIG. 22B

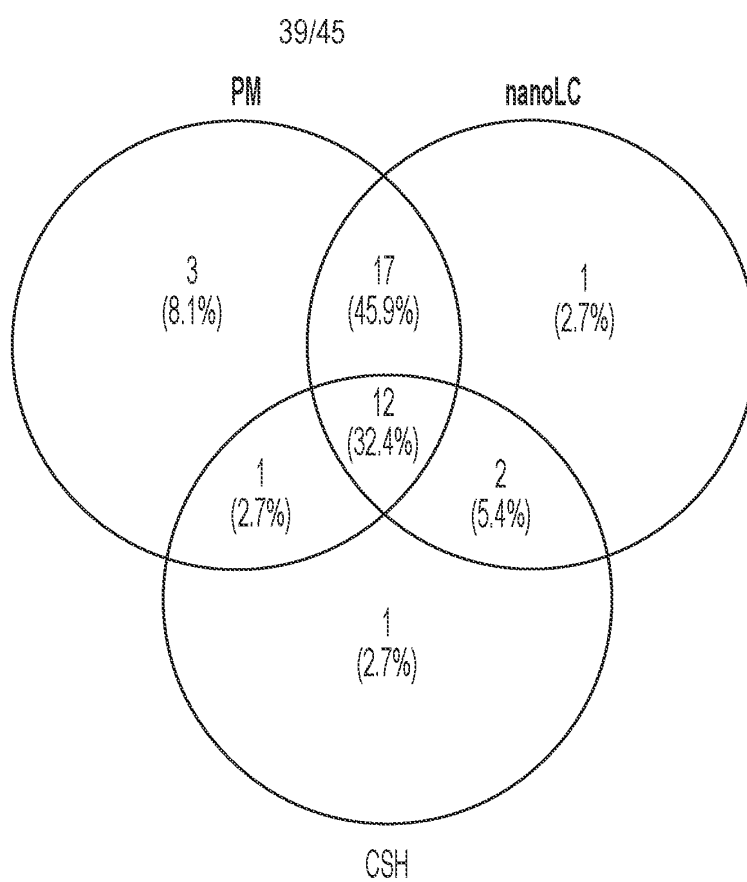


FIG. 22C

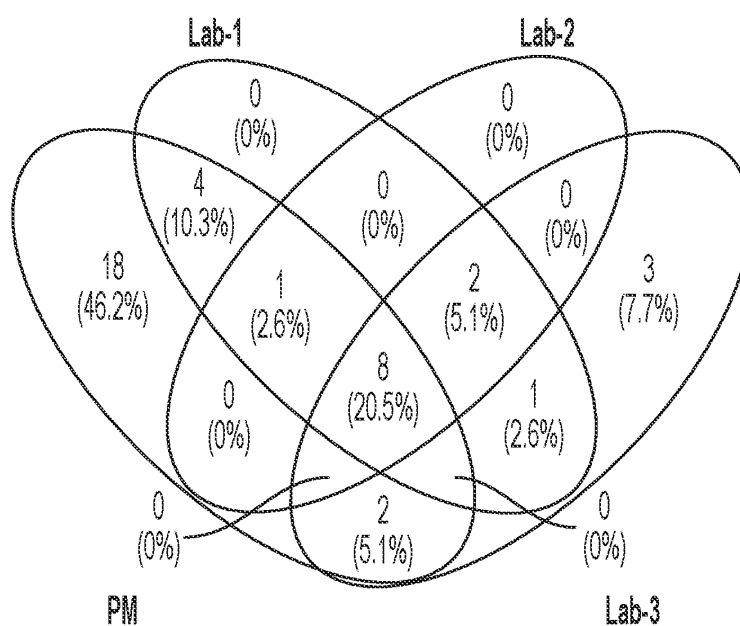


FIG. 22D

AA Substitution	Chain	Position	ProteoMiner™ 1 nanoLC	ProteoMiner™ 2 nanoLC	ProteoMiner™ 3 nanoLC	Direct Digest nanoLC	Direct Digest CSH LC Lab-1	Direct Digest CSH LC Lab-2	Direct Digest CSH LC Lab-3
Ala → Thr	HC	132	0.01	0.01		0.01	0.01	0.01	0.01
	HC	381					0.01	0.02	
Arg → Lys	LC	60	0.02	0.02	0.02	0.01	0.01	0.01	
	HC	125	0.004	0.003	0.005	0.004	0.01	0.01	0.01
Gly → Asp	HC	141	0.03	0.03	0.03	0.03	0.03	0.04	0.01
	HC	284	0.02	0.03	0.02	0.02	0.02	0.03	0.03
	LC	199	0.07	0.09	0.14	0.09	0.07	0.07	0.02
Lys → Arg	HC	150	0.05	0.05	0.05	0.05	0.04	0.04	0.02
	HC	30					0.03		0.02
Ser → Asn	LC	170/173	0.07	0.08	0.08	0.06	0.07	0.09	0.05
	LC	181			0.03	0.02	0.07	0.03	0.04
	HC	270	0.04	0.02	0.02	0.03	0.01	0.02	0.10
	HC	307	0.04	0.04	0.04	0.04	0.02	0.04	
	HC	411	0.19	0.29	0.22	0.22	0.08	0.06	0.02
Val → Ile or Leu	LC	57	0.02	0.02	0.02	0.02	0.01	0.02	0.03
	HC	128	0.10	0.06	0.07	0.04		0.01	0.00
	HC	308/311		0.06	0.08	0.05	0.06	0.06	0.04

SUBSTITUTE SHEET (RULE 26)

FIG. 22E

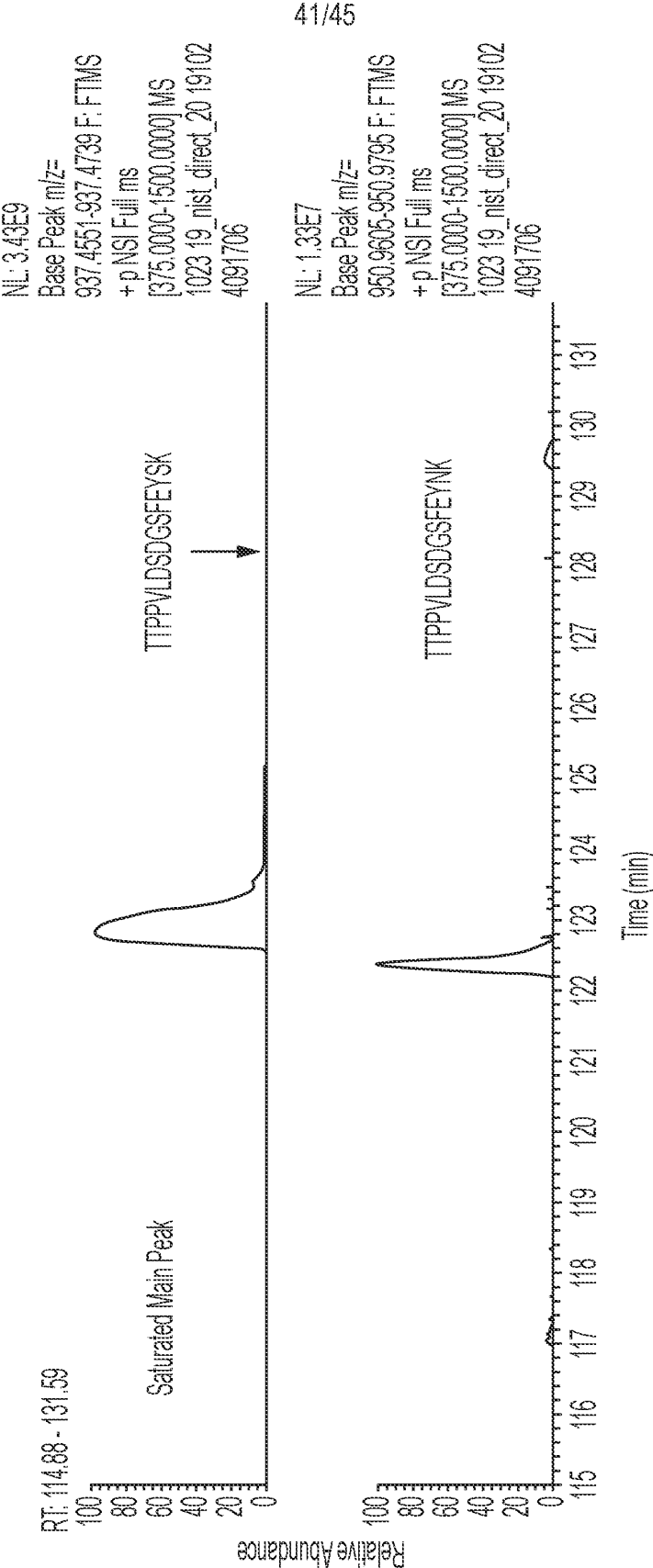


FIG. 23

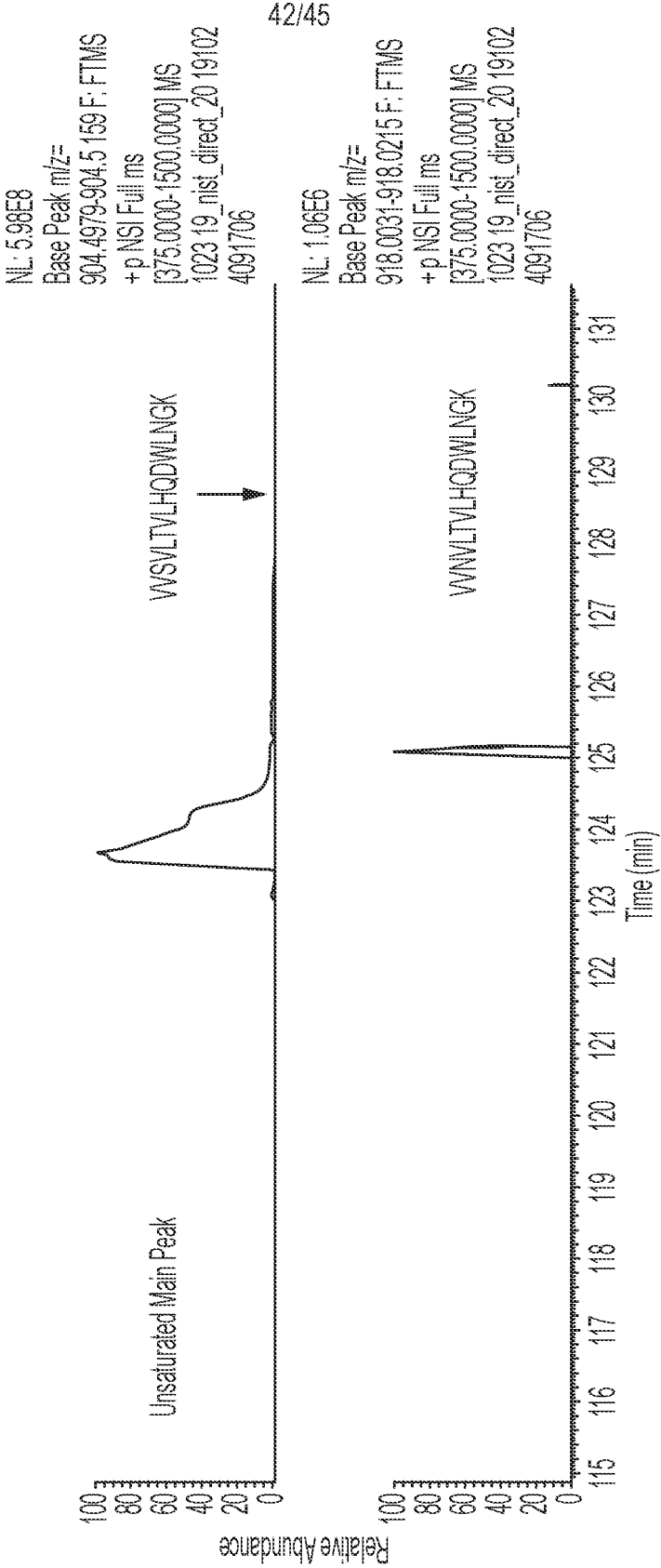


FIG. 23
CONTINUED

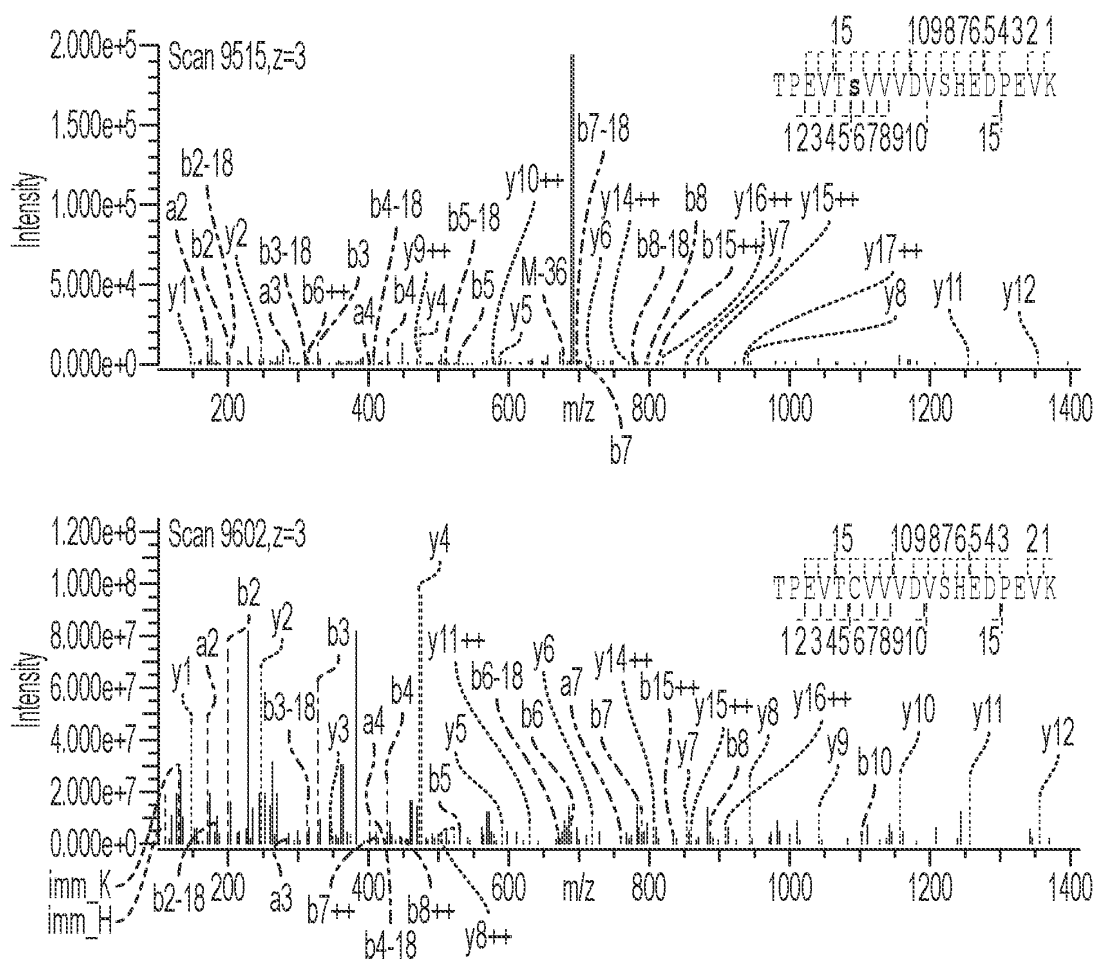
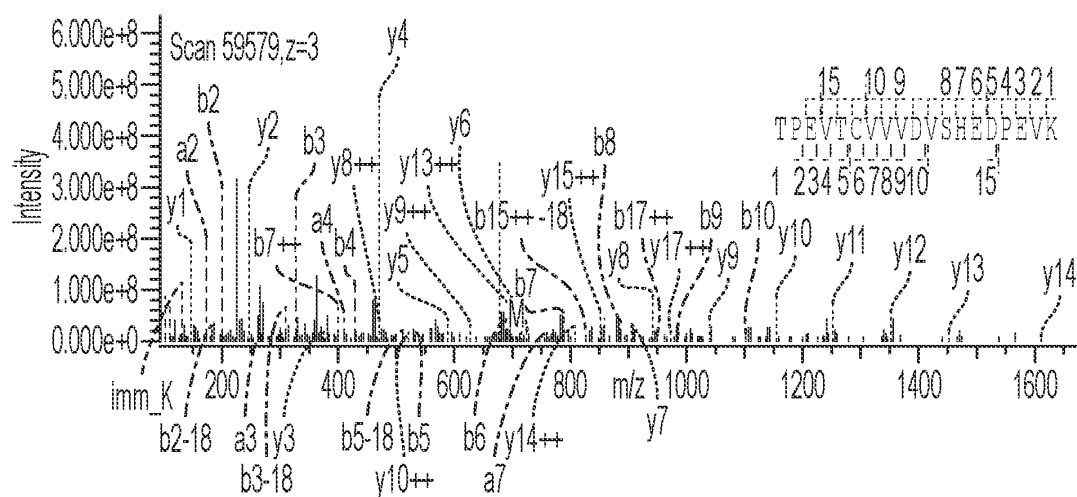
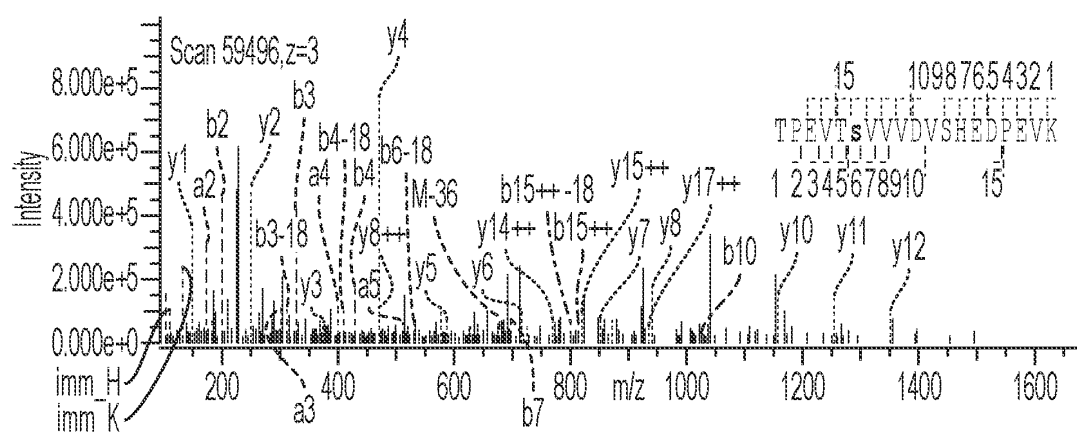


FIG. 24

FIG. 24
CONTINUED

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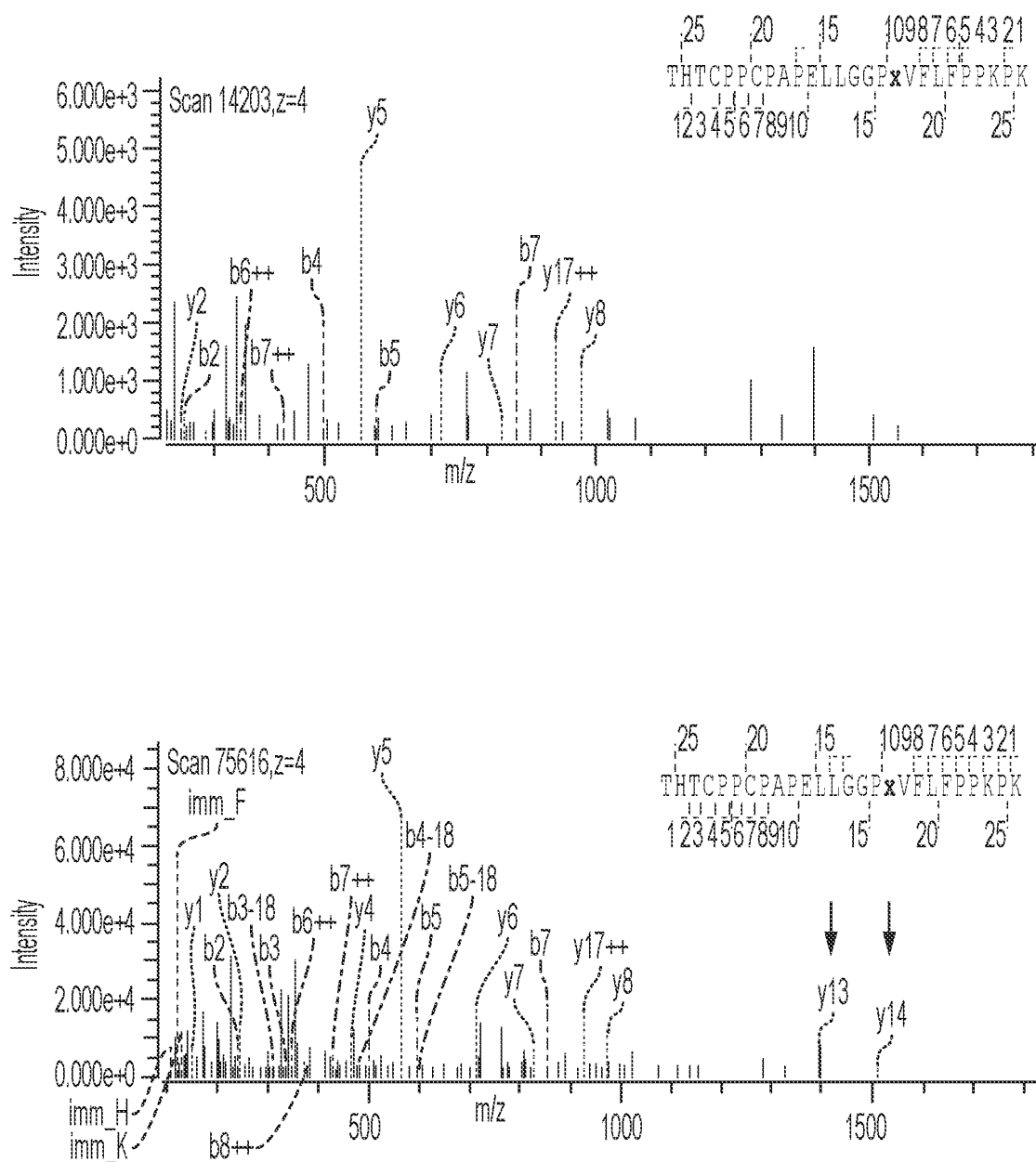


FIG. 25

SUBSTITUTE SHEET (RULE 26)