

Serial Affinity Chromatography as a Selection Tool in Glycoproteomics

Supporting Information

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Figure S-1: Structures of Lewis x (Le^x) and sialy-Lewis x (sLe^x)

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Table S-1. Protein lists identified from the first combination of SAC; the LEL→HPA→anti-Le^x Ab→anti-sLe^x Ab serial columns. Proteins are listed by alphabetical order, based on its captured type; such as whether the protein was captured by four selectors, three selectors, two selectors, or only one selector.

	LEL	HPA	anti-Le ^x Ab	anti-sLe ^x Ab	Swiss-Prot Accession no. and entry name	Name
1	✓	✓	✓	✓	P02765 FETUA_HUMAN	Alpha-2-HS-glycoprotein
2	✓	✓	✓	✓	P01023 A2MG_HUMAN	Alpha-2-macroglobulin
3	✓	✓	✓	✓	P02647 APOA1_HUMAN	Apolipoprotein A-I
4	✓	✓	✓	✓	P02654 APOC1_HUMAN	Apolipoprotein C-I
5	✓	✓	✓	✓	P02656 APOC3_HUMAN	Apolipoprotein C-III
6	✓	✓	✓	✓	P05090 APOD_HUMAN	Apolipoprotein D
7	✓	✓	✓	✓	P10909 CLUS_HUMAN	Clusterin
8	✓	✓	✓	✓	P02746 C1QB_HUMAN	Complement C1q subcomponent subunit B
9	✓	✓	✓	✓	P00736 C1R_HUMAN	Complement C1r subcomponent
10	✓	✓	✓	✓	P02671 FIBA_HUMAN	Fibrinogen alpha chain
11	✓	✓	✓	✓	P02675 FIBB_HUMAN	Fibrinogen beta chain
12	✓	✓	✓	✓	P02679 FIBG_HUMAN	Fibrinogen gamma chain
13	✓	✓	✓	✓	P00738 HPT_HUMAN	Haptoglobin
14	✓	✓	✓	✓	P01859 IGHG2_HUMAN	Ig gamma-2 chain C region
15	✓	✓	✓	✓	P01834 IGKC_HUMAN	Ig kappa chain C region
16	✓	✓	✓	✓	P18136 KV313_HUMAN	Ig kappa chain V-III region HIC
17	✓	✓	✓	✓	P01871 IGHM_HUMAN	Ig mu chain C region
18	✓	✓	✓	✓	P51884 LUM_HUMAN	Lumican
19	✓	✓	✓	✓	P02743 SAMP_HUMAN	Serum amyloid P-component
20	✓	✓	✓	✓	P04004 VTNC_HUMAN	Vitronectin
21	✓	✓	✓		P04003 C4BPA_HUMAN	C4b-binding protein alpha chain
22	✓	✓	✓		P02790 HEMO_HUMAN	Hemopexin
23	✓	✓	✓		P01857 IGHG1_HUMAN	Ig gamma-1 chain C region

24	✓	✓	✓		P06314 KV404_HUMAN	Ig kappa chain V-IV region B17
25	✓	✓	✓		P01591 IGJ_HUMAN	Immunoglobulin J chain
26	✓	✓	✓		P01042 KNG1_HUMAN	Kininogen-1
27	✓	✓			P08603 CFAH_HUMAN	Complement factor H
28	✓		✓	✓	P02649 APOE_HUMAN	Apolipoprotein E
29	✓		✓	✓	P01764 HV303_HUMAN	Ig heavy chain V-III region VH26
30	✓		✓	✓	P01776 HV315_HUMAN	Ig heavy chain V-III region WAS
31	✓		✓	✓	P0CG05 LAC2_HUMAN	Ig lambda-2 chain C regions
32	✓		✓	✓	Q14624 ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4
33	✓		✓		Q15485 FCN2_HUMAN	Ficolin-2
34	✓		✓		Q08380 LG3BP_HUMAN	Galectin-3-binding protein
35	✓		✓		P01766 HV305_HUMAN	Ig heavy chain V-III region BRO
36	✓		✓		P06316 LV107_HUMAN P01701 LV103_HUMAN	Ig lambda chain V-I region BL2 Ig lambda chain V-I region NEW
37	✓		✓		P01701 LV103_HUMAN	Ig lambda chain V-I region NEW OS=Homo sapiens PE=1 SV=1
38	✓		✓		P02768 ALBU_HUMAN	Serum albumin
39	✓			✓	P02652 APOA2_HUMAN	Apolipoprotein A-II
40	✓			✓	P27169 PON1_HUMAN	Serum paraoxonase/arylesterase 1
41	✓				P02763 A1AG1_HUMAN	Alpha-1-acid glycoprotein 1
42	✓				P19652 A1AG2_HUMAN	Alpha-1-acid glycoprotein 2
43	✓				P04114 APOB_HUMAN	Apolipoprotein B-100
44	✓				O75882 ATRN_HUMAN	Attractin
45	✓				P02749 APOH_HUMAN	Beta-2-glycoprotein 1
46	✓				O43866 CD5L_HUMAN	CD5 antigen-like
47	✓				P09871 C1S_HUMAN	Complement C1s subcomponent
48	✓				Q92496 FHR4_HUMAN	Complement factor H-related protein 4
49	✓				Q9BXR6 FHR5_HUMAN	Complement factor H-related protein 5

50	✓				P16581 LYAM2_HUMAN	E-selectin
51	✓				Q9UGM5 FETUB_HUMAN	Fetuin-B
52	✓				P23142 FBLN1_HUMAN	Fibulin-1
53	✓				O75636 FCN3_HUMAN	Ficolin-3
54	✓				P00739 HPTR_HUMAN	Haptoglobin-related protein
55	✓				P06326 HV107_HUMAN	Ig heavy chain V-I region Mot
56	✓				P01781 HV320_HUMAN	Ig heavy chain V-III region GAL
57	✓				P01602 KV110_HUMAN	Ig kappa chain V-I region HK102 (Fragment)
58	✓				P01614 KV201_HUMAN	Ig kappa chain V-II region Cum
59	✓				P04220 MUCB_HUMAN	Ig mu heavy chain disease protein
60	✓				Q9Y6R7 FCGBP_HUMAN	IgG Fc-binding protein
61	✓				P05362 ICAM1_HUMAN	Intercellular adhesion molecule 1
62	✓				P48740 MASP1_HUMAN	Mannan-binding lectin serine protease 1
63	✓				Q99784 NOE1_HUMAN	Noelin
64	✓				P55058 PLTP_HUMAN	Phospholipid transfer protein
65	✓				P05155 IC1_HUMAN	Plasma protease C1 inhibitor
66	✓				P07998 RNAS1_HUMAN	Ribonuclease pancreatic
67	✓				P02787 TRFE_HUMAN	Serotransferrin
68	✓				P35542 SAA4_HUMAN	Serum amyloid A-4 protein
69	✓				P0CG04 LAC1_HUMAN	Ig lambda-1 chain C regions
70		✓	✓	✓	P01024 CO3_HUMAN	Complement C3
71		✓	✓		P06309 KV205_HUMAN	Ig kappa chain V-II region GM607 (Fragment)
72		✓	✓		P01714 LV301_HUMAN	Ig lambda chain V-III region SH
73		✓	✓		P01717 LV403_HUMAN	Ig lambda chain V-IV region Hil
74		✓		✓	P01777 HV316_HUMAN	Ig heavy chain V-III region TEI
75		✓			P03950 ANGI_HUMAN	Angiogenin
76		✓			Q03591 FHR1_HUMAN	Complement factor H-related protein 1
77		✓			P01742 HV101_HUMAN	Ig heavy chain V-I region EU

78		√			P01779 HV318_HUMAN	Ig heavy chain V-III region TUR
79		√			P01593 KV101_HUMAN	Ig kappa chain V-I region AG
80		√			P04208 LV106_HUMAN	Ig lambda chain V-I region WAH
81		√			P80748 LV302_HUMAN	Ig lambda chain V-III region LOI
82		√			P0CG06 LAC3_HUMAN	Ig lambda-3 chain C regions
83		√			P22692 IBP4_HUMAN	Insulin-like growth factor- binding protein 4
84		√			P04259 K2C6B_HUMAN	Keratin, type II cytoskeletal 6B
85		√			P03952 KLKB1_HUMAN	Plasma kallikrein
86		√			P00747 PLMN_HUMAN	Plasminogen
87		√			P27918 PROP_HUMAN	Properdin
88			√	√	P02747 C1QC_HUMAN	Complement C1q subcomponent subunit C
89			√	√	P01861 IGHG4_HUMAN	Ig gamma-4 chain C region
90			√	√	P19823 ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2
91			√		P01772 HV311_HUMAN	Ig heavy chain V-III region KOL
92			√		P04432 KV124_HUMAN	Ig kappa chain V-I region Daudi
93			√		P35527 K1C9_HUMAN	Keratin, type I cytoskeletal 9
94			√		P20742 PZP_HUMAN	Pregnancy zone protein
95				√	P01009 A1AT_HUMAN	Alpha-1-antitrypsin
96				√	P06727 APOA4_HUMAN	Apolipoprotein A-IV
97				√	P02655 APOC2_HUMAN	Apolipoprotein C-II
98				√	P00450 CERU_HUMAN	Ceruloplasmin
99				√	P02745 C1QA_HUMAN	Complement C1q subcomponent subunit A
100				√	P0C0L5 CO4B_HUMAN P0C0L4 CO4A_HUMAN	Complement C4-B Complement C4-A
101				√	P02751 FINC_HUMAN	Fibronectin
102				√	P01877 IGHA2_HUMAN	Ig alpha-2 chain C region

“√” means that the protein was identified in the eluent from the selector among the serial affinity columns.

Table S-2. Protein lists identified from the second combination of SAC; the anti-sLe^x Ab→anti-Le^x Ab→HPA→LEL serial columns. Proteins are listed by alphabetical order, based on its captured type; such as whether the protein was captured by four selectors, three selectors, two selectors, or only one selector.

	anti-sLe ^x Ab	anti-Le ^x Ab	HPA	LEL	Swiss-Prot Accession no. and entry name	Names
1	√	√	√	√	P01876 IGHA1_HUMAN	Ig alpha-1 chain C region
2	√	√	√	√	P01834 IGKC_HUMAN	Ig kappa chain C region
3	√	√	√	√	P01842 LAC_HUMAN	Ig lambda chain C regions
4	√	√	√	√	P01871 IGHM_HUMAN	Ig mu chain C region
5	√	√	√	√	P02787 TRFE_HUMAN	Serotransferrin
6	√	√	√	√	P04004 VTNC_HUMAN	Vitronectin
7	√	√		√	P01023 A2MG_HUMAN	Alpha-2-macroglobulin
8	√	√		√	P02647 APOA1_HUMAN	Apolipoprotein A-I
9	√	√		√	P02654 APOC1_HUMAN	Apolipoprotein C-I
10	√	√		√	P05090 APOD_HUMAN	Apolipoprotein D
11	√	√		√	P10909 CLUS_HUMAN	Clusterin
12	√	√		√	P02671 FIBA_HUMAN	Fibrinogen alpha chain
13	√	√		√	P02675 FIBB_HUMAN	Fibrinogen beta chain
14	√	√		√	P02679 FIBG_HUMAN	Fibrinogen gamma chain
15	√	√		√	P01857 IGHG1_HUMAN	Ig gamma-1 chain C region
16	√	√		√	P01861 IGHG4_HUMAN	Ig gamma-4 chain C region
17	√	√		√	P01591 IGJ_HUMAN	Immunoglobulin J chain
18	√	√		√	P02743 SAMP_HUMAN	Serum amyloid P-component
19	√			√	P02763 A1AG1_HUMAN	Alpha-1-acid glycoprotein 1
20	√			√	P01009 A1AT_HUMAN	Alpha-1-antitrypsin
21	√			√	P02765 FETUA_HUMAN	Alpha-2-HS-glycoprotein
22	√			√	P02652 APOA2_HUMAN	Apolipoprotein A-II
23	√			√	P06727 APOA4_HUMAN	Apolipoprotein A-IV

24	✓			✓	P02656 APOC3_HUMAN	Apolipoprotein C-III
25	✓			✓	P02649 APOE_HUMAN	Apolipoprotein E
26	✓			✓	P08519 APOA_HUMAN	Apolipoprotein(a)
27	✓			✓	P02749 APOH_HUMAN	Beta-2-glycoprotein 1
28	✓			✓	P02745 C1QA_HUMAN	Complement C1q subcomponent subunit A
29	✓			✓	P02746 C1QB_HUMAN	Complement C1q subcomponent subunit B
30	✓			✓	P02747 C1QC_HUMAN	Complement C1q subcomponent subunit C
31	✓			✓	P00736 C1R_HUMAN	Complement C1r subcomponent
32	✓			✓	P01024 CO3_HUMAN	Complement C3
33	✓			✓	P0C0L5 CO4B_HUMAN	Complement C4-B
34	✓			✓	P02751 FINC_HUMAN	Fibronectin
35	✓			✓	P00738 HPT_HUMAN	Haptoglobin
36	✓			✓	P01859 IGHG2_HUMAN	Ig gamma-2 chain C region
37	✓			✓	P01860 IGHG3_HUMAN	Ig gamma-3 chain C region
38	✓			✓	P01766 HV305_HUMAN	Ig heavy chain V-III region BRO
39	✓			✓	P01602 KV110_HUMAN	Ig kappa chain V-I region HK102 (Fragment)
40	✓			✓	P06313 KV403_HUMAN; P06312 KV401_HUMAN	Ig kappa chain V-IV region JI Ig kappa chain V-IV region (Fragment)
41	✓			✓	P80748 LV302_HUMAN	Ig lambda chain V-III region LOI
42	✓			✓	P35527 K1C9_HUMAN	Keratin, type I cytoskeletal 9
43	✓			✓	P04264 K2C1_HUMAN	Keratin, type II cytoskeletal 1
44	✓			✓	P01042 KNG1_HUMAN	Kininogen-1
45	✓			✓	Q09666 AHNK_HUMAN	Neuroblast differentiation-associated protein AHNAK
46	✓			✓	P27918 PROP_HUMAN	Properdin
47	✓			✓	Q6PID6 TTC33_HUMAN	REVERSED Tetratricopeptide repeat protein 33
48	✓			✓	P02768 ALBU_HUMAN	Serum albumin
49	✓				P01776 HV315_HUMAN	Ig heavy chain V-III region WAS
50	✓				P04431 KV123_HUMAN	Ig kappa chain V-I region Walker

51	✓				P01620 KV302_HUMAN	Ig kappa chain V-III region SIE
52	✓				P63123 VPK7_HUMAN	REVERSED HERV-K_1q23.3 provirus ancestral Pro protein
53		✓			P01762 HV301_HUMAN	Ig heavy chain V-III region TRO
54		✓			P01617 KV204_HUMAN	Ig kappa chain V-II region TEW
55		✓			P06314 KV404_HUMAN P06313 KV403_HUMAN P06312 KV401_HUMAN	Ig kappa chain V-IV region B17 Ig kappa chain V-IV region JI Ig kappa chain V-IV region (Fragment)
56		✓			P01699 LV101_HUMAN	Ig lambda chain V-I region VOR
57		✓			P13645 K1C10_HUMAN	Keratin, type I cytoskeletal 10
58		✓			P20742 PZP_HUMAN	Pregnancy zone protein
59			✓		P04206 KV307_HUMAN	Ig kappa chain V-III region GOL
60				✓	P04114 APOB_HUMAN	Apolipoprotein B-100
61				✓	O14791 APOL1_HUMAN	Apolipoprotein L1
62				✓	O75882 ATRN_HUMAN	Attractin
63				✓	P55774 CCL18_HUMAN	C-C motif chemokine 18
64				✓	O43866 CD5L_HUMAN	CD5 antigen-like
65				✓	P06276 CHLE_HUMAN	Cholinesterase
66				✓	P06681 CO2_HUMAN	Complement C2
67				✓	Q02985 FHR3_HUMAN	Complement factor H-related protein 3
68				✓	Q9BXR6 FHR5_HUMAN	Complement factor H-related protein 5
69				✓	Q9UGM5 FETUB_HUMAN	Fetuin-B
70				✓	P23142 FBLN1_HUMAN	Fibulin-1
71				✓	Q15485 FCN2_HUMAN	Ficolin-2
72				✓	Q08380 LG3BP_HUMAN	Galectin-3-binding protein
73				✓	P00739 HPTR_HUMAN	Haptoglobin-related protein
74				✓	P69905 HBA_HUMAN	Hemoglobin subunit alpha
75				✓	P02790 HEMO_HUMAN	Hemopexin
76				✓	P01772 HV311_HUMAN	Ig heavy chain V-III region KOL
77				✓	P01764 HV303_HUMAN	Ig heavy chain V-III region

						VH26
78				√	P01609 KV117_HUMAN	Ig kappa chain V-I region Scw
79				√	P18136 KV313_HUMAN	Ig kappa chain V-III region HIC
80				√	P14151 LYAM1_HUMAN	L-selectin
81				√	P51884 LUM_HUMAN	Lumican
82				√	P11226 MBL2_HUMAN	Mannose-binding protein C
83				√	P05155 IC1_HUMAN	Plasma protease C1 inhibitor
84				√	P63119 VPK1_HUMAN	REVERSED HERV-K_12q14.1 provirus ancestral Pro protein
85				√	P35542 SAA4_HUMAN	Serum amyloid A-4 protein
86				√	P27169 PON1_HUMAN	Serum paraoxonase/arylesterase 1

“√” means that the protein was identified in the eluent from the selector among the serial affinity columns.

Figure S-1.

