PROTEOMICS

Supporting Information for Proteomics

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A systematic analysis of the effects of increasing degrees of serum immunodepletion in terms of depth of coverage and other key aspects in top-down and bottom-up proteomic analyses

Supplementary Data 1. Examples of proteins identified from the tryptic digest of significantly different spots in the 2D-DIGE experiment. Proteins quoted were seen in more than one preparatory gel, or no conflicting identities were apparent if seen in a single preparatory gel; multiple identities from one spot are separated by "/"; fragments of immunoglobulins observed are not listed

Increased in:	Number of spots	Examples of proteins identified
MARS14 and Prot20 compared to MARS6	19	Hemopexin, Proteoglycan 4, Inositol polyphosphate 5-phosphatase, Neutrophil defensin 1, Complement factor B (Fragment)
Prot20 compared to MARS14 and MARS6	40	Proteoglycan 4, Complement factor H, Inositol polyphosphate 5-phosphatase, Neutrophil defensin 1
Prot20 compared to MARS6	49	Hemopexin, CD5 antigen-like protein / vitamin D-binding protein precursor, Proteoglycan 4, Complement factor H, Inositol polyphosphate 5-phosphatase, Neutrophil defensin 1
Prot20 compared to MARS14	29	Hemopexin, Inositol polyphosphate 5-phosphatase
Stepwise rise (increasing progressively from MARS6 to MARS14 to Prot20)	7	Alpha-1B-glycoprotein / Inter-alpha-trypsin inhibitor heavy chain H4 / Afamin

Supplementary Data 2: Details of immunodepleted proteins seen with LTQ-Orbitrap Velos

Expected depletions			**Proteins seen in depleted sample on 1D-LC MS/MS Orbitrap Velos analysis (Y = evidence of the protein seen in the sample ; N= evidence of the protein not seen)**					
			≥2 peptide level			**1 peptide level**		
MARS 6	MARS 14	ProteoPrep20	MARS 6	MARS 14	ProteoPrep20	MARS 6	MARS 14	ProteoPrep20
Albumin	Albumin	Albumin	Y	Y	Y	Y	Y	Y
Transferrin	Transferrin	Transferrin	N	Y	Y	N	Y	Y
Fibrinogen	Fibrinogen	Fibrinogen	Y	Y	Y	Y	Y	Y
Alpha-1-antitrypsin	Alpha-1-antitrypsin	Alpha-1-antitrypsin	Y	Y	Y	Y	Y	Y
Haptoglobin	Haptoglobin	Haptoglobin	Y	Y	Y	Y	Y	Y
X	Alpha-2-macroglobulin	Alpha-2-macroglobulin	-	Y	Y	-	Y	Y
X	Alpha-1-acid glycoprotein	Alpha-1-acid glycoprotein	-	Y	N[1]	-	Y	Y
X	Apo-A-I	Apo-A-I	-	Y	Y	-	Y	Y
X	Apo-A-II	Apo-A-II	-	Y	Y	-	Y	Y
X	C3	C3	-	Y	Y	-	Y	Y
X	Transthyretin	Transthyretin	-	N	N	-	Y	N
X	X	Ceruloplasmin	-	-	Y	-	-	Y
X	X	Apo-B	-	-	N[2]	-	-	Y
X	X	C1q	-	-	N	-	-	Y[3]
X	X	C4	-	-	N	-	-	N
X	X	Plasminogen	-	-	N	-	-	Y
IgG	IgG	IgG	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]
IgA	IgA	IgA	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]
X	IgM	IgM	-	Y[4]	Y[4]	-	Y[4]	Y[4]
X	X	IgD	-	-	Y[4]	-	-	Y[4]

Notes

1. Alpha-1-acid glycoprotein 1 is seen in the Prot20 depleted sample at the 1+ peptide level, but not at the 2+ peptide level. Alpha-1-acid glycoprotein 2 is seen at the 2+ peptide level in all depletions
2. Refers to ApoB (IPI00894122); peptides from (either) Apo B-100 / ApoB-48 are seen at 2+ peptide level - these don't appear to be depleted
3. Complement C1q subcomponent subunit A is seen at the 1 peptide level. Subunits B and C are not seen even at that level
4. Multiple immunoglobulin fragments remain in all depletions at the 2+ peptide level. Distinguishing which are from which immunoglobulin subfamily is difficult

Supplementary Data 3: Number of unique peptides seen in each peak 1 sample for the immunodepleted proteins. ≤1 peptide detections are shown in red

Immunodepleted Proteins[1]	WS	MARS 6	MARS 14	Prot 20
Alpha-1-antitrypsin	31	30	31	7
Fibrinogen alpha chain	14	27	25	12
Fibrinogen beta chain	15	24	16	7
Fibrinogen gamma chain	4	13	6	4
Haptoglobin	22	17	14	12
Serotransferrin	93	**0**	15	8
Serum albumin	113	5	28	45
Alpha-1-acid glycoprotein 1 (Orosomucoid-1)	11	14	3	**1**
Alpha-1-acid glycoprotein 2 (Orosomucoid-2)	9	11	4	2
Transthyretin(Prealbumin)	5	8	**1**	**0**
Alpha-2-macroglobulin	55	73	54	56
Apolipoprotein A-I	31	58	49	41
Apolipoprotein A-II	13	15	14	10
Apolipoprotein(a)	14	33	38	13
Complement C3	22	36	2	4
Apolipoprotein B-100;Apolipoprotein B-48[2]	52	154	174	157
APOB protein	2	4	4	**1**
Plasminogen	47	70	74	**1**
Ceruloplasmin	7	11	11	2
Complement C1q subcomponent subunit A	4	8	9	**1**
Complement C1q subcomponent subunit B	4	7	6	**0**
Complement C1q subcomponent subunit C	4	8	7	**0**
Complement component 4B	**0**	**1**	**1**	**0**

1. These are groupings of all the protein names referring to the same protein or fragments / isoforms thereof
2. The number of unique peptides seem for these suggests the immunodepletion column antibodies are not directed against these forms