

Quantitative Host Cell Protein Analysis using Two Dimensional Data Independent LC-MS^E

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Supplementary Information

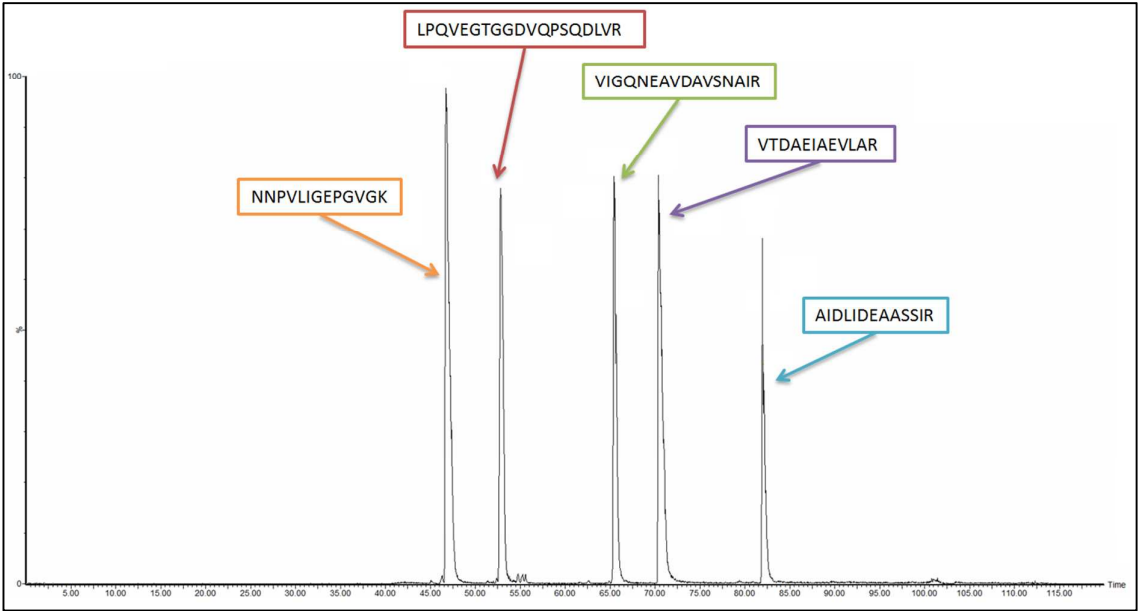
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Supplementary Table 1: P-value result for each Hi3 standard peptide calculated from peak height of the peptide ion peak in extracted ion chromatograms from three separate experiments, each containing 20 2D-LC-MS^E samples. Peptide sequence, average molecular weight and typical m/z value are also outlined.

Hi3 Standard Peptide Sequence	Average Molecular Weight	m/z	P-value
NNPVLIGEPGVGK	1293.4863	647.38	0.13770
LPQVEGTGGDVQPSQDLVR	1995.1776	998.54	0.12817
VIGQNEAVDAVSNAIR	1655.8292	828.47	0.07979
VTDAEIAEVLAR	1286.4484	643.88	0.55885
AIDLIDEAASSIR	1373.5266	687.39	0.14309

Supplementary Figure 1: Extracted ion chromatogram showing five Hi3 standards used for Hi3 quantitation of HCPs identified in purified mAb samples.



Supplementary Table 2: Summary of identified HCPs and related concentrations in combined sample sets processed from Protein A resin with different elution buffers.

List of HCPs identified with >2 unique peptides		Concentration of HCP in combined sample sets (ppm - ng/mg)				PLGS protein score Range	Protein average mass (kDa)	Protein Sequence Coverage Range (%)	Number of Peptides Identified
Protein Entry	Protein Description	100 mM Acetate	100 mM Arginine	100 mM Citrate	100 mM Glycine				
CH10	10 kDa heat shock protein mitochondrial	ND	ND	ND	*	542.0	11.032	23.53	2
RL20	60S ribosomal protein L30	ND	44.1	*	ND	780.6 - 2090.1	12.955	20.80 - 26.09	2 – 3
GRP78	78 kDa glucose-regulated protein	73.7	126.3	205.6	ND	419.3 - 503.4	72.550	11.93 - 14.53	7 – 12
ACTC	Actin alpha cardiac muscle 1	*	ND	ND	ND	307.7	42.361	14.59	2
ACTB	Actin cytoplasmic 1	*	ND	ND	113.7	470.9 - 569.6	42.080	20.27 - 36.27	2 – 11
ACTG	Actin cytoplasmic 2	19.5	ND	ND	ND	627.4	33.391	17.69	7
ANXA2	Annexin A2	*	ND	ND	232.6	396.4 - 771.0	38.946	9.73 - 25.37	2 – 5
ANXA5	Annexin A5	95.1	ND	ND	*	346.7 - 536.4	36.122	10.28 - 19.31	2 – 6
SLPI	Antileukoproteinase	1088.3	819.4	3727.5	4528.7	2531.1 - 21214.76	16.745	30.56 - 59.72	4 – 10
ACTBL	Beta-actin-like protein 2	153.0	ND	ND	ND	257.1	37.484	15.57	5
CALR	Calreticulin	208.1	ND	360.8	380.5	819.7 - 1882.2	48.414	30.46 - 37.41	8 – 13
CATB	Cathepsin B	99.4	ND	*	261.1	479.2 - 2732.9	38.417	10.03 - 40.12	2 – 8
CATD	Cathepsin D	ND	ND	ND	951.6	285.7	44.567	7.60	8
CCL2	C-C motif chemokine 2	ND	*	*	ND	635.6 - 703.7	16.201	5.59	2
CLUS	Clusterin	445.9	ND	ND	ND	535	52.385	20.58	12
CXCL3	C-X-C motif chemokine 3	72.7	ND	ND	*	2258.0 - 3765.8	11.264	40.59	2 - 5
DLDH	Dihydrolipoyl dehydrogenase mitochondrial	*	ND	ND	ND	342.5	54.702	5.89	2
FIBB	Fibrinogen beta chain	*	ND	ND	ND	335.0	54.893	3.55	2
G3PT	Glyceraldehyde-3-phosphate dehydrogenase	*	ND	ND	ND	407.4	35.976	6.91	2

H12	Histone H1.2	ND	ND	ND	82	1212.3	21.285	17.92	5
H13	Histone H1.3	*	ND	ND	ND	1196.2	20.512	17.87	2
H2B1F	Histone H2B type 1-F/J/L	ND	ND	*	ND	2297.5	11.158	27.45	2
H3	Histone H3	ND	ND	*	ND	951.4	24.371	11.93	2
H4	Histone H4	ND	ND	*	86.3	782.6 - 1301.0	8.983	24.69 - 37.04	2 – 3
MFGM	Lactadherin	ND	207.1	419.6	ND	2639.8 - 3598.1	48.284	43.56 - 52.22	9 – 15
MMP19	Matrix metalloproteinase-19	*	ND	210.0	449.8	462.7 - 584.3	59.170	12.57 - 20.38	2 – 8
TIMP2	Metalloproteinase inhibitor 2	358.7	298.0	346.9	1583.4	507.5 - 2285.2	27.486	17.62 - 52.46	6 – 9
PHOCN	MOB-like protein phocein isoform X2	627.4	ND	ND	*	540.8 - 2205.8	32.759	20.28 - 25.17	2 – 8
NELFE	Negative elongation factor E	ND	ND	*	ND	755.8	42.788	26.26	2
NID1	Nidogen-1	3357.9	*	ND	2516	308.9 - 744.5	135.143	14.19 - 25.91	2 – 26
YBOX1	Nuclease-sensitive element-binding protein 1	318.6	ND	ND	ND	565.7	32.776	31.03	8
PPIB	Peptidyl-prolyl cis-trans isomerase B	ND	*	85.3	ND	619.8 - 1464.6	23.692	21.76 - 22.22	2 – 6
PRDX1	Peroxiredoxin-1	*	ND	ND	*	210.0 - 653.6	22.548	9.17 - 16.08	2
PCOC1	Procollagen C-endopeptidase enhancer	98.8	ND	ND	1210	222.8 - 1109.1	51.131	16.45 - 33.55	7 – 11
PRC2B	Proline-rich protein 2-like	1940.1	ND	*	ND	1310.9 - 1512.2	52.585	62.63 - 63.93	2 - 52
BROMI	Protein Broad minded isoform X3	ND	*	ND	*	374.5 - 415.7	146.423	4.77 - 5.16	2
S10A6	Protein S100-A6	1.4	ND	ND	ND	1187.4	10.108	61.80	6
PLBL2	Putative phospholipase B-like 2	*	ND	ND	ND	313.2	65.940	9.40	2
RCN1	Reticulocalbin-1	*	ND	ND	ND	741.6	19.539	55.76	2
HTRA1	Serine Protease HTRA1	247.6	781.0	1247.9	2119.5	592.1.2 - 1530.7	48.697	24.94 - 38.98	7 - 13
Total concentration (ppm - ng HCP/mg IgG)		9206.2	2275.9	6603.6	14515.2				

HCPs identified using a minimum of 2 peptides are denoted by the symbol ‘*’, while undetected HCPs are represented by ‘ND’.

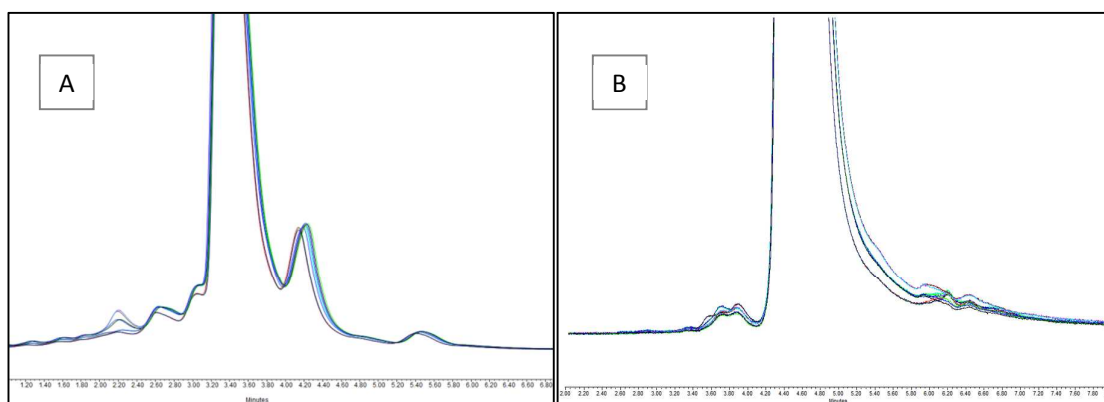
Supplementary Table 3: Whole protein immunogenicity score reported by the CHOPPI web tool for assessment of immunogenicity risk from HCPs in CHO-based protein production. Proteins with an immunogenicity score of > 20 are considered to be a high risk for immunogenicity, while proteins with a score < -20 are reportedly immunologically inert.

Protein Entry	Protein Description	Immunogenicity score reported by CHOPPI
YBOX1	Nuclease-sensitive element-binding protein 1	-65.86
RCN1	Reticulocalbin-1	-57.94
PRDX1	Peroxiredoxin-1	-51.76
GRP78	78 kDa glucose-regulated protein	-48.51
G3PT	Glyceraldehyde-3-phosphate dehydrogenase	-39.89
H13	Histone H1.3	-39.18
CALR	Calreticulin	-37.79
ACTB	Actin cytoplasmic 1	-34.19
CATB	Cathepsin B	-32.6
NID1	Nidogen-1	-32.42
ACTC	Actin alpha cardiac muscle 1	-32.34
H12	Histone H1.2	-28.81
PCOC1	Procollagen C-endopeptidase enhancer	-28.49
ACTBL	Beta-actin-like protein 2	-26.78
TIMP2	Metalloproteinase inhibitor 2	-25.28
FIBB	Fibrinogen beta chain	-24.24
DLDH	Dihydrolipoyl dehydrogenase mitochondrial	-22.06
PPIB	Peptidyl-prolyl cis-trans isomerase B	-13.65
HTRA1	Serine Protease HTRA1	-13.02
CATD	Cathepsin D	-11.36
ACTG	Actin cytoplasmic 2	-9.36
MFGM	Lactadherin	-9.16
CH10	10 kDa heat shock protein mitochondrial	-7.1
CLUS	Clusterin	-3.55
MMP19	Matrix metalloproteinase-19	-2.49
SLPI	Antileukoproteinase	0.01
PHOCN	MOB-like protein phocein isoform X2	4.44
H2B1F	Histone H2B type 1-F/J/L	6.77
H4	Histone H4	7.67
NELFE	Negative elongation factor E	10.59
ANXA2	Annexin A2	14.87
CCL2	C-C motif chemokine 2	18.31
H3	Histone H3	25.79
PLBL2	Putative phospholipase B-like 2	32.89
S1046	Protein S100-A6	52.84
ANXA5	Annexin A5	52.91
RL20	60S ribosomal protein L30	57.41
CXCL3	C-X-C motif chemokine 3	91.84

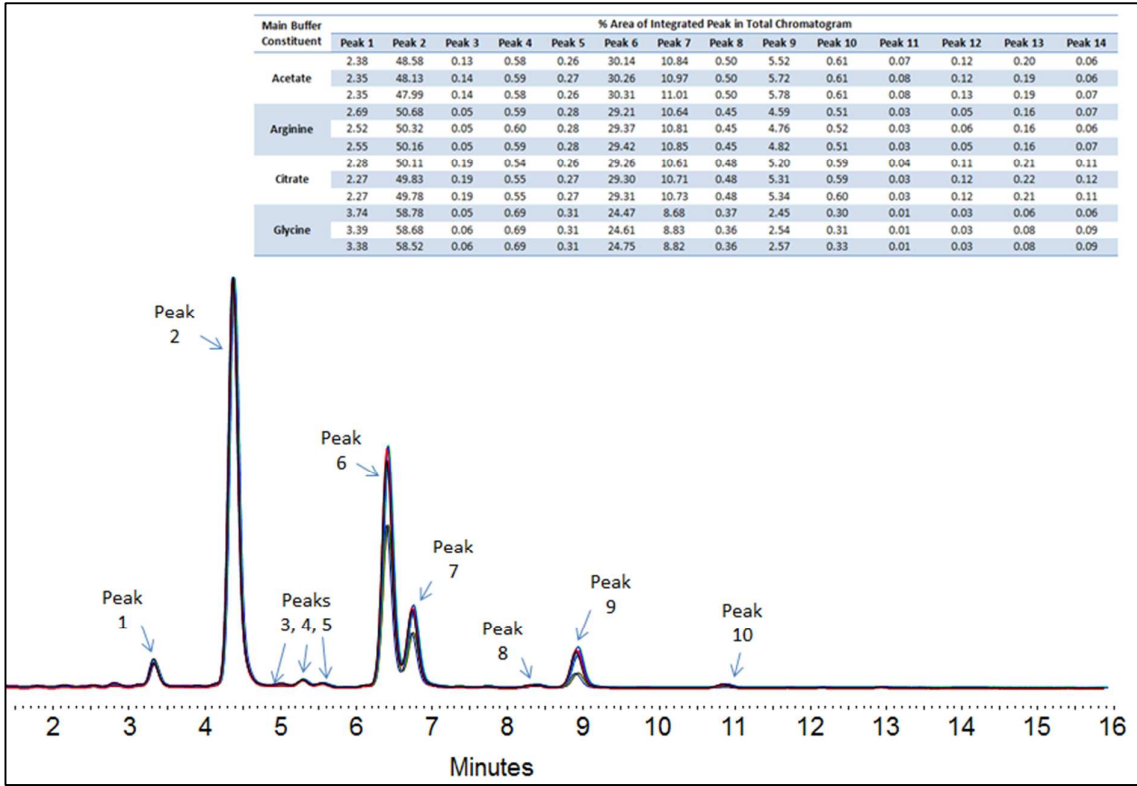
Supplementary Table 4: Analytical results from charge variant and size exclusion analysis of intact mAb1.

Buffer under evaluation	Charge variant analysis			Size exclusion analysis		
	% acidic variants	% main	% basic variants	% aggregates	% monomer	% fragment
100 mM sodium acetate, pH3.5	2.75 ± 0.23	87.61 ± 0.14	9.64 ± 0.94	0.10 ± 0.94	99.87 ± 0.12	0.03 ± 10.57
100 mM arginine, pH3.5	2.14 ± 1.64	88.72 ± 0.14	9.14 ± 0.59	0.10 ± 3.08	99.87 ± 0.06	0.03 ± 15.59
100 mM citrate, pH3.5	3.43 ± 1.36	87.21 ± 0.21	9.37 ± 0.26	0.19 ± 0.95	99.76 ± 0.10	0.05 ± 13.74
100 mM glycine, pH3.5	1.94 ± 2.15	88.76 ± 0.22	9.30 ± 0.57	0.12 ± 3.24	99.84 ± 0.05	0.04 ± 3.49

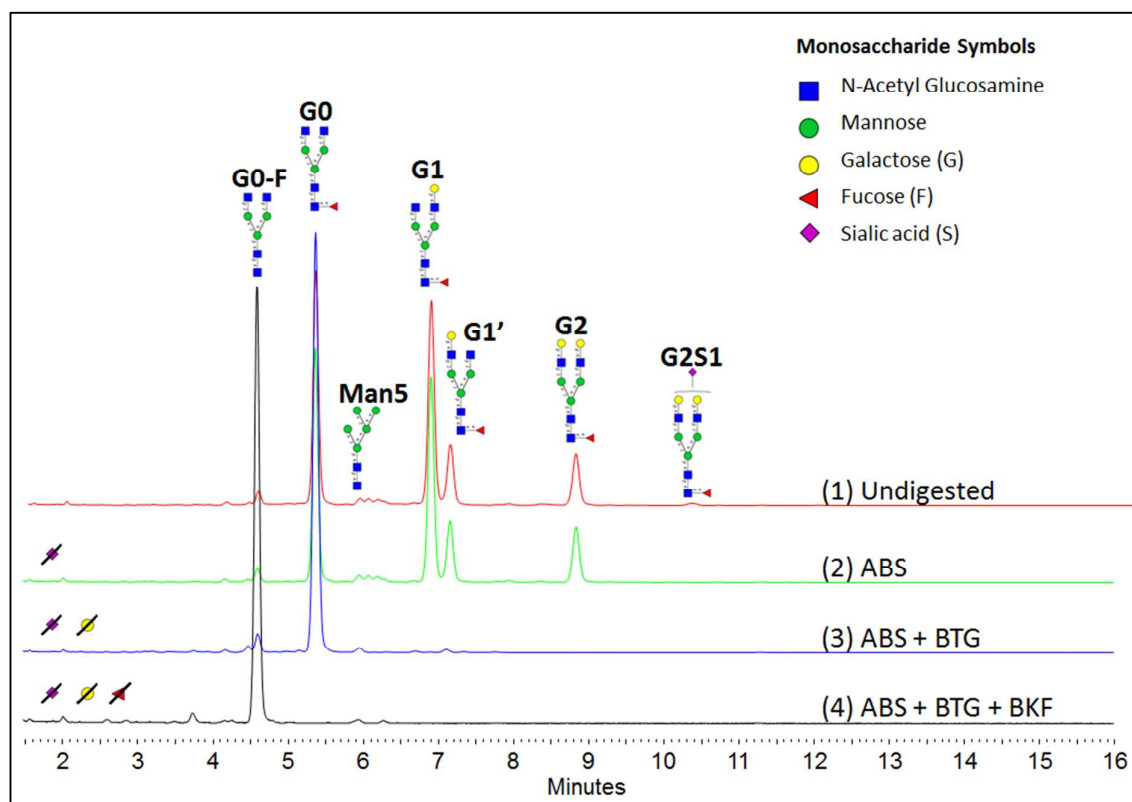
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Supplementary Figure 3: Overlay of chromatograms displaying *N*-glycan profiles of mAb1 processed from Protein A resin using elution buffers 100 mM sodium acetate, pH 3.5, 100 mM arginine, pH 3.5, 100 mM citrate, pH 3.5 and 100 mM glycine, pH 3.5.



Supplementary Figure 4: Annotation of 2AB-labelled mAb1 *N*-glycan profiles following exoglycosidase digestion. The top profile (1) shows undigested pool of *N*-glycan, followed by a series of exoglycosidase digestions with (2) *Arthrobacter ureafaciens* sialidase, ABS, (3) ABS and Bovine testis galactosidase, BTG and (4) ABS, BTG and Bovine kidney fucosidase, BKF.



Supplementary Figure 5: Graphic of anti-Interleukin 8 IgG1 (mAb1) structure. MAb1 peptides, which were identified in sample fractions also containing Calreticulin peptides, are highlighted in the heavy chain (blue) and light chain (green) portions of the structure.

