

# HCP Analysis of a Small Drug-Protein in Process Sample

## by Combining Platform Immunoassays and Mass Spectrometry

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### Introduction

Among various evaluation methods to detect host cell protein (HCP) impurities, 2D-gel based separation of drug samples followed by Western blotting with anti-HCP antibodies offers visual confirmation of immunodetected proteins. In addition to these visual results, ELISA based assays provide a valuable high throughput tool to determine product purity in terms of total HCP content relative to the drug protein itself. Mass spectrometry based orthogonal methods, such as GeLC-MS/MS enables identification of individual host cell proteins, and provides exact protein name, database entry number, amino acid sequence as well as theoretical pI and molecular weight for each identified HCP.

Here, we combine a high coverage platform immunoassay with highly sensitive GeLC-MS/MS for HCP analysis of both an in process drug protein and the corresponding *E. coli* null cell lysate. This combination of methods enables maximum coverage and information details on each HCP.

### Methods

- Western blot and ELISA assays were performed utilizing generic HCP antibodies generated by immunization of host cell proteins fractionated by size. Total protein stains were performed after separation by 2D-PAGE using Oriole stain.
- Nano flow LC-MS/MS were performed on peptide digests based on proteins separated by 1D-PAGE and separated into eight fractions (GeLC-MS/MS). MS data was searched against a database containing the *E. coli* proteome and relevant contaminants.

### Conclusion

Combining the strengths of platform ELISA and MS orthogonal HCP detection methods enabled a convenient, high throughput detection strategy for drug protein impurities with capabilities to visualize, quantify and identify host cell proteins in the in process sample as well as the null cell lysate.



### Immunoassay Results - *E. coli* Null Cell Lysate

HCP proteins in the null cell lysate were detected using a generic antibody (200-401-M61) showing a total coverage of 46%. Detection of high molecular weight (HMW,  $\geq 20$  kDa) proteins was 58% and 23% for low molecular weight (LMW,  $\leq 20$  kDa) proteins (Fig. 1-3).

Figure 1: *E. coli* null cell lysate 2D-PAGE (A) and Western blot (B) analysis

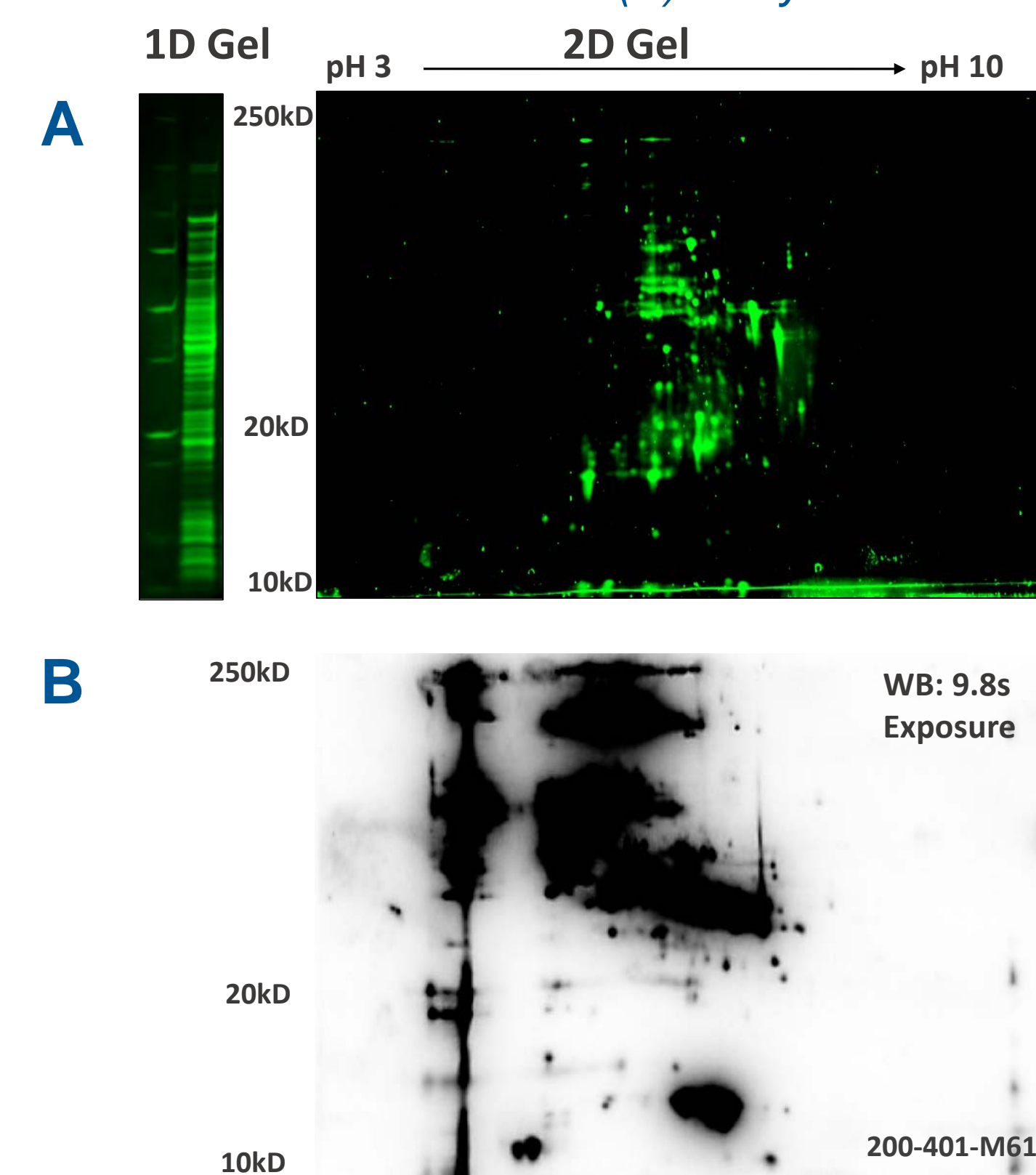


Figure 2: Coverage and detection analysis

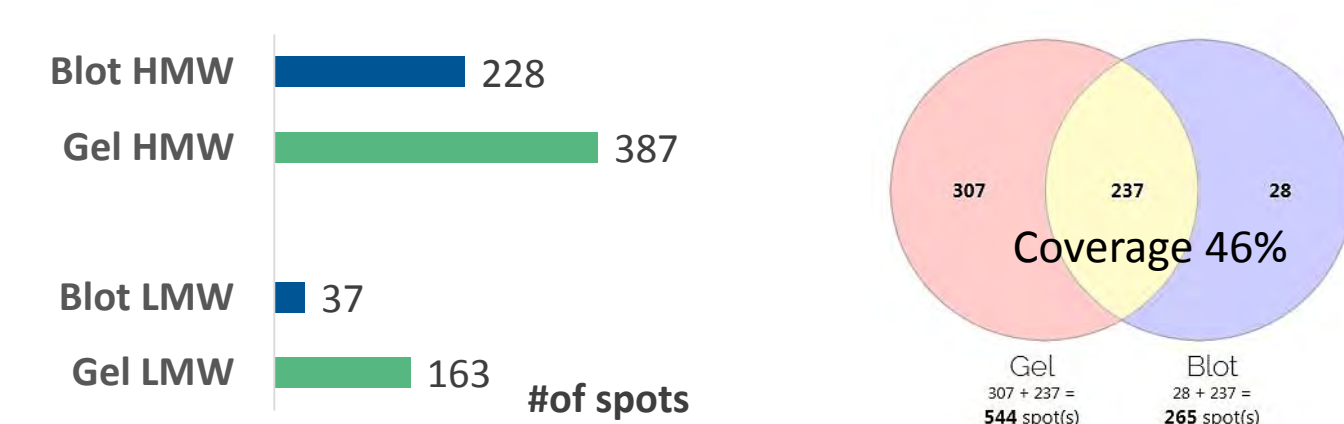
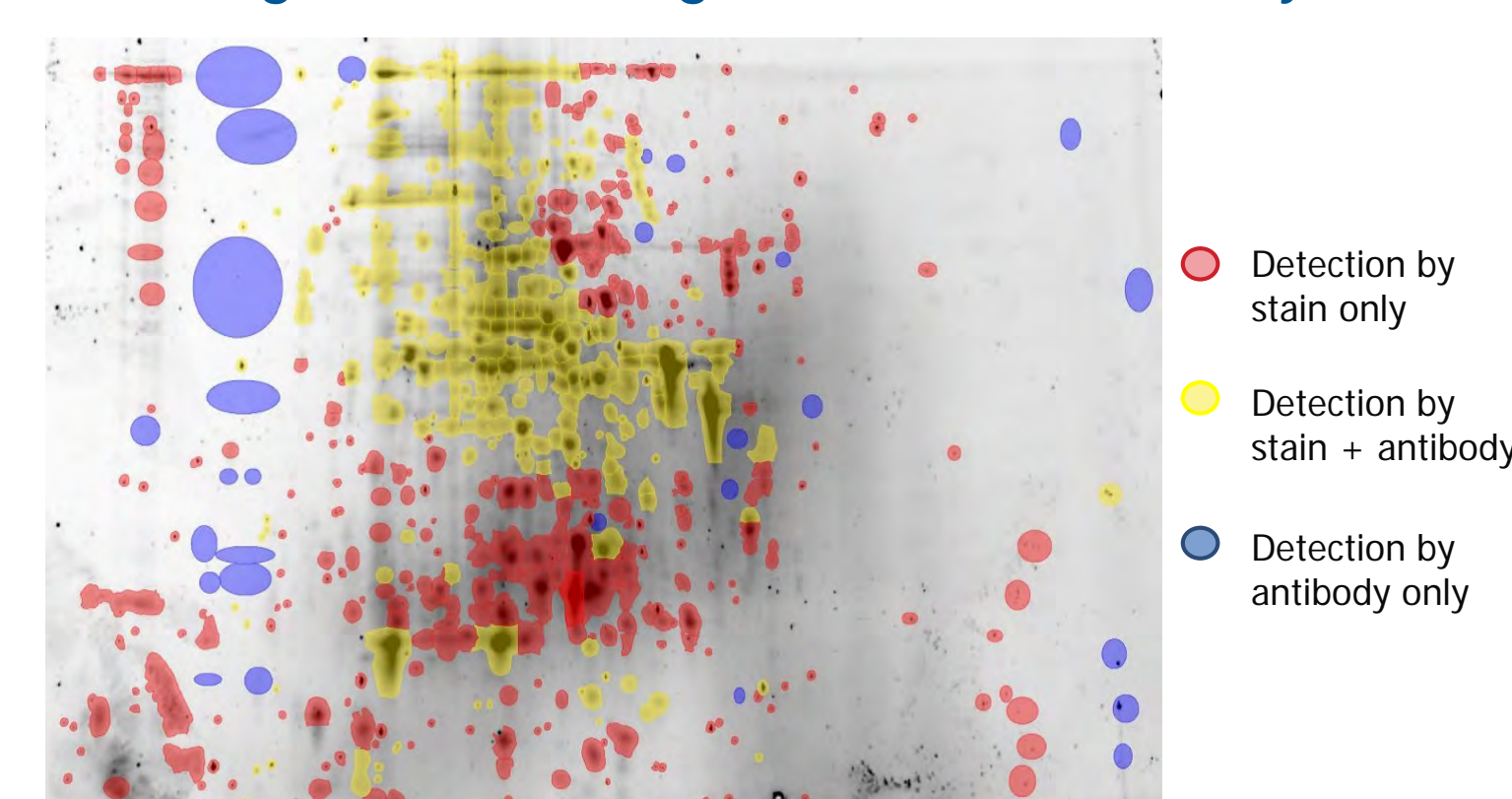
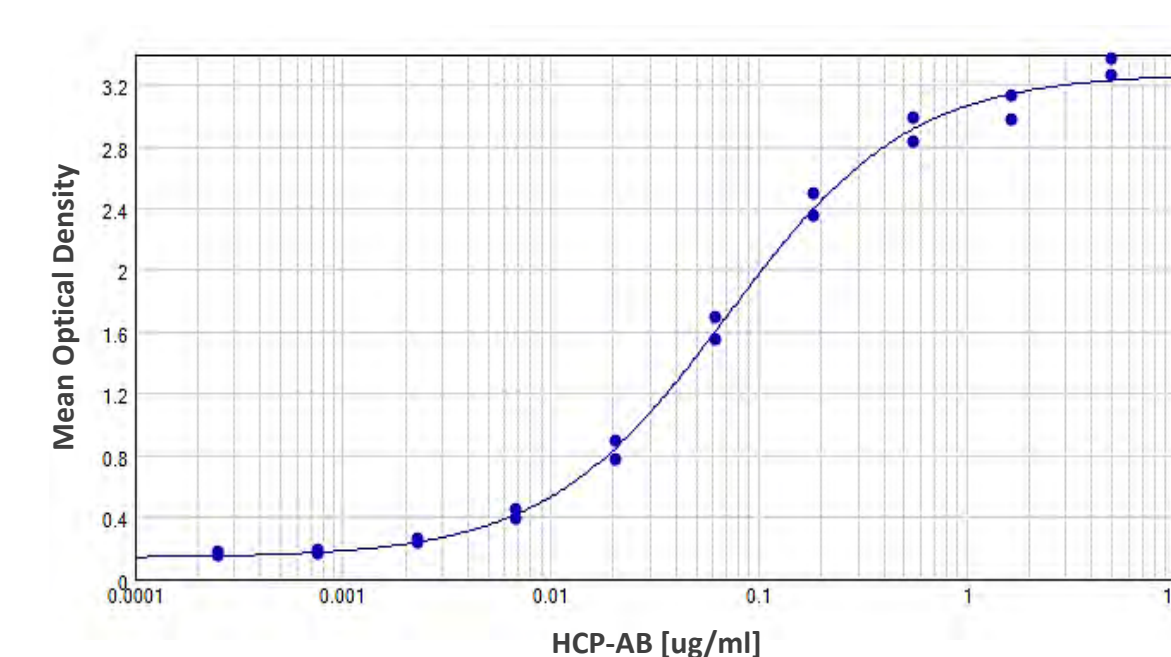


Figure 3: Detection of HCP by ELISA



### Immunoassay Results - In Process Sample

HCP impurities in the in process sample (drug protein theoretical molecular weight: 12kDa; pI 9.1) were detected using the same generic antibody. A total coverage of 32% was shown. The detection of HMW proteins was 92% and 5% for LMW proteins (Fig 4-6).

Figure 4: Process sample (A) 2D-PAGE and Western blot (B) analysis

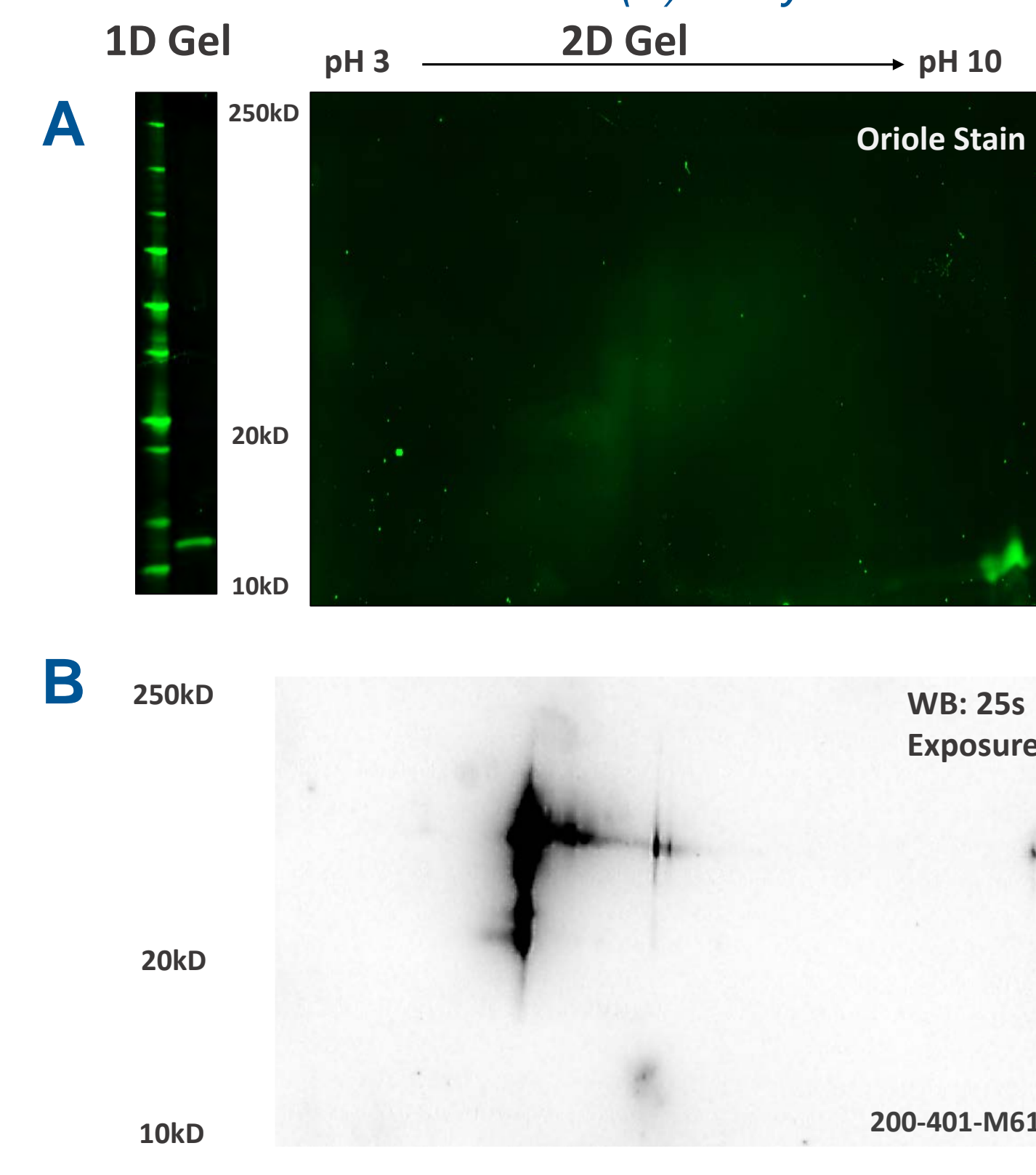


Figure 5: Coverage and detection analysis

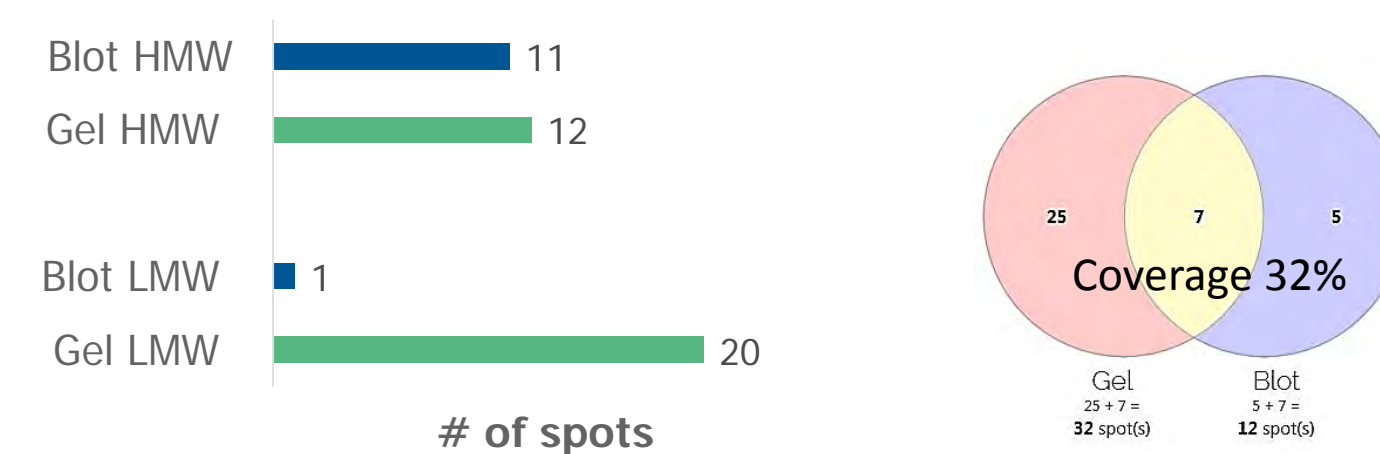
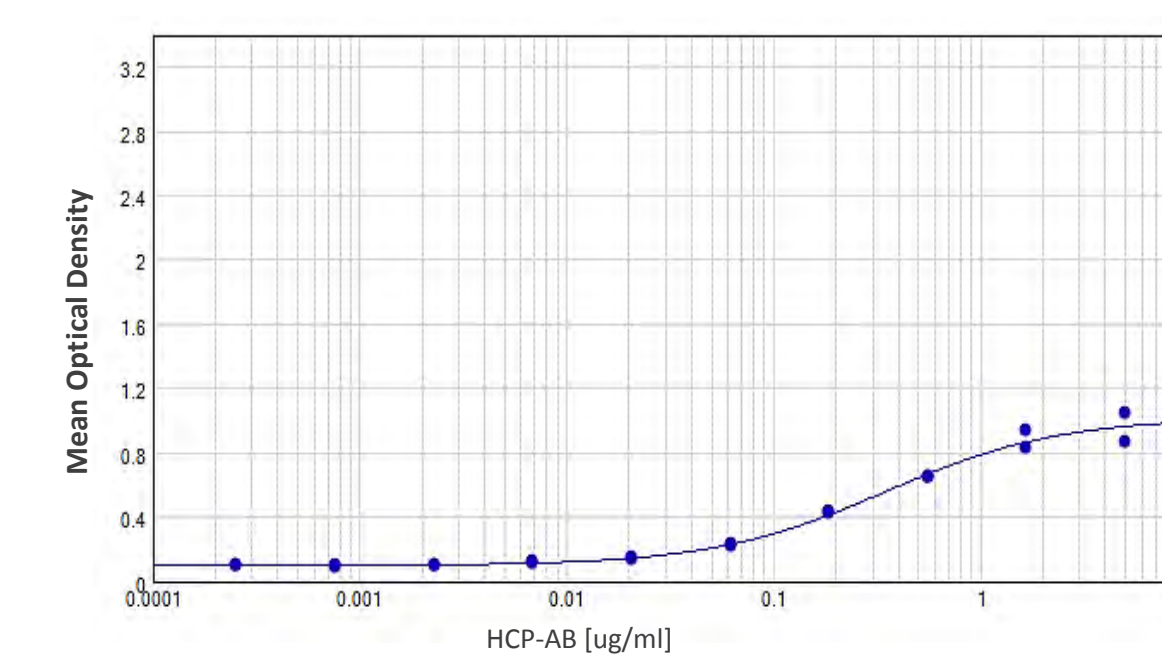


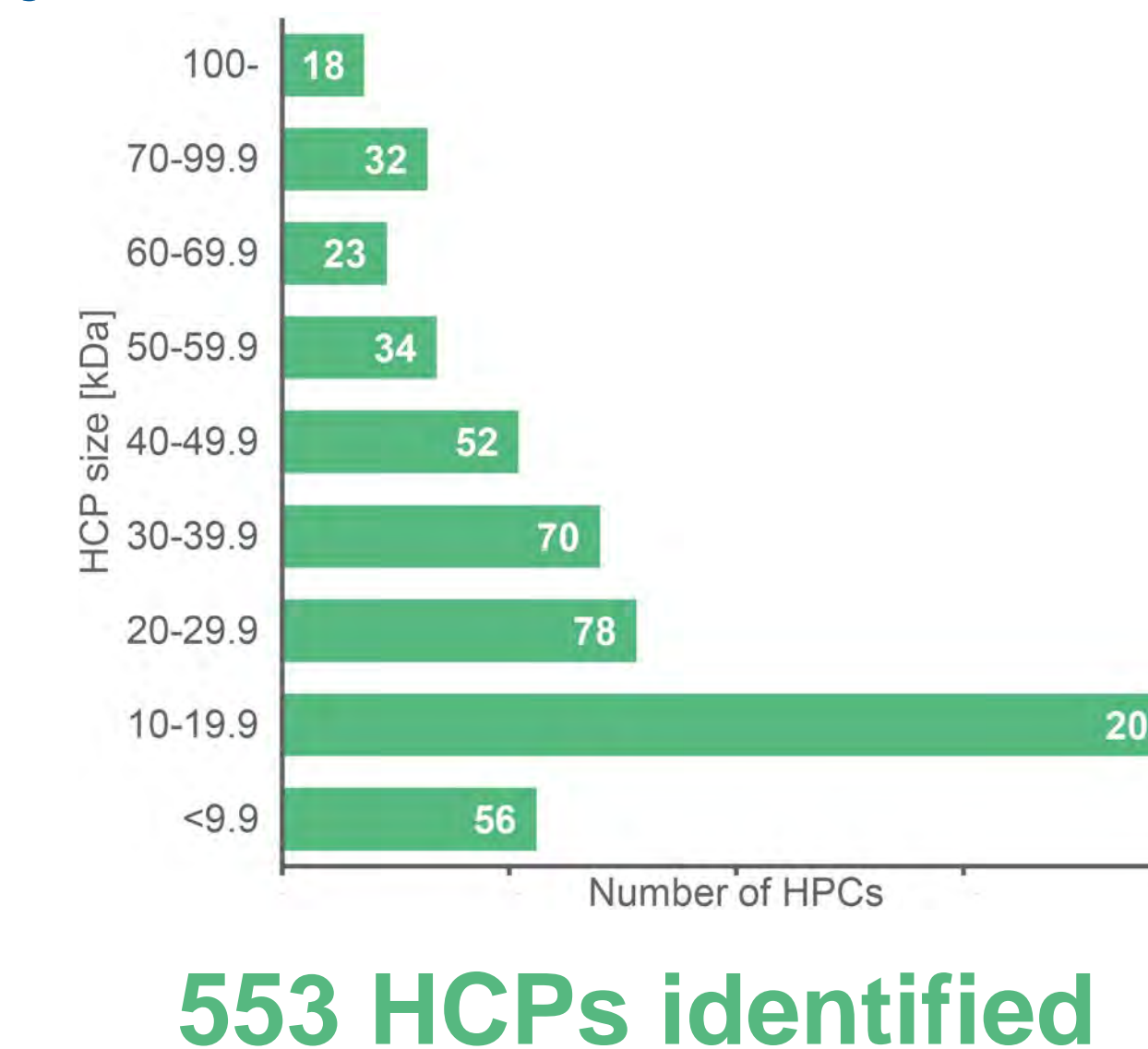
Figure 6: Detection of HCP by ELISA



### GeLC-MS/MS Results

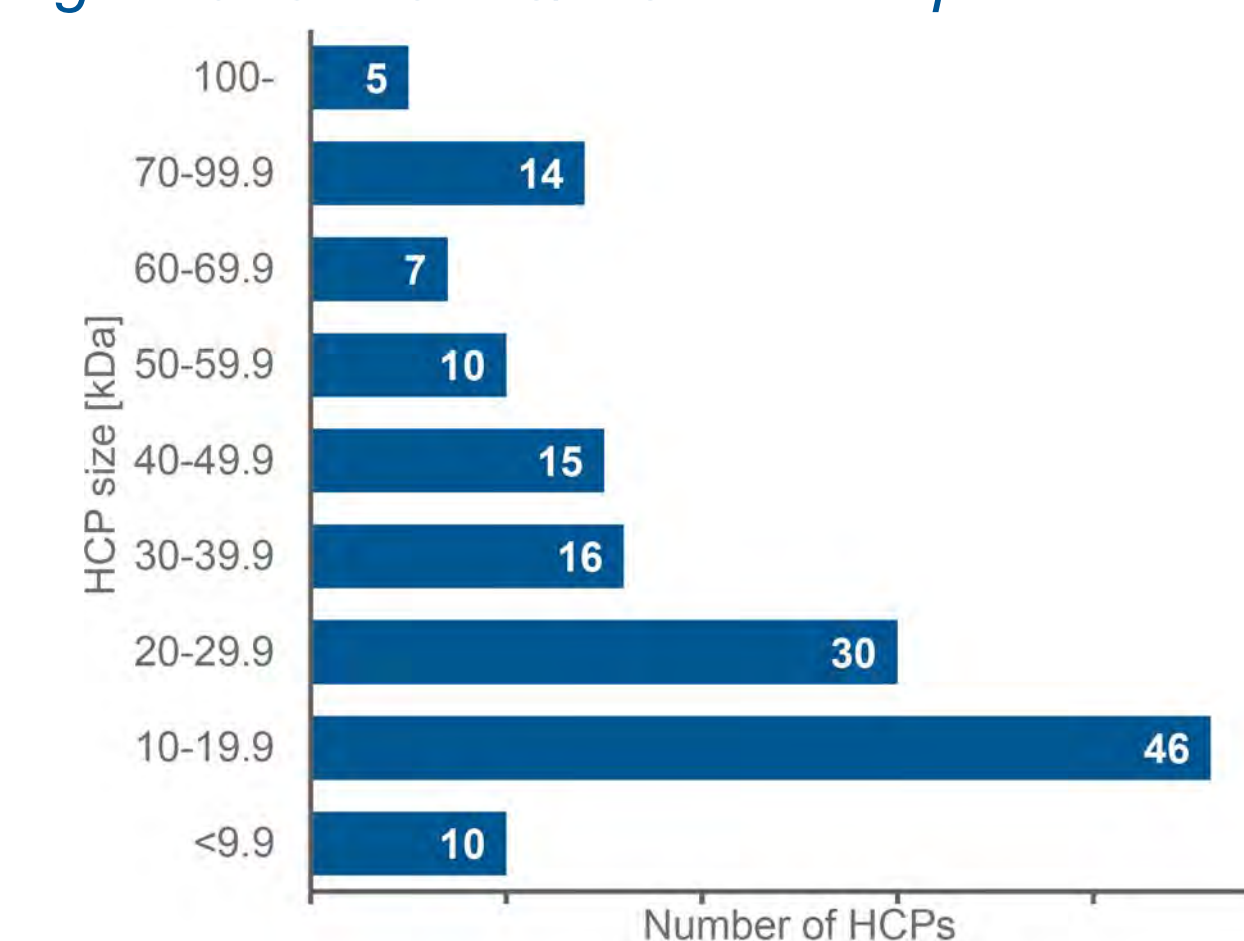
GeLC-MS/MS identified 553 proteins in the *E. coli* null cell lysate and 152 host cell proteins in the in process sample all at high confidence. High percentages of LMW HCPs were identified: 46% (256 of 553) and 37% (56 of 152) in the *E. coli* null cell lysate and in the in process sample, respectively (Fig. 7-8). The HCPs identified cover a wide mass as well as pI range. 76% (116 of 152) of the HCPs identified in the in process sample were also identified in the *E. coli* null cell lysate (Fig. 9).

Figure 7: GeLC-MS/MS of the *E. coli* null cell lysate



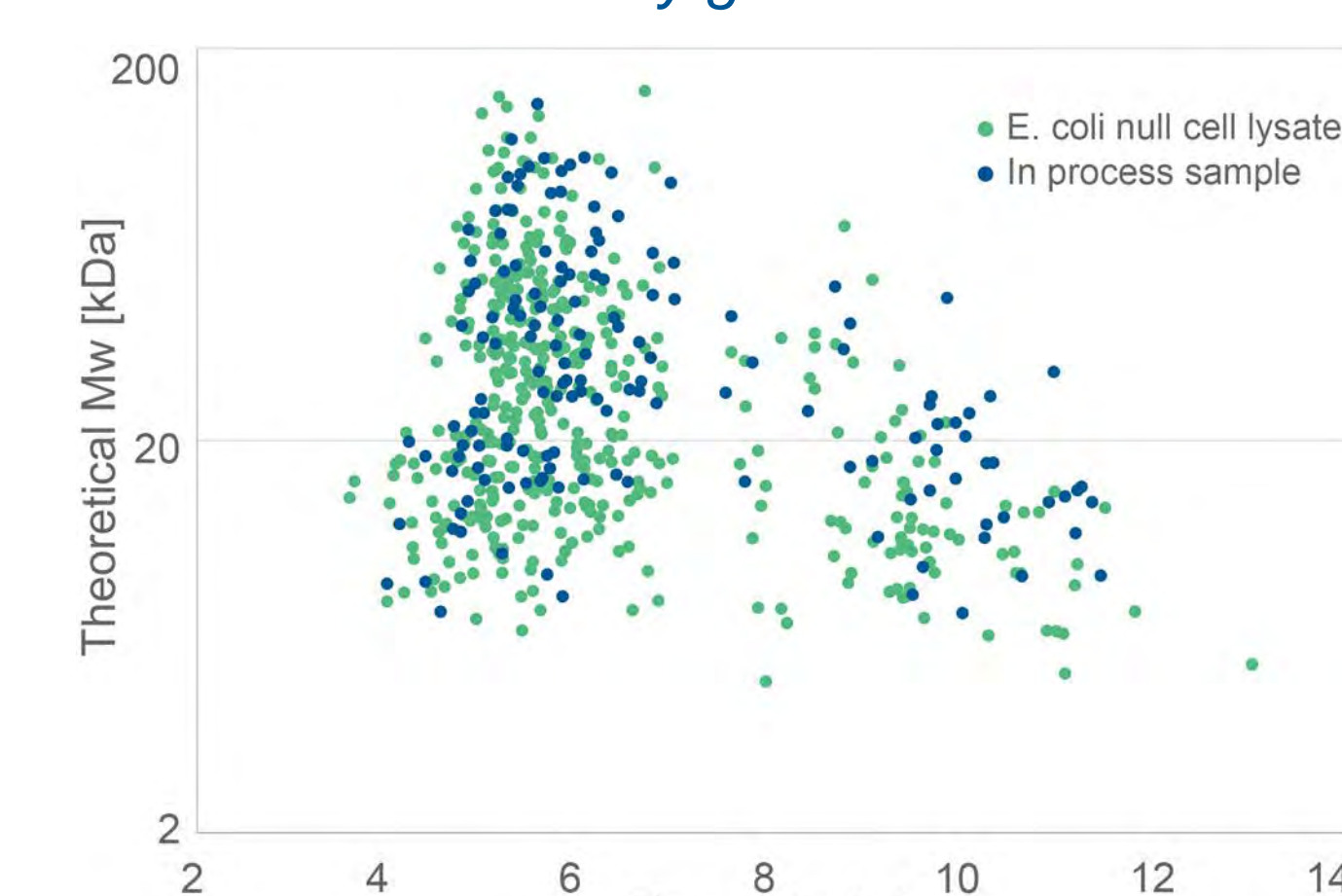
553 HCPs identified

Figure 8: GeLC-MS/MS of the in process sample



152 HCPs identified

Figure 9: Molecular weight and pI of the HCPs identified by geLC-MS/MS



### HCP Characteristics

GeLC-MS/MS provides database entry number, exact protein name, amino acid sequence, theoretical molecular weight and pI for each identified HCP. These are important properties for evaluation of HCP characteristics.

The 30 highest scoring HCPs identified by GeLC-MS/MS from the *E. coli* null cell lysate and the in process sample are listed below (Table 1 and 2). The list includes database accession number, exact protein name, theoretical molecular weight and pI.

The accession numbers can be used to retrieve additional information for HCP characterization and individual risk assessment.

Table 1: Top 30 HCPs identified by GeLC-MS/MS in the *E. coli* null cell lysate

HCP no	Accession no	Protein Name	Mass	pI	Score
1	tr C6E8E1	DNA-directed RNA polymerase, beta subunit	150928	6.67	8620
2	tr C6E8G8	Aldehyde-alcohol dehydrogenase	96380	6.32	8750
3	tr C6E8U3	Pyruvate dehydrogenase E1 component	99978	5.46	8381
4	tr C6E8E2	DNA-directed RNA polymerase, beta subunit	150937	5.15	8614
5	tr C6E8F7	Alpha-1,4-glucan phosphorylase	96865	6.94	8757
6	tr C6E8E1	Protein-Gram-negative type	89309	4.76	7230
7	tr C6E8T4	Alanine-tRNA ligase	96314	5.61	8157
8	tr C6E8T9	Aconitate hydratase B	94009	5.24	7299
9	tr C6E8J2	2-oxoglutarate dehydrogenase, E1 subunit	105566	6.04	7483
10	tr C6E8C9	Valine-tRNA ligase	106336	5.2	7439
11	tr C6E8K5	Glycine dehydrogenase (decarboxylating)	105078	5.62	7529
12	tr C6E8J5	DNA-directed RNA polymerase	99477	6.77	8014
13	tr C6E8S7	Translation initiation factor IF-2	97461	5.8	7557
14	tr C6E8K1	Leucyl-tRNA synthetase	97814	6.11	7467
15	tr C6E8L6	Beta-galactosidase	117321	5.28	7134
16	tr C6E8Z7	Isoleucine-tRNA ligase	105042	5.7	7046
17	tr C6E8I9	Carbamoyl-phosphate synthase (glu-hydrolyzing)	118594	5.23	7912
18	tr C6E8E3	Phosphoenolpyruvate carboxylase	99470	5.52	7161
19	tr C6E8W2	NADH:guanine oxidoreductase	101078	5.89	7984
20	tr C6E8G2	Ribosomal protein S3	25967	10.27	1076
21	tr C6E8Z9	Elongation factor Tu	43457	5.3	6632
22	tr C6E8V6	Maltopilin	40995	4.81	7230
23	tr C6E8G4	Translation elongation factor G	77704	5.24	5771
24	tr C6E8K5	Cytochrome bd ubiquinol oxidase subunit I	58338	6.35	777
25	tr C6E8C0	Glycine-tRNA ligase beta subunit	76936	5.29	779
26	tr C6E8D9	Formyl-tRNA synthetase	85586	5.69	1638
27	tr C6E8F0	DNA polymerase I	103168	5.4	1290
28	tr C6E8E5	Methionine synthase	136639	4.97	254
29	tr C6E8I7	Transcriptional regulator, LacI family	39049	6.39	4449
30	tr C6E8F6	Phosphate acetyltransferase	77466	5.28	2333

Table 2: Top 30 HCPs identified by GeLC-MS/MS in the in process sample

HCP no	Accession no	Protein Name	Mass	pI	Score
1	tr C6E8I7	Chaperone protein DnaK	69130	4.83	6120
2	tr C6E8Q1	Uroporphyrin III C/tetrapyrrole methyltransferase	31500	5.83	3484
3	tr C6E8N7	Ferric uptake regulator, Fur family	17012	5.68	3455
4	tr C6E8E8	Transcriptional regulator, LysR family	33266	6.05	3404
5	tr C6E8D0	Glycyl-tRNA synthetase	85686	6.97	3034
6	tr C6E8H3	Peptide deformylase	19430	5.23	2765
7	tr C6E8E9	Bifunctional protein PutA	144393	5.55	2696
8	tr C6E8G0	Elongation factor Tu	43457	5.3	2522
9	tr C6E8Z7	GTP cyclohydrolase 1	24929	6.79	2389
10	tr C6E8A4	Transcriptional regulator, LacI family	39049	6.39	2389
11	sp P10145	Pseudouridine synthase	25963	5.75	2370
12	tr C6E8F2	ATP synthase F1, alpha subunit	55416	5.8	2005
13	tr C6E8C1	Ribosomal protein L3	25967	10.27	1918
14	tr C6E8C0	Methionine S-sulfide reductase	15783	5.58	1881
15	tr C6E8E6	Ribose-phosphate pyrophosphokinase	36854	5.48	1842
16	tr C6E8G4	SOS ribosomal protein L3	22230	9.91	1701
17	tr C6E8I7	Translation elongation factor G	77704	5.24	1555
18	sp P62577	Transcriptional regulator, LacI family	39049	6.39	1407
19	tr C6E8A4	Chloramphenicol acetyltransferase	25931	10.01	1371
20	tr C6E8E3	SOS ribosomal protein L10	17757	9.04	1367
21	tr C6E8C3	Histidine biosynthesis bifunctional protein HisB	40591	5.76	1144
22	tr C6E8W2	Ribosomal protein S7	17593	10.3	1082
23	tr C6E8E3	NADH:quinone oxidoreductase	101078	5.89	1059
24	tr C6E8W3	Uncharacterized protein GN-ECB_3349	25539	4.96	1040
25	tr C6E8L9	Polyribonucleotide nucleotidyltransferase	77110	5.11	1038
26	tr C6E8I4	Trigger factor	48163	4.83	1006
27	tr C6E8I7	Tyrosine recombinase XerD	34225	8.74	998
28	tr C6E8A4	ATP synthase F1, beta subunit	53531	4.9	997
29	tr C6E8B5	Acyl-ACP-UDP-N-acetylglucosamine O-acetyltransferase	28348	6.63	982
30	tr C6E8U9	Peptidyl-prolyl cis trans isomerase	21182	4.86	967