

HCP ANALYSIS for

PROCESS DEVELOPMENT

BATCH PURITY

ELISA COVERAGE

Identity and quantity of
each Host Cell Protein

Total HCP content in ng/mg



LC-MS PROVIDES THE MISSING DETAILS

HCP ELISAs do not provide any details about the individual HCPs in your samples - and product specific ELISAs take a long time to set up.

HCP analysis by LC-MS (Mass Spectrometry) gives you a list of individual HCPs and their quantity. The method is applicable for all process steps from harvest sample to final drug substance.

Our generic workflow works on any expression system with a sequenced genome; CHO, Yeast, Adenovirus, *E.coli*. It is very advantageous for small proteins, where ELISA may underestimate low MW HCPs. For purified mAbs, the detection limit is < 1 ppm.

BENEFITS COMPARED TO ELISA

- ✓ **FASTER** - No need for costly immunization. Can be applied directly to your samples.
- ✓ **ROBUST** - Reproducible data is obtained by microflow SWATH® LC-MS.
- ✓ **DETAILED** - You get a list of individual HCPs with their pI, MW, and quantity in ppm, - and of course total HCP content in ng/mg drug substance.

Process Development

When production processes change, the assay can quickly be adjusted. You get precise information about clearance of each HCP for each process step.

Batch Purity

With LC-MS it is easy to compare HCP profiles of clinical batches and monitor individual HCPs of concern. Also for comparison of Biosimilar HCP profile with originator.

ELISA Evaluation

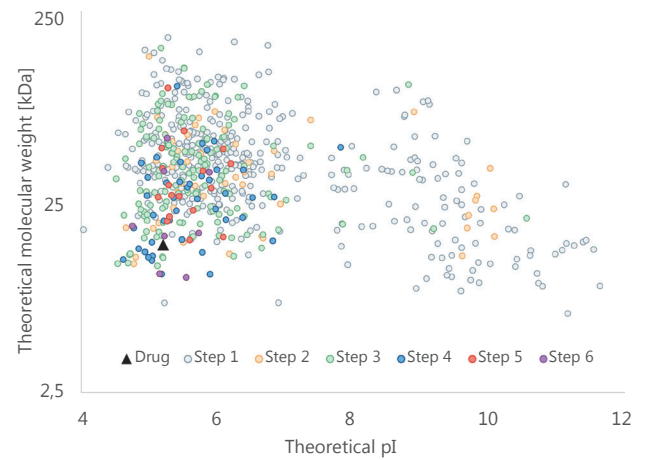
Get the coverage of ELISA for individual HCPs in your process and drug substance. Evaluation by immunoaffinity & LC-MS, alternative to 2D PAGE - Western blot.

HCPs in process steps quantified by SWATH® LC-MS

Host Cell Protein	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Mass	pI	Protein name
sp P0C058 IBPB_ECOLI	4,274	2,905	2,154	186	229	111	16,093	5,2	Small heat shock protein IbpB
sp P0A9A9 FUR_ECOLI	158	284	296	142	147	94	16,795	5,7	Ferric uptake regulation protein
sp P0ABK5 CYSK_ECOLI	597	913	711	618	200	68	34,490	5,8	Cysteine synthase A
sp P69783 PTGA_ECOLI	33	250	378	256	185	62	18,251	4,7	PTS system glucose-specific EIIA component
sp P0A8J4 YBED_ECOLI	432	215	253	222	112	21	9,827	5,5	UPF0250 protein YbeD
sp P02930 TOLC_ECOLI	41	283	187	417	57	11	53,741	5,2	Outer membrane protein TolC
sp P62623 ISPH_ECOLI	312	1,146	855	231	62	15	34,775	5,2	4-hydroxy-3-methylbut-2-enyl diphosphate reductase
sp P0ADP9 YIHD_ECOLI	33	28	32	11	10	18	10,273	5,1	Protein YihD
sp P0A763 INDK_ECOLI	106	240	100	349	113		15,463	5,6	Nucleoside diphosphate kinase
sp P35340 AHPF_ECOLI	67	291	171	174	48		56,177	5,5	Alkyl hydroperoxide reductase subunit F
sp P08200 IDH_ECOLI	390	271	166	355	42		45,757	5,2	Isocitrate dehydrogenase
sp P69797 PTNAB_ECOLI	284	339	240	25	26		35,048	5,7	PTS system mannose-specific EIIB component
sp P0A717 RIBA_ECOLI	345	297	106	48	45		21,836	5,6	GTP cyclohydrolase-2
sp P0AEN1 FRE_ECOLI	741	870	849	1,404	33		26,242	5,3	NAD(P)H-flavin reductase
sp P36683 JACNB_ECOLI	129	87	82	9	8		93,498	5,2	Aconitate hydratase B
sp P0ADE8 YGFZ_ECOLI	18	113	45	150	26		36,094	5,2	tRNA-modifying protein YgfZ
sp P0AB91 AROG_ECOLI	56	231	188	42	22		38,010	6,1	Phospho-2-dehydro-3-deoxyheptonate aldolase
sp P0A825 GLYA_ECOLI	128	332	204	263	13		45,317	6,0	Serine hydroxymethyltransferase
sp P0A6K3 DEF_ECOLI	129	136	55	61	26		19,328	5,2	Peptide deformylase
sp P0ABP8 DEOD_ECOLI	33	113	78	19	20		25,950	5,4	Purine nucleoside phosphorylase DeoD-type
Number of HCPs									
	562	245	206	67	25	8			
Total HCP ppm (w/w)	193.169	48.548	33.391	9.599	1.493	401			
HCP % (w/w)	19,32%	4,85%	3,34%	0,96%	0,15%	0,04%			

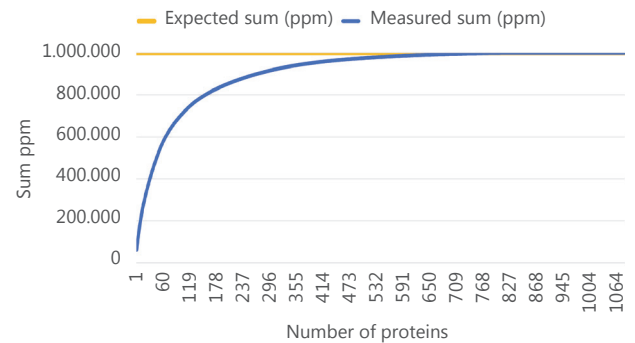
Virtual 2D gel of HCPs

in each process step



1000 Proteins quantified by SWATH® LC-MS

in *E. coli* mock lysate



Examples of results

SWATH® LC-MS provides a list of proteins and quantities in each process step for HCP clearance analysis in virtual 2D gel.

Label-free quantification of 1.000 proteins in mock cell lysates.

WHAT CUSTOMERS SAY:

“Targovax is an immuno-oncology company developing highly targeted immunotherapies for cancer patients. We used the Alphalyse SWATH® LC-MS analysis as a complementary characterization method to ELISA. The aim was to gain knowledge about identity and quantity of the HCPs in our adenovirus product.

Alphalyse handled the project professionally and rapidly, and we received a final report that was very well written, clearly explaining the findings.”

Targovax ASA, Finland
Kristiina Hyvärinen - Director, QC

“Alphalyse provided additional information to the total content of HCPs obtained from our process-specific ELISA. The analysis provided identity and quantity of individual HCPs, allowing us to identify differences between batches.

The method is also applicable for process development – giving detailed knowledge about amounts of individual HCPs and how these were eliminated during our downstream purification. A knowledge not obtained from any ELISA assay.”

SSI, Vaccine Development, Denmark
Max Kristiansen – Assay Development

WHY WORK WITH US ?



- 15 years of experience helping biotech companies in Europe and USA.
- Analysis adjusted to your requirements.
- High level of expertise.



CONTACT US

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