APPLICATION NOTE

Streamline the CHO HCP impurity quantification workflow with new high-throughput, no-wash HTRF™ and AlphaLISA™ CHO Host Cell Protein Kits

Authors

Sonia Brun
Stéphane Martinez
Alexis Aquilina
Revvity, Inc., Codolet, France

Summary

Host cell proteins (HCPs) are process-related protein impurities found in drug products derived from host organisms (bacterial, yeast, or mammalian production cell lines) during biotherapeutics manufacturing and purification. Among protein expression cell lines, the most commonly used mammalian hosts for the industrial production of recombinant protein therapeutics are Chinese Hamster Ovary (CHO) cells. During the expression of a recombinant protein drug, CHO cells can express many endogenous proteins, called HCPs. Despite downstream processing of biopharmaceuticals to remove most of these HCP contaminants, there are concerns about the presence of residual HCPs in the final product that could induce potentially adverse clinical effects and decrease drug product potency and stability. Hence the ability to detect and quantify HCP impurities is critical for biopharmaceutical companies, in conformity with regulatory agency guidelines. Revvity HTRF and AlphaLISA CHO HCP kits are designed to quantitatively measure CHO HCP contaminants in routine bioprocess operations using CHO expression systems, from the very crude harvest material to the final product, in a highly sensitive, quantitative, reproducible, and user-friendly mode (Table 1).

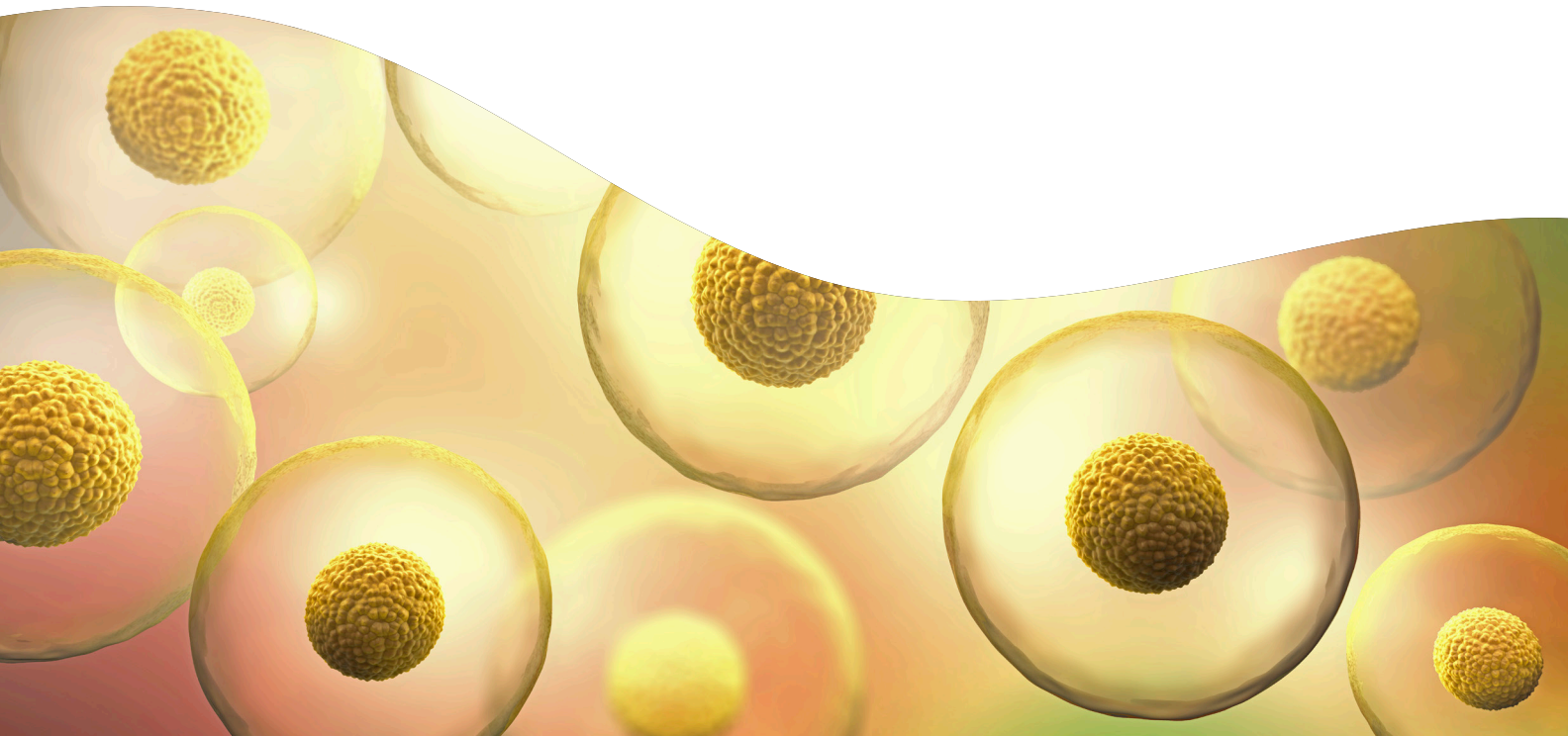

Table 1: Specifications of HTRF and AlphaLISA CHO HCP detection kits.

	HTRF - 64CHOPEG/H	AlphaLISA - AL3176HV/C/F
Format	Homogeneous assay	Homogeneous assay
LOD (ng/mL)	0.8 ng/mL*	0.5 ng/mL**
LOQ (ng/mL)	3.6 ng/mL***	1.8 ng/mL****
Standard range (ng/mL)	6.25-400 ng/mL	0.03-10,000 ng/mL
Quantitative range (ng/mL)	3.6-400 ng/mL	1.8-3,000 ng/mL
Time to results	O/N	3h30
Precision	< 20% intra- and inter assay variability	
Antibody coverage (2D-DIBE)	> 90 % for CHO-S, CHO-K1 and CHO-DG44 supernatant samples	

**The LOD (Limit of Detection) is calculated by interpolating the average background counts + 2 x standard deviation (SD) value on the standard curve*

*** The LOD is calculated by interpolating the average background counts + 3 x SD value on the standard curve*

**** The LOQ (Limit of Quantification) is calculated by interpolating a S/N (signal / noise) ratio of 1.2 on the standard curve*

***** The LOQ is calculated by interpolating the average background counts + 10 x SD value on the standard curve*

HTRF CHO Host Cell Protein Detection Kit

In the HTRF (Homogeneous Time-Resolved Fluorescence) assay, CHO HCPs are detected in a sandwich assay format using a qualified anti-CHO HCP polyclonal antibodies pool, labeled with Europium Cryptate (donor) and d2 (acceptor) (Figure 1). When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the CHO HCP concentration.

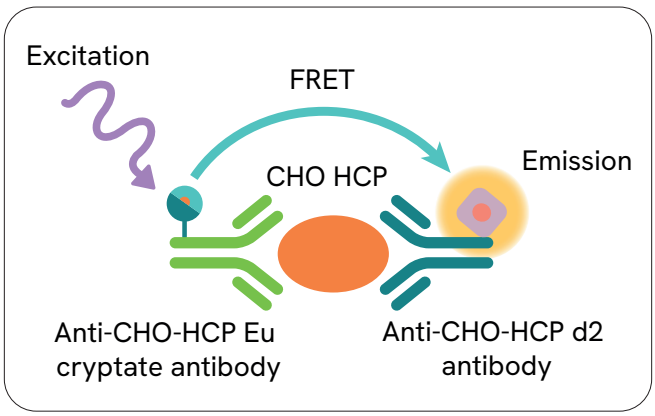

Figure 1: Principle of HTRF CHO HCP detection kit.

AlphaLISA CHO Host Cell Protein Detection Kit

In the AlphaLISA assay, the anti-CHO HCP polyclonal antibodies pool is biotinylated on one side to bind the streptavidin coated AlphaLISA Donor beads and conjugated to AlphaLISA Acceptor beads on the other side. In the presence of CHO HCPs, the beads come into proximity. The excitation of the Donor beads provokes a release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λmax at 615 nm (Figure 2).

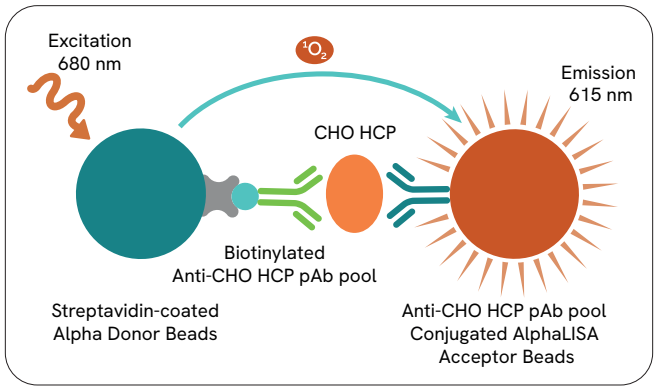

Figure 2: Principle of AlphaLISA CHO HCP detection kit.

Good dilutional linearity and antigen spike recovery

Dilutional linearity and antigen spike recovery experiments are important methods for assessing and validating the performance of immunoassays.

Dilutional linearity

Dilutional linearity experiments are performed to demonstrate that the immunoassay and its optimized diluent can be used to dilute highly concentrated samples down to the standard curve, and still give reliable expected results by multiplying the measured concentration by the dilution factor. This is particularly important when immunoassays are intended to measure complex analytes. Taking into consideration the diverse nature of HCPs, and in some cases the presence of very high concentrations of HCPs beyond the maximum quantitation assay range, dilutional linearity is an important factor to be validated.

Table 2: HTRF CHO HCP detection kit: Dilutional recovery (%) for two samples corresponding to CHO-S and CHO-K1 cell culture supernatants and diluted as indicated with Diluent #5.

HTRF CHO HCP assay - CHO-S HCP sample			
Dilution Factor	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Dilutional recovery**
Neat	426	426	100%
2	213	226	106%
4	106	111	104%
8	53	57	106%
16	27	28	107%
32	13	13	98%
64	7	7	101%
128	3	4	106%
Linearity R^2 = 0.9991			

HTRF CHO HCP assay - CHO-K1 HCP sample			
Dilution Factor	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Dilutional recovery**
Neat	344	344	100%
2	172	173	100%
4	86	88	102%
8	43	45	105%
16	22	24	111%
32	11	13	119%
Linearity R^2 = 1.000			

To assess the dilutional linearity of these two Revvity CHO HCP detection kits, two samples corresponding to CHO-S and CHO-K1 cell culture supernatants were prepared with the appropriate assay buffers (Diluent #5 for HTRF, and Hiblock buffer for AlphaLISA), and serial dilutions of samples were made with the assay buffer. Concentrations of CHO HCPs in diluted samples were determined by interpolating concentrations with the standard curve. Excellent dilution linearity was achieved using the two Revvity CHO HCP detection kits, with a global mean % dilution recovery higher than 90%. The determination coefficient of the linear regression between measured and expected concentrations was also evaluated. The results obtained are shown in the tables below (Tables 2 and 3).

Table 3: AlphaLISA CHO HCP detection kit: Dilutional recovery (%) for two samples corresponding to CHO-S and CHO-K1 cell culture supernatants and diluted as indicated with Hiblock buffer.

AlphaLISA CHO HCP assay - CHO-S HCP sample			
Dilution Factor	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Dilutional recovery**
Neat	2525	2525	100%
2	1263	1170	93%
4	631	533	84%
8	316	274	87%
16	158	140	89%
32	79	68	86%
64	39	35	88%
128	20	16	82%
256	10	8	85%
Linearity R^2 = 0.9979			

AlphaLISA CHO HCP assay - CHO-K1 HCP sample			
Dilution Factor	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Dilutional recovery**
Neat	1972	1972	100%
2	986	909	92%
4	493	437	89%
8	247	233	94%
16	123	113	92%
32	62	55	89%
64	31	26	83%
128	15	12	79%
256	8	6	81%
Linearity R^2 = 0.9983			

Antigen spike recovery

An antigen spike recovery test is another metric of immunoassay performance applied to investigate if there are substances in the sample interfering with the measurement, and to determine how the assay can recover added/spiked analytes. This test also checks that identical responses are observed between the standards and the biological samples.

To do so, various levels of CHO HCP standard were independently mixed with three different concentrations of two samples (cell culture supernatant from the biomanufacturing process using two cell lines, CHO-DG44 and CHO-K1). Concentrations of CHO HCPs in samples (= measured concentrations) were determined by interpolating concentrations with the standard curve. The total measured concentrations were compared to the theoretical ones (= expected concentration) and expressed as % Antigen recovery. Excellent antigen spike recovery was achieved with the two Revvity CHO HCP detection assays in all the test conditions (acceptance criteria: 80-120%) (Tables 4 and 5).

Table 4: HTRF CHO HCP detection kit: Antigen recovery (%) for a mixture of CHO HCP standard with samples derived from CHO-DG44 or CHO-K1 cell culture supernatant.

HTRF CHO HCP assay				
[CHO HCP Standard], ng/mL	**[CHO-DG44 HCP Sample], ng/mL**	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Antigen recovery**
13.51	7.04	20.55	21.38	104%
	14.07	27.58	27.92	101%
	23.38	36.89	41.25	112%
45.55	26.04	71.59	69.45	97%
	45.06	90.61	94.08	104%
	88.02	133.57	137.62	103%
95.11	45.06	140.17	150.64	107%
	88.02	183.13	202.40	111%
	161.56	256.67	279.14	109%

HTRF CHO HCP assay				
[CHO HCP Standard], ng/mL	**[CHO-K1 HCP Sample], ng/mL**	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Antigen recovery**
95.22	158.10	253.32	276.41	109%
	80.82	176.04	180.68	103%
	42.16	137.38	137.75	100%
47.35	80.82	128.17	131.37	102%
	42.16	89.51	89.17	100%
	26.28	73.63	66.71	91%
14.47	21.39	35.86	37.97	106%
	12.87	27.34	26.47	97%
	7.91	22.38	21.20	95%

Table 5: AlphaLISA CHO HCP detection kit: Antigen recovery (%) for a mixture of CHO HCP standard with samples derived from CHO-DG44 or CHO-K1 cell culture supernatant.

AlphaLISA CHO HCP assay				
[CHO HCP Standard], ng/mL	[CHO-DG44 HCP Sample], ng/mL	Expected [CHO HCP], ng/mL	Measured [CHO HCP], ng/mL	% Antigen recovery
15.61	10.70	26.31	29.98	114%
	21.21	36.82	40.36	110%
	42.16	57.77	49.47	86%
185.03	95.07	280.10	257.21	92%
	185.04	370.07	362.39	98%
	322.31	507.34	524.89	103%
337.13	151.26	488.39	421.14	86%
	310.06	647.19	560.02	87%
	476.30	813.43	851.36	105%

AlphaLISA CHO HCP assay				
[CHO HCP Standard], ng/mL	**[CHO-K1 HCP Sample], ng/mL**	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Antigen recovery**
18.74	7.80	26.54	27.92	105%
	13.19	31.93	31.05	97%
	25.22	43.96	45.73	104%
159.92	50.53	210.45	229.13	109%
	97.69	257.61	299.84	116%
	197.06	356.98	401.47	112%
443.99	144.24	588.23	671.72	114%
	285.95	729.94	798.10	109%
	462.45	906.44	841.07	93%
890.17	285.95	1176.13	962.25	82%
	462.45	1352.62	1071.21	79%
	1050.21	1940.38	1427.27	74%

Precision

As shown in Tables 6-9, the two Revvity CHO HCP detection kits are robust assays with reproducible results (%CV intra and %CV inter on concentrations < 15%).

Intra-assay precision (reproducibility)

The intra-assay precision of the two kits was determined for each sample using a total of at least 22 replicates. Each replicate was interpolated in concentration on the standard curve and the variability data are shown in percentage of the coefficient of variation (CV%) (Tables 6 and 7).

Table 6: Intra-assay precision of HTRF CHO HCP detection kit.

HTRF CHO HCP assay			
Sample Number	[CHO HCP], ng/mL	CV% intra	n =
1	10	6%	24
2	29	3%	24
3	97	3%	24
4	306	4%	24

Table 7: Intra-assay precision of AlphaLISA CHO HCP detection kit.

AlphaLISA CHO HCP assay			
Sample Number	[CHO HCP], ng/mL	CV% intra	n =
1	10	3%	27
2	31	2%	27
3	98	3%	27
4	282	2%	26
5	911	4%	27
6	2739	14%	22

Inter-assay precision (repeatability)

The inter-assay precision of the two kits was determined for each sample using a total of at least 3 independent experiments with at least 3 replicates per experiment. Each replicate was interpolated in concentration on the standard curve and variability data are shown in CV% (Tables 8 and 9).

Table 8: Inter-assay precision of HTRF CHO HCP detection kit.

HTRF CHO HCP assay			
Sample Number	[CHO HCP], ng/mL	CV% inter	n =
1	10	9%	4
2	29	5%	4
3	97	4%	4
4	292	5%	4

Table 9: Inter-assay precision of AlphaLISA CHO HCP detection kit.

AlphaLISA CHO HCP assay			
Sample Number	[CHO HCP], ng/mL	CV% inter	n =
1	10	5%	4
2	31	4%	4
3	105	5%	4
4	306	6%	4
5	1019	8%	4
6	3733	12%	3

Compatibility with buffers commonly used in biotherapeutics manufacturing

Some components used in biotherapeutics manufacturing buffers (low pH, high salt concentration, excipients…) may slightly interfere with the immunoassay. So, when first used, we recommend you evaluate the potential matrix effect of each buffer tested. This could be determined by comparing the standard curve in the assay buffer and the investigated manufacturing buffer. Concentrations of the standard curve prepared in the investigated buffer were compared with the expected ones (= concentration of the standard curve in the assay buffer) and expressed as % Antigen recovery. We consider that antigen recovery should be between 80% and 120% to be acceptable.

Case 1: Matrix effect is highlighted, and sample contains high HCPs concentration

If a matrix effect is highlighted and the sample contains a high HCPs concentration, we advise you to dilute it in the assay buffer (Diluent #5 for HTRF and Hiblock buffer for AlphaLISA) to reduce possible matrix effects. The optimal dilution factor should be determined by preparing a CHO HCP sample spiked in the buffer of interest and performing a series of dilutions of the sample in the assay buffer. Concentrations of CHO HCPs in these samples (= measured concentrations) could be determined by interpolating concentrations with the standard curve (CHO HCP standard diluted in the assay buffer). Measured concentrations can be compared to the expected ones (= concentration of CHO HCP sample spiked in the assay buffer) and expressed as % Antigen recovery. We consider that the dilution factor is optimal when antigen recovery is between 80% and 120%. The Tables 10 and 11 below show examples of data for some commonly used biotherapeutics manufacturing buffers assessed with HTRF and AlphaLISA CHO HCP detection kits. For each manufacturing buffer investigated, the dilution factor selected is highlighted.

Table 10: HTRF CHO HCP detection kit: Antigen recovery (%) for four CHO HCP samples spiked in some buffers commonly used in biotherapeutics manufacturing (25 mM Phosphate Buffer, pH 7.5; 25 mM Citrate Buffer, pH 3.5; 25 mM Histidine, 100 mM Trehalose, pH 6; 25 mM Histidine, 0.1% PS80, pH 6), and serially diluted with Diluent #5 as indicated.

HTRF CHO HCP assay	Dilution Factor	Expected [CHO HCP], ng/mL	Measured [CHO HCP], ng/mL	% Antigen recovery
25 mM Phosphate Buffer, pH 7.5	1	319	178	56%
	2	155	122	79%
	10	29	25	86%
25 mM Citrate Buffer, pH 3.5	1	319	17	5%
	2	155	76	49%
	4	76	61	79%
	10	29	26	92%
25 mM Histidine, 100 mM Trehalose, pH 6	1	319	153	48%
	2	155	123	79%
	10	29	31	109%
25 mM Histidine, 0.1% PS80, pH 6	1	319	255	80%
	2	155	166	107%

Table 11: AlphaLISA CHO HCP detection kit: Antigen recovery (%) for four CHO HCP samples spiked with some buffers commonly used in biotherapeutics manufacturing (25 mM Phosphate Buffer, pH 7.5; 25 mM Citrate Buffer, pH 3.5; 25 mM Histidine, 100 mM Trehalose, pH 6; 25 mM Histidine, 0.1% PS80, pH 6), and serially diluted with Hiblock buffer as indicated.

AlphaLISA CHO HCP assay	Dilution Factor	Expected [CHO HCP], ng/mL	Measured [CHO HCP], ng/mL	% Antigen recovery
25 mM Phosphate Buffer, pH 7.5	Neat	707	179	25%
	2	370	165	45%
	5	178	125	70%
	20	55	47	85%
25 mM Citrate Buffer, pH 3.5	Neat	707	79	11%
	2	370	146	39%
	5	178	125	70%
	20	55	49	90%
25 mM Histidine, 100 mM Trehalose, pH 6	Neat	707	436	62%
	2	370	456	123%
	10	98	81	83%
25 mM Histidine, 0.1% PS80, pH 6	Neat	707	291	41%
	2	370	224	60%
	10	98	116	118%

After screening the optimal dilution factor for the biotherapeutics manufacturing buffer of interest, it is recommended to further validate this dilution factor by spiking various CHO HCP concentrations in the buffer under investigation, then diluting them by the determined dilution factor with the assay buffer (Diluent #5 for HTRF and Hiblock buffer for AlphaLISA). Then calculate the antigen recovery (acceptance criteria: 80-120%). The Tables 12 and 13 below show examples of data for some commonly used biotherapeutics manufacturing buffers assessed with HTRF and AlphaLISA CHO HCP detection kits.

Table 12: HTRF CHO HCP detection kit: Antigen recovery (%) of two CHO HCP concentrations spiked with seven buffers commonly used in biotherapeutics manufacturing, then diluted with Diluent #5.

HTRF CHO HCP assay				
Sample buffer	Dilution Factor	Expected [CHO HCP], ng/mL	Measured [CHO HCP], ng/mL	% Antigen recovery
25 mM Phosphate Buffer, pH 7.5	10	155	130	84%
		307	281	91%
25 mM Citrate Buffer, pH 3.5	10	155	136	88%
		307	282	92%
25 mM Citrate Buffer, 320 mM NaCl, pH 5	20	151	120	79%
		308	251	82%
25 mM Histidine, 100 mM Trehalose, pH 6	10	155	129	83%
		307	286	93%
25 mM Histidine, 0.1% PS80, pH 6	2	158	181	114%
		324	348	107%
25 mM Histidine, 50 mM L-Methionine, pH 6	2	158	164	103%
		324	356	110%
25 mM Histidine, 200 mM Arginine, pH 6	20	151	120	80%
		308	266	86%

Table 13: AlphaLISA CHO HCP detection kit: Antigen recovery (%) of two CHO HCP concentrations spiked with seven buffers commonly used in biotherapeutics manufacturing, then diluted with Hiblock buffer.

AlphaLISA CHO HCP assay				
Sample buffer	**Dilution Factor**	**Expected [CHO HCP], ng/mL**	**Measured [CHO HCP], ng/mL**	**% Antigen recovery**
25 mM Phosphate Buffer, pH 7.5	20	340	277	81%
		189	200	106%
		83	97	118%
25 mM Citrate Buffer, pH 3.5	20	340	312	92%
		189	183	97%
		83	73	88%
25 mM Citrate Buffer, 320 mM NaCl, pH 5	30	324	292	90%
		156	144	92%
		85	80	94%
25 mM Histidine, 100 mM Trehalose, pH 6	10	624	597	96%
		239	253	106%
		150	124	83%
25 mM Histidine, 0.1% PS80, pH 6	10	624	509	82%
		239	259	109%
		150	108	72%
25 mM Histidine, 50 mM L-Methionine, pH 6	10	624	544	87%
		239	266	112%
		150	134	90%
25 mM Histidine, 200 mM Arginine, pH 6	30	324	290	89%
		156	144	92%
		85	80	95%

Case 2: Matrix effect is highlighted, and the sample cannot be diluted due to HCPs concentration close to the assay's lowest limit of detection

If a matrix effect is found and the sample cannot be diluted as the HCPs content is close to the assay's limit of detection, we recommend running the standard curve in the same buffer as that used in the sample.

Lack of cross-reactivity between CHO HCP detection and drug substance presence

The two Revvity kits were checked for their putative cross-reactivity with some drug substances. Two monoclonal antibodies used in clinics (Cetuximab and Trastuzumab) were spiked in CHO HCP samples with different HCPs concentrations. As reported in Tables 14 and 15, no significant impact on antigen recovery (≤ 15%) was observed with the two assays.

Table 14: HTRF CHO HCP detection kit: Antigen recovery (%) for CHO HCP samples in presence of two monoclonal antibodies (Cetuximab and Trastuzumab) at 2.5 mg/mL.

HTRF CHO HCP assay	% Antigen recovery	
[CHO HCP], ng/mL	2.5 mg/mL Cetuximab	2.5 mg/mL Trastuzumab
28	90%	86%
100	85%	85%
295	92%	85%

Table 15: AlphaLISA CHO HCP detection kit: Antigen recovery (%) for CHO HCP samples in presence of two monoclonal antibodies (Cetuximab and Trastuzumab) at 2.5 mg/mL.

AlphaLISA CHO HCP assay	% Antigen recovery	
[CHO HCP], ng/mL	2.5 mg/mL Cetuximab	2.5 mg/mL Trastuzumab
11	103%	
20		89%
42	106%	
78		102%
150	102%	
267		109%
568	103%	
931		115%

Antibody coverage analysis

An important analysis to validate HCPs detection is the determination of antibody coverage. This provides a means to estimate the percentage of total HCPs that can be detected by the assay.

Whereas regulatory authorities do not provide guidance concerning a minimum coverage percentage, it is beneficial to demonstrate that the polyclonal antibodies pool used in the assay displays the broadest coverage percentage. For a generic kit, 60% coverage is considered to be sufficient, even though regulatory guidelines recommend obtaining the highest coverage percentage for your manufacturing workflow.

We performed a coverage analysis by 2D-DIBE (2D Differential In Blot Electrophoresis) on cell supernatants from three CHO cell lines commonly used in biotherapeutics manufacturing (CHO-S, CHO-K1, and CHO-DG44) with the Revvity anti-CHO HCP antibodies pool. Supernatant proteins from the three cell lines were independently labeled with Cy3. The labeled protein samples were then separated based on their isoelectric point and by their molecular weight, using the 2-dimensional SDS-PAGE technique. 2D gels were then transferred to a Western Blot membrane and incubated with the Revvity anti-CHO HCP antibodies pool. The detection of bound antibodies was carried out using Cy5-labeled secondary antibodies raised against the host species of the primary antibody pool. The membranes were scanned on an imager, and an image was acquired for each Cy3 and Cy5 channels. Spot detection and antibody coverage were assessed using a dedicated software. As shown in Figure 3 and Table 16, the Revvity anti-CHO HCP antibodies pool has an excellent coverage of CHO HCPs on the three CHO cell lines tested (CHO-S, CHO-K1, and CHO- DG44).

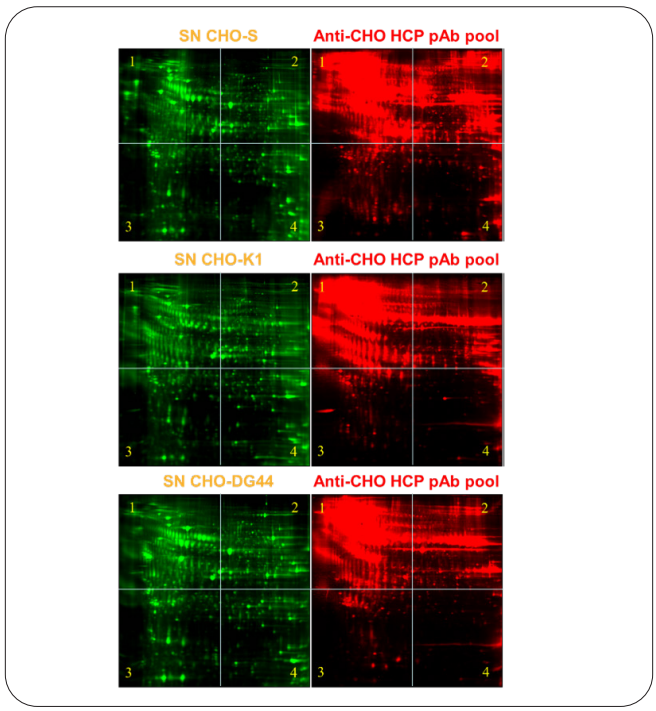

Figure 3: Coverage of CHO HCPs of three supernatant samples derived from three CHO cell lines commonly used in biotherapeutics manufacturing (CHO-S, CHO-K1, and CHO-DG44) with the Revvity anti-HCP antibodies pool.

Table 16: % coverage of CHO HCPs of three supernatant samples derived from three CHO cell lines commonly used in biotherapeutics manufacturing (CHO-S, CHO-K1, and CHO-DG44) with the Revvity anti-HCP antibodies pool.

	CHO supernatant		
	CHO-S	CHO-K1	CHO-DG44
% total coverage of CHO HCPs by 2D-DIBE	99.3%	98.5%	98.5%

Conclusion

This application note demonstrates that the ready-to-use HTRF™ and AlphaLISA™ no-wash HCP detection assay kits are designed to quickly and easily detect and quantify CHO HCP impurities during biopharmaceutics manufacturing.

These off-the-shelf kits are designed to deliver a streamlined workflow, a broader dynamic range, and higher sensitivity than traditional multi-step ELISA assays. The assays also display excellent dilutional linearity and antigen spike recovery, with robust reproducibility, and are compatible with the most commonly used biotherapeutics manufacturing buffers and in presence of the drug substance.

The excellent coverage of CHO HCPs demonstrated here makes these innovative HTRF and AlphaLISA assay kits more effective and efficient within existing workflows, and contributes to quality control and successful biotherapeutics.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com