MANUAL

AlphaLISA™ CHO Host Cell Protein Detection Kit

Product number: AL3176

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed for the quantitative determination of Chinese Hamster Ovary cells Host Cell Protein (CHO HCP) using a homogeneous no wash AlphaLISA assay.

Kit contents: The kit contains 5 components: AlphaLISA Acceptor beads coated with anti-CHO HCP polyclonal antibodies (pAb) pool, Streptavidin-coated Donor beads, Biotinylated anti-CHO HCP pAb pool, Lyophilized CHO cell culture supernatant and 10X AlphaLISA Hiblock.

Sensitivity: Lower Detection Limit (LDL): 0.52 ng/mL
Lower Limit of Quantification (LLOQ): 1.80 ng/mL
EC_{50}: 947 ng/mL

Dynamic Range: 0.52 – 3 000 ng/mL

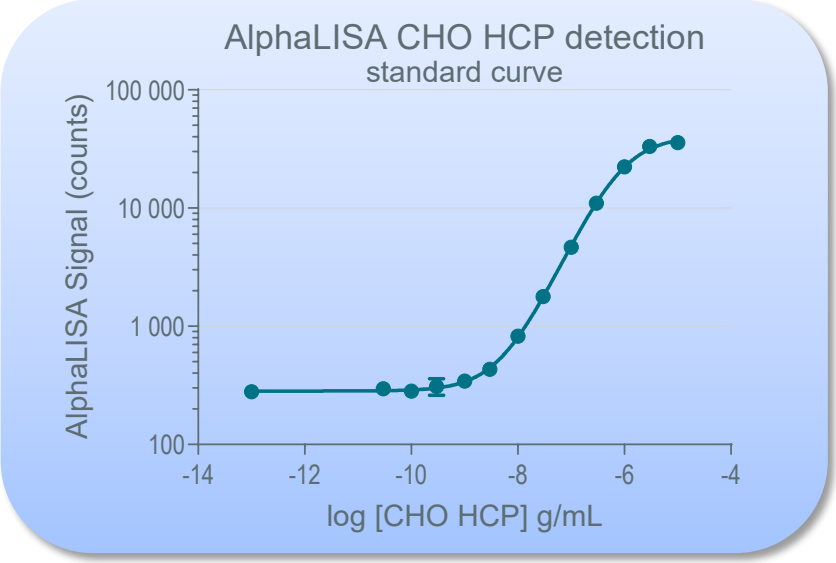


Figure 1. Typical sensitivity curve in AlphaLISA Hiblock Buffer. The data was generated using a grey AlphaPlate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit in the dark at 4 ºC. For reconstituted analyte, aliquot and store at -20 ºC (or below) for up to 3 months. It has been validated that three freeze-thaw cycles don't impact kit performance.

Stability: This kit is stable for at least 18 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

Host cell proteins (HCP) are process-related protein impurities found in drug product derived from host organisms (bacterial, yeast or mammalian production cell lines) during biotherapeutic manufacturing and purification. Among protein expression cell lines, the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics are Chinese hamster ovary (CHO) cells. During expression of a recombinant protein drug, CHO cells can express many endogenous proteins, called HCP. Despite downstream processing of biopharmaceuticals removes the majority of these HCP contaminants, there are concerns about the presence of residual HCP in the final product due to potential adverse clinical effects, decrease in drug product efficacy and stability. Hence, detection and quantification of HCP impurities is critical for biopharmaceutical companies in agreement with regulatory agency guidelines.

AlphaLISA CHO Host Cell Protein Detection Kit is designed to quantitatively measure HCP contamination in drug products expressed in CHO expression systems.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-CHO HCP pAb pool binds to the streptavidin coated AlphaLISA Donor beads, while the anti-CHO HCP pAb pool is conjugated to AlphaLISA Acceptor beads. In the presence of CHO HCP, the beads come into proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λ_{max} at 615 nm (Figure 2).

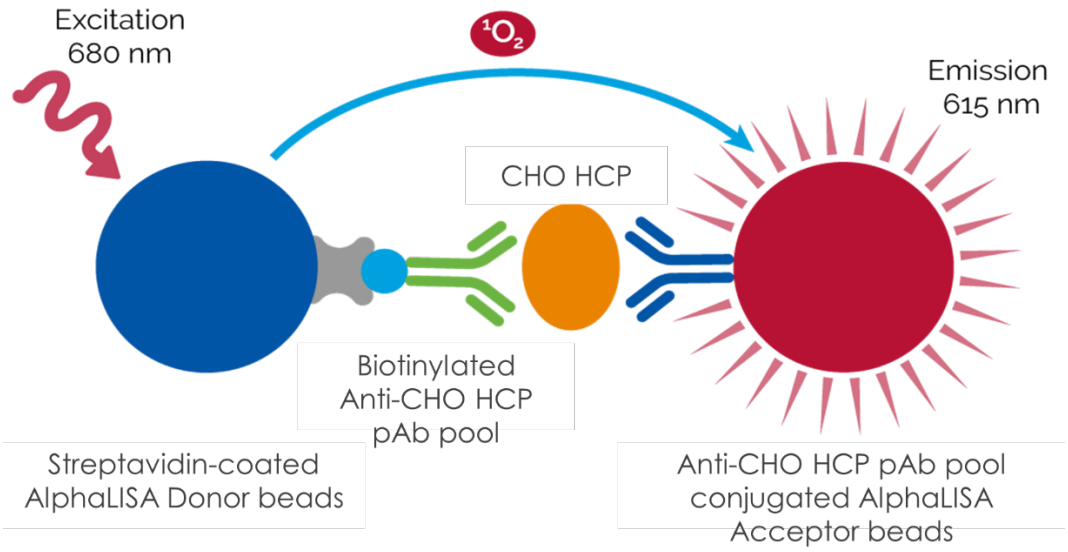


Figure 2. AlphaLISA CHO Host Cell Protein Detection Kit Detection Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated anti-CHO HCP pAb pool contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3176HV 100 assay points***	AL3176C 500 assay points***	AL3176F 5000 assay points***
AlphaLISA Anti-CHO HCP pAb pool Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	100 µL @ 5 mg/mL (1 brown tube, white cap)	1000 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	1000 µL @ 5 mg/mL (2 brown tubes, black cap)
Biotinylated Anti-CHO HCP pAb pool stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	40 µL @ 500 nM (1 tube, black cap)	200 µL @ 500 nM (1 tube, black cap)	1000 µL @ 500 nM (2 tubes, black cap)
Lyophilized – CHO HCP Standard*	10 µg (1 tube, clear cap)	10 µg (1 tube, clear cap)	10 µg (1 tube, clear cap)
AlphaLISA HiBlock Buffer (10X) **	2 mL, 1 small bottle	10 mL, 1 middle bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20 °C for future experiments. The aliquoted analyte at -20 °C (or below) is stable up to 1 month. It has been validated that three freeze-thaw cycles don't impact kit performance. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL3176S).

** Extra buffer can be ordered separately (cat # AL004C: 10 mL, cat # AL004F: 100 mL).

*** The number of assay points is based on an assay volume of 50 µL in 96-well or 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001% final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the biotinylated anti-CHO HCP pAb pool stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.	6050185
EnVision®-Alpha Reader	Revvity Inc.	-

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000*g*, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA HiBlock Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.
- CHO HCP standard (for standard curve) must be prepared in Hiblock Buffer or in the same medium as the samples. Some components used for biotherapeutics manufacturing may yield to slight interference in the assay. So, to reduce possible matrice effects, it is advised to dilute samples containing high HCP concentration in Hiblock Buffer. When testing samples without dilution, it is recommended to compare standard curves in both Hiblock Buffer and sample's buffer. If the sample's buffer impairs standard curve results, we recommend to run it in sample buffer instead of Hiblock Buffer.

Assay Procedure

- The manual described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The manuals also include testing samples in 452 wells. If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.

- The standard dilution manual is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

Format	# of data points	Volume					Plate recommendation
		Final	Sample	AlphaLISA pAb Acceptor Beads	AlphaLISA biotinylated pAb	SA-Donor beads	
AL3176HV	100	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560)
AL3176C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3176F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

The 3-Step manual described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Hiblock Buffer:
Add 5 mL of 10X AlphaLISA Hiblock Buffer to 45 mL Milli-Q® grade H_2O.

2) Preparation of CHO HCP analyte standard dilutions:

a. Reconstitute lyophilized CHO HCP Standard in 100 µL Milli-Q® grade H_2O. The remaining reconstituted analyte should be aliquoted immediately and stored at -20 ºC (or below) for future assays (see page 4 for more details).
b. Prepare standard dilutions as follows in 1X AlphaLISA Hiblock Buffer (change tip between each standard dilution):

Tube	Vol. of CHO HCP (µL)	Vol. of diluent (µL) *	[CHO HCP] in standard curve	
			(g/mL in 5 µL)	(ng/mL in 5 µL)
A	10 µL of reconstituted CHO HCP standard	90	10.00E-06	10 000
B	60 µL of tube A	140	3.00E-06	3 000
C	60 µL of tube B	120	1.00E-06	1 000
D	60 µL of tube C	140	3.00E-07	300
E	60 µL of tube D	120	1.00E-07	100
F	60 µL of tube E	140	3.00E-08	30
G	60 µL of tube F	120	1.00E-08	10
H	60 µL of tube G	140	3.00E-09	3
I	60 µL of tube H	120	1.00E-09	1
J	60 µL of tube I	140	3.00E-10	0.3
K	60 µL of tube J	120	1.00E-10	0.1
L	60 µL of tube K	140	3.00E-11	0.03
M ** (background)	0	50	0	0
N ** (background)	0	50	0	0
O ** (background)	0	50	0	0
P ** (background)	0	50	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Hiblock Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-CHO HCP pAb pool Acceptor beads (100 µg/mL):

a. Prepare just before use
b. Add 100 µL of 5 mg/mL AlphaLISA Anti-CHO HCP pAb pool Acceptor Bead to 4900 µL of 1X AlphaLISA Hiblock Buffer.

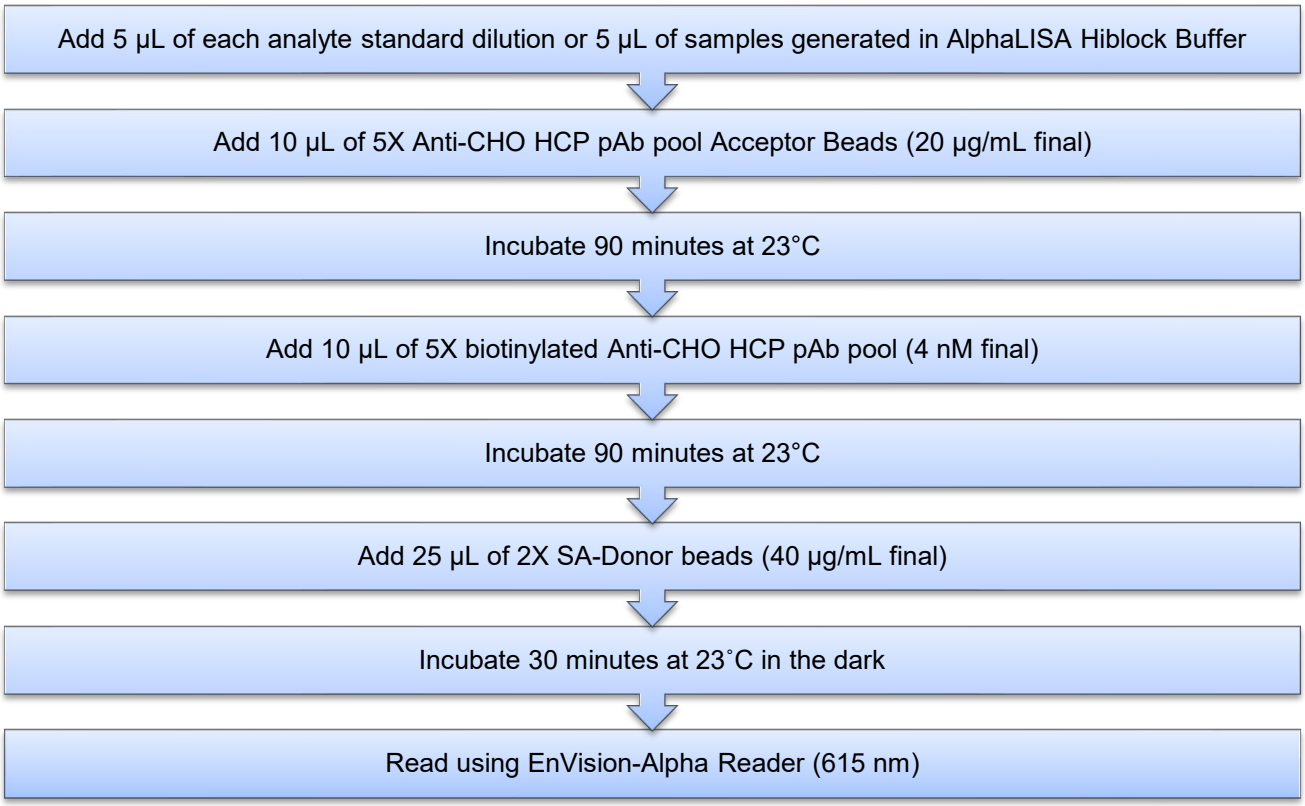
4) Preparation of 5X biotinylated Anti-CHO HCP pAb pool (20 nM)

a. Prepare just before use
b. Add 200 µL of 500 nM biotinylated Anti-CHO HCP pAb pool to 4800 µL of 1X AlphaLISA Hiblock Buffer

5) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):

a. Prepare just before use.
b. Keep the beads under subdued laboratory lighting.
c. Add 200 µL of 5 mg/mL SA-Donor beads to 12300 µL of 1X AlphaLISA Hiblock Buffer.

6) In a Light Gray AlphaPlate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined to be a 3-step manual using AlphaLISA Hiblock buffer as assay buffer. All components, including standard curve, were also prepared in AlphaLISA Hiblock buffer. The assays were performed in 384-well plate format.

- Assay Sensitivity:

The LDL was calculated as described above (average background counts + (3xSD)). The value corresponds to the lowest concentration of analyte that can be detected in a volume of 5 µL sample using the recommended assay conditions.

LDL (ng/mL)	(Analyte diluent)	# of assesments
0.52	AlphaLISA Hiblock buffer	24

- Assay Precision:
    - Intra-assay precision:

The intra-assay precision was determined for each sample using a total of at least 22 replicates. Each replicate is interpolated in concentration on the standard curve and data of variability are shown in CV% on concentration.

Sample #	[CHO HCP], ng/mL	CV intra on concentration
1	10	3%
2	31	2%
3	98	3%
4	282	2%
5	911	4%
6	2739	14%

    - Inter-assay precision:

The inter-assay precision was determined for each sample using a total of at least 3 independent experiments with at least 6 replicates. Each replicate is interpolated in concentration on the standard curve and data of variability are shown in CV% on concentration.

Sample #	[CHO HCP], ng/mL	CV inter on concentration
1	10	5%
2	31	4%
3	105	5%
4	306	6%
5	1019	8%
6	3733	12%

- Antigen Spike Recovery:

Assay equilibrium between standard and sample was assessed using an antigen spike recovery experiment. Three different levels of CHO HCP standard were independently mixed with three different sample (cell culture supernatant from a biomanufacturing process using the CHO-DG44 cell line) concentrations. All assay components, including standard curve, were diluted in AlphaLISA Hiblock buffer. Concentrations of CHO HCP in samples (= measured concentrations) were determined by interpolating concentrations to the standard curve. Total measured concentrations were compared to the theoretical ones (= expected concentration) and expressed as % antigen recovery. Excellent antigen spike recovery was achieved in all tested conditions (acceptance criteria: 85-115%). The results are shown in the table below.

[CHO HCP Standard], ng/mL	[CHO-DG44 HCP Sample], ng/mL	Expected concentration, ng/mL	Measured concentration, 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%

- Dilution Linearity:

Two samples corresponding to cell culture supernatant from CHO-S and CHO-K1 cell lines were prepared with AlphaLISA Hiblock buffer and serial dilutions of the samples were made with a 2- to 256-fold dilution using AlphaLISA Hiblock buffer. All assay components, including standard curve, were prepared in AlphaLISA Hiblock buffer. Concentrations of CHO HCP in diluted samples were determined by interpolating concentrations to the standard curve. Excellent dilution linearity was achieved in the two samples (2- to 256-fold dilution) with a global mean % dilution recovery closed to 90%. The results are shown in the tables below.

CHO-S HCP sample			
Dilution Factor (x)	**Expected CHO HCP concentration (ng/mL)**	**Measured CHO HCP concentration (ng/mL)**	**% dilution 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			

CHO-K1 HCP sample			
Dilution Factor (x)	**Expected CHO HCP concentration (ng/mL)**	**Measured CHO HCP concentration (ng/mL)**	**% dilution 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			

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: www.revvity.com

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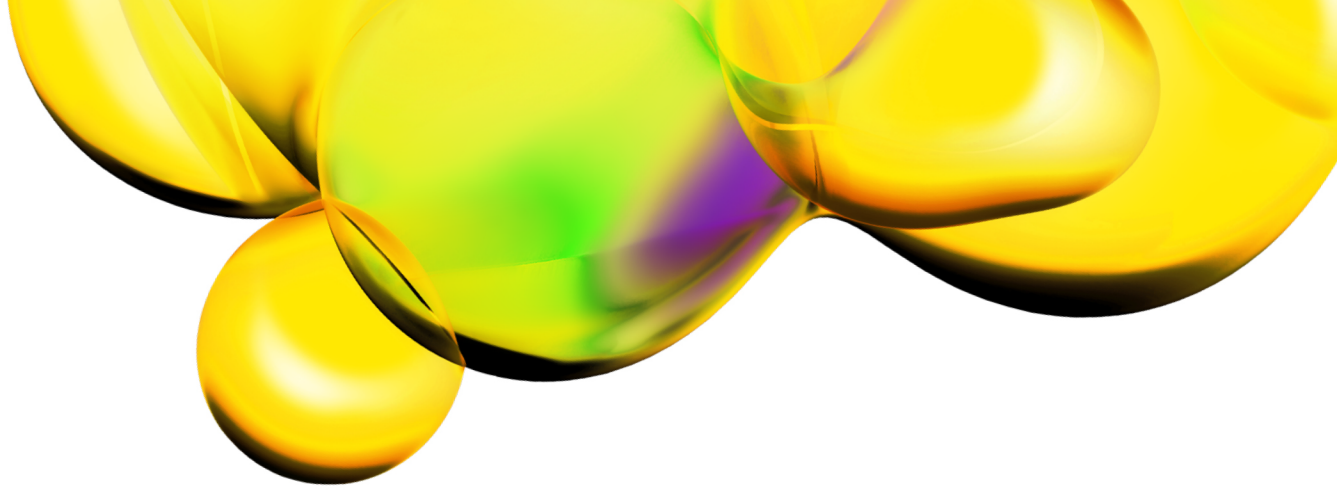

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