



## AlphaLISA<sup>®</sup> BCL-2 (Human) Detection Kit

**Product number:** AL384 HV/C/F

Research Use Only. Not for use in diagnostic procedures.

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### Product Information

- Application:** This kit is designed for the quantitative determination of hBcl-2 in serum, buffered solution and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). This kit is not cross reactive with mouse Bcl-2.
- Sensitivity:** Lower Detection Limit (LDL): 30 pg/mL  
Lower Limit of Quantification (LLOQ): 72 pg/mL  
EC<sub>50</sub>: 20 ng/mL
- Dynamic range:** 30 – 1 000 000 pg/mL (Figure 1).

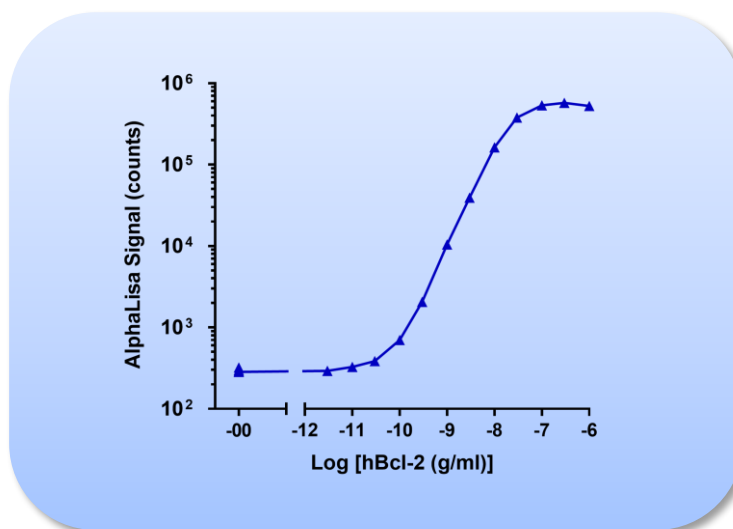


Figure1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplat<sup>™</sup> 384 microplate and the EnVision<sup>®</sup> Multilabel Plate Reader 2103 with Alpha option.

- Storage:** Store kit in the dark at +4°C. Store reconstituted analyte at -20°C. Avoid freeze-thaw cycles.
- Stability:** This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

## Analyte of Interest

Bcl-2 (B-cell lymphoma 2) is an oncogenic protein that inhibits apoptosis. It is a protein with a MW of 24-26 kDa, located within the mitochondrial membrane, endoplasmic reticulum and nuclear envelope. Bcl-2 is considered an important anti-apoptotic protein and is thus classified as a proto-oncogene. Bcl-2 overexpression has been implicated in a wide variety of malignancies including (but not limited to) breast, prostate, skin, colon and pancreatic cancers as well as neurodegenerative and autoimmune diseases. Related anti-apoptotic proteins include Bcl-xL, Mcl-1 and Bcl-w. Pro-apoptotic proteins in the Bcl-2 family include Bax, BAD, Bak, Blk and Bid.

## Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a biotinylated anti-analyte antibody binds to the Streptavidin-coated Alpha Donor beads, while another anti-analyte antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

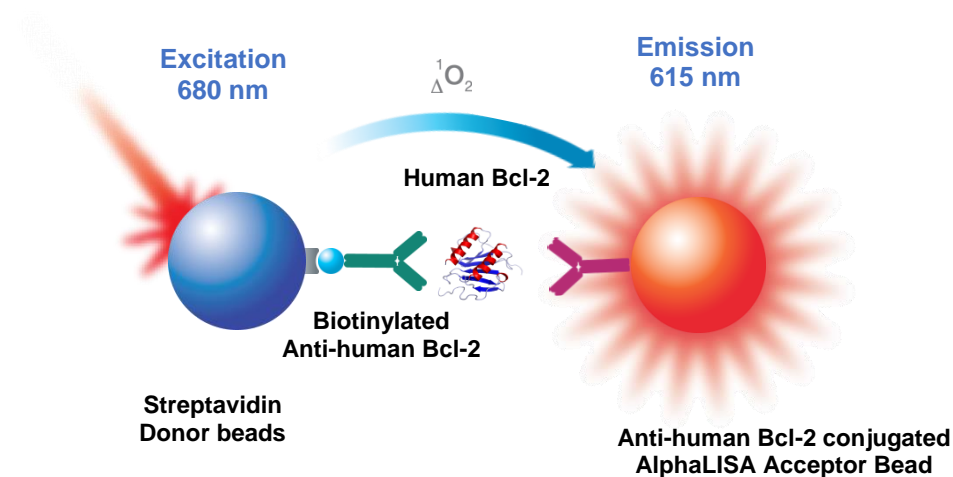


Figure 2. AlphaLISA Assay Principle.

## 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.
- All blood components and biological materials should be handled as potentially hazardous.
- Some analytes are present in blood. Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

## Kit Content: Reagents and Materials

Kit components	AL384HV (100 assay points)***	AL384C (500 assay points)***	AL384F (5000 assay points)***
AlphaLISA Anti-hBcl-2 Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	50 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	500 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	100 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	1 mL @ 5 mg/mL (1 brown tube, <u>black</u> cap)
Biotinylated Anti-hBcl-2 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN <sub>3</sub> , pH 7.4	40 µL @ 500 nM (1 tube, <u>black</u> cap)	100 µL @ 500 nM (1 tube, <u>black</u> cap)	1 mL @ 500 nM (1 tube, <u>black</u> cap)
Human Bcl-2 Analyte lyophilized*	1 µg 1 tube, <u>clear</u> cap	1 µg 1 tube, <u>clear</u> cap	1 µg 1 tube, <u>clear</u> cap
AlphaLISA Immunoassay Buffer (10X)**	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

\* Reconstitute hBcl-2 in 100 µL Milli-Q® grade H<sub>2</sub>O. The reconstituted analyte should be used within 60 minutes, or can be aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid repeated freezing and thawing. One vial contains an amount of hBcl-2 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL384S).

\*\* Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100 and 0.5% Kathon. Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

\*\*\* The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 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.

### 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

- 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 (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H<sub>2</sub>O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay 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 Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus Film.
- The AlphaLISA signal is detected with an EnVision Multilabel 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. The standard curve should be performed in the Immunoassay buffer for serum and/or plasma samples.

## Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- 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 a 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.

		Volume					
Format	# of data points	Final	Sample	AlphaLISA beads	Biotin Antibody	Donor beads	Plate recommendation
AL384HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL384C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) 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)
AL384F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4µL	4µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	2µL	2µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

## The manual for hBcl-2 AlphaLISA Assay:

**3-Step Manual — Dilution of standards in 1X AlphaLISA Immunoassay Buffer or cell culture medium.** The manual described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Immunoassay Buffer:

Add 10 mL of 10X AlphaLISA Immunoassay Buffer to 90 mL H<sub>2</sub>O.

2) Preparation of hBcl-2 analyte standard dilutions:

- Reconstitute lyophilized hBcl-2 (1 µg) in 100 µL H<sub>2</sub>O.
- Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of hBcl-2 (µL)	Vol. of diluent (µL) *	[hBcl-2] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted hBcl-2	90	1.00E-06	1 000 000
B	60 µL of tube A	140	3.00E-07	300 000
C	60 µL of tube B	120	1.00E-07	100 000
D	60 µL of tube C	140	3.00E-08	30 000
E	60 µL of tube D	120	1.00E-08	10 000
F	60 µL of tube E	140	3.00E-09	3 000
G	60 µL of tube F	120	1.00E-09	1 000
H	60 µL of tube G	140	3.00E-10	300
I	60 µL of tube H	120	1.00E-10	100
J	60 µL of tube I	140	3.00E-11	30
K	60 µL of tube J	120	1.00E-11	10
L	60 µL of tube K	140	3.00E-12	3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

\* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay 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-hBcl-2 Acceptor beads (50 µg/mL)

- Add 50 µL of 5 mg/mL AlphaLISA Anti-hBcl-2 Acceptor beads to 4950 µL of 1X AlphaLISA Immunoassay Buffer.
- Prepare just before use.

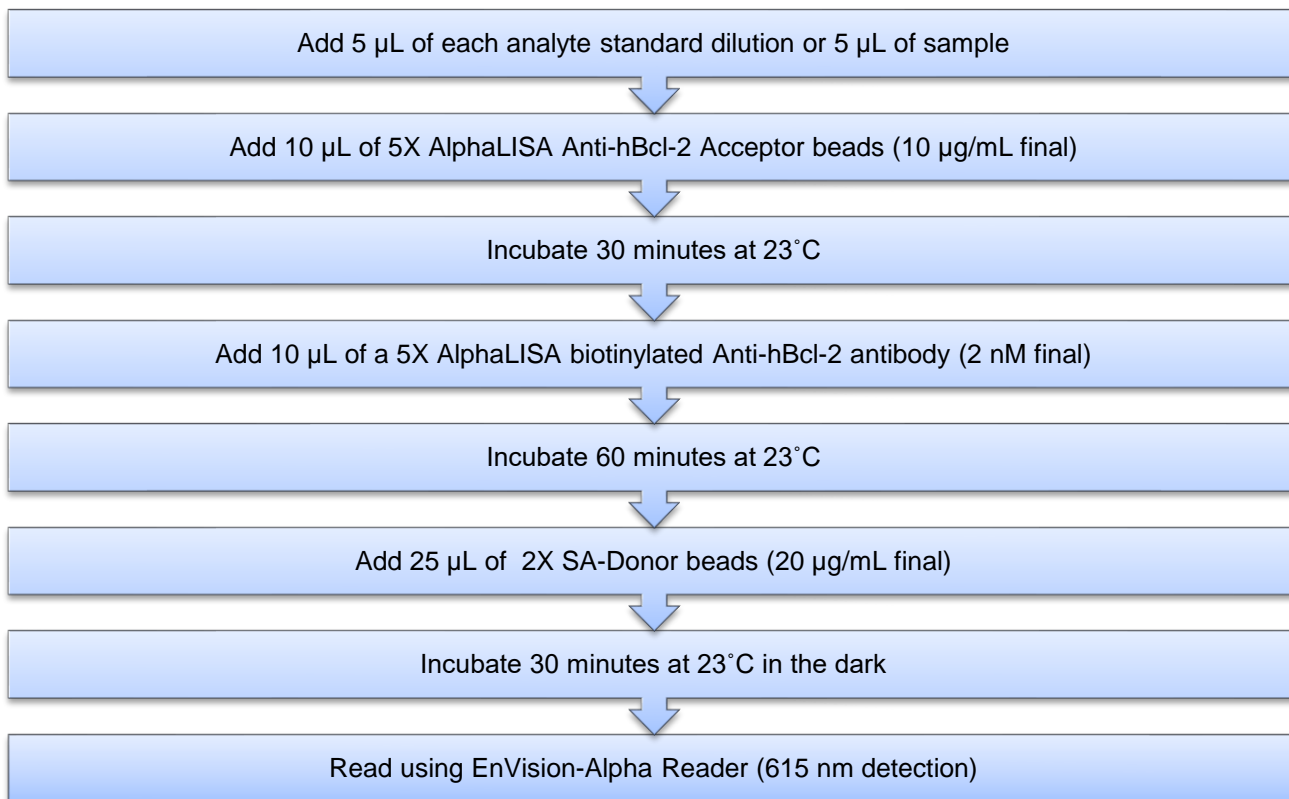
4) Preparation of 5X AlphaLISA Biotinylated Anti-hBcl-2 (10 nM)

- Add 100 µL of 500 nM Biotinylated Anti-hBcl-2 antibody to 4900 µL of 1X AlphaLISA Immunoassay Buffer.
- Prepare just before use.

5) Preparation of 2X Streptavidin (SA) Donor beads (40 µg/mL):

- a. Prepare just before use and keep the beads under subdued laboratory lighting.
- b. Add 100 µL of 5 mg/mL SA-Donor beads to 12400 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white Optiplate (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 using the 3 step manual performed in AlphaLISA Immunoassay Buffer (IAB).

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5  $\mu$ L using the recommended assay conditions. The assay performs well in cell culture medium supplemented with 10% FBS.

LDL (pg/mL)	Buffer/Media	# of experiments
30	IAB	8
72	DMEM +10% FBS	6
612	RPMI +10% FBS	6

\* The standard was prepared in these diluents. Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10  $\mu$ L of analyte in a final assay volume of 50  $\mu$ L).

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in IAB, DMEM or RPMI supplemented with 10% FBS. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format using IAB.

- Intra-assay precision:

The intra-assay precision was determined using a total of 6 independent determinations in triplicate, shown as CV%.

hBcl-2	IAB	DMEM with 10% FBS	RPMI with 10% FBS
CV%	3.1	5.5	8.5

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements at 100 ng/mL. Shown as CV%.

hBcl-2 (100 ng/mL)	IAB	DMEM with 10% FBS	RPMI with 10% FBS
CV%	4.8	5.9	10.6



- Spike Recovery:

Three known concentrations of analyte were spiked into IAB. All samples, including non-spiked Immunoassay Buffer were measured in the assay. The average recovery from three independent measurements is reported.

Spiked hBcl-2 (ng/mL)	% of Recovery		
	IAB	DMEM with 10% FBS	RPMI with 10% FBS
100	112	95	80
10	103	87	86
1	107	76	78

- Specificity:

Cross-reactivity of the hBcl-2 AlphaLISA Kit was tested using the following protein at 10 ng/mL in IAB.

Protein	% Cross-reactivity
Mouse Bcl-2	0

## Human Serum Experiments

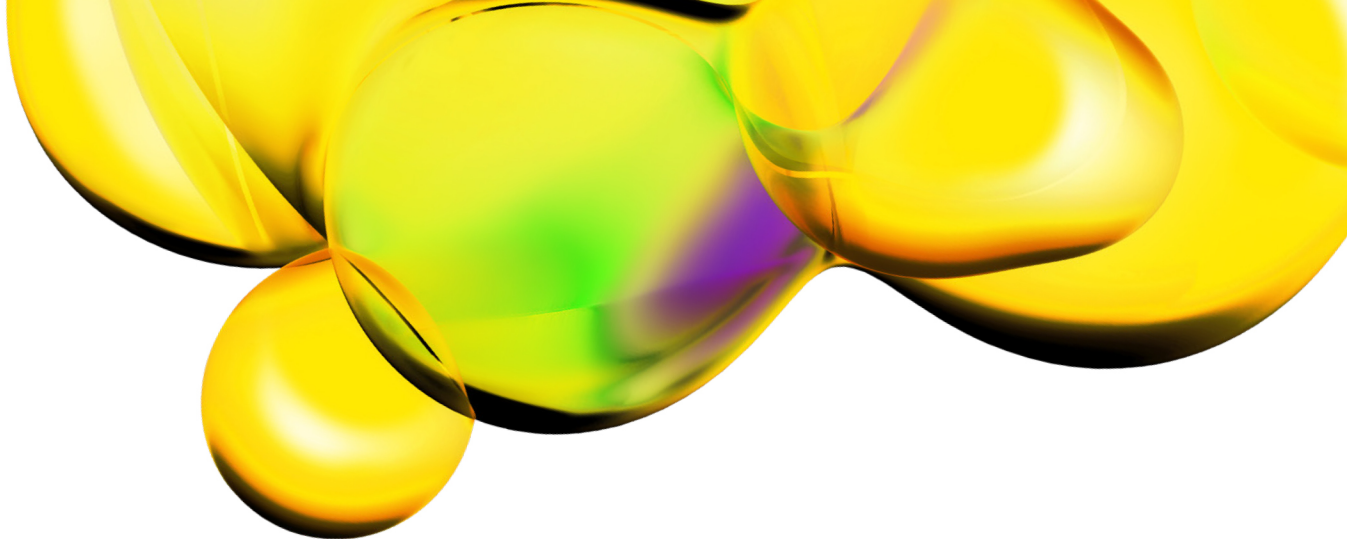
To validate the assay kit, hBcl-2 is spiked to commercially available normal human pooled serum. The recovery is shown as below.

Human serum dilution fold	Spike (ng/mL)	% Recovery
16	62.5	48
32	31.25	54
64	15.6	57
128	7.81	60

## Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: [www.revity.com](http://www.revity.com)

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