TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

AlphaLISA PD-L1 (Human) Detection Kit

Product number: AL355 HV/C/F

Lot number: 2711460

Manufacturing date: April 17, 2020

Research Use Only. Not for use in diagnostic procedures.

Contents

Product Information

Application: This kit is designed for the quantitative determination of Human PDL-1 (CD274/B7-H1) in immunoassay buffer, human serum, plasma, and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The assay shows negligible cross-reactivity with Human PDL-2, B2-H2, B7-H3, B7-1, B7-1, and Mouse PDL-1. Other species have not been tested.

Sensitivity: Lower Detection Limit (LDL): 2 pg/mL

Lower Limit of Quantification (LLOQ): 10 pg/mL

EC_{50}: 25 ng/mL

Dynamic range: 2 – 300 000 pg/mL (Figure 1).

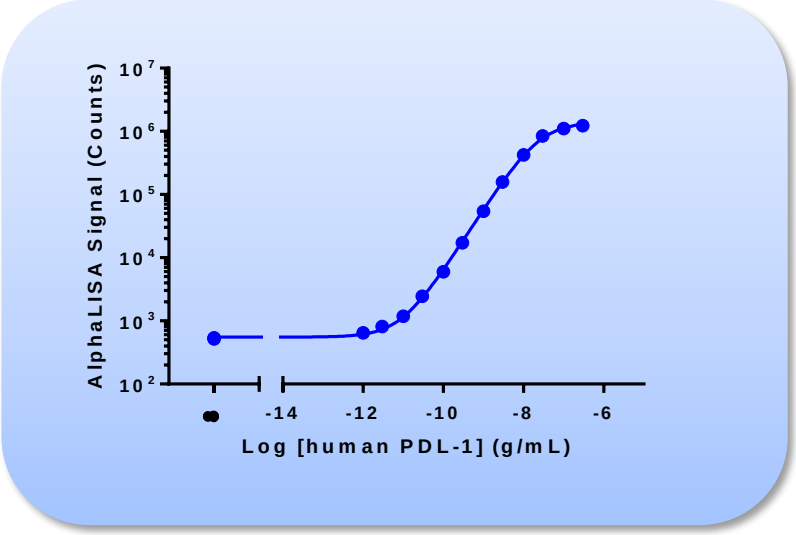


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

Storage: Store kit in the dark at +4˚C. Store reconstituted analyte at -20°C.

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

EC_{50}:	33.990 ng/mL
LDL:	7.448 pg/mL
LLOQ	23.860 pg/mL
Min Counts:	359
Max Counts:	331,994

Analyte of Interest

Programmed death ligand 1 (PDL-1), also known as cluster of differentiation 274 (CD274) or B7 homolog1 (B7-H1) belongs to the growing B7 family of immune proteins and has been demonstrated to play a role in the regulation of immune responses and peripheral tolerance. Human PDL-1 is constitutively expressed in several organs such as heart, skeletal muscle, placenta and lung, and in lower amounts in thymus, spleen, kidney and liver. PDL-1, together with PDL-2, are two ligands for PD-1 (programmed death 1), a member of the CD28 family of immunoreceptors. By binding to PD-1 on activated T-cells and B-cells, PDL-1 may inhibit ongoing T-cell responses by inducing apoptosis and arresting cell-cycle progression. Accordingly, it leads to growth of immunogenic tumor growth by increasing apoptosis of antigen specific T cells and may contribute to immune evasion by cancers. PDL-1 thus is regarded as promising therapeutic target for human autoimmune disease and malignant cancers.

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).

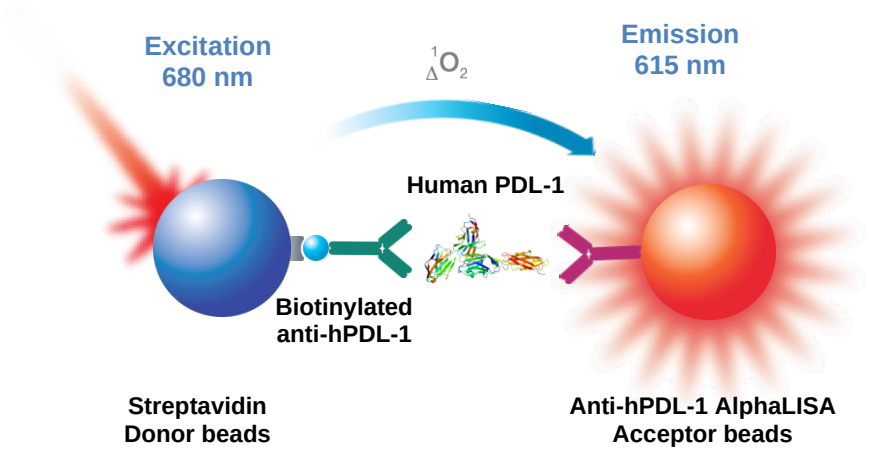


Figure 2. AlphaLISA Assay Principle.

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. The analyte included in this kit is from a human source.
- Some analytes are present in saliva. 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	AL355HV (100 assay points)	AL355C (500 assay points)	AL355F (5000 assay points)
AlphaLISA Anti-hPDL-1 Acceptor beads stored in PBS, 0.05% Kathon , pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 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 , pH 7.4	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 x 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Anti-hPDL-1 Antibody in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	30 µL @ 50 µg/mL (1 tube, black cap)	75 µL @ 50 µg/mL (1 tube, black cap)	750 µL @ 50 µg/mL (1 tube, black cap)
Human PDL-1	0.3 µg, lyophilized (1 tube, clear cap)	0.3 µg, lyophilized (1 tube, clear cap)	0.3 µg, lyophilized (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X)	2 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute hPDL-1 in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. One vial contains an amount of hPDL-1 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL355S).

** 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 (AL355HV) or 50 µL in 96- 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 Antibody 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 Adhesive Sealing Film	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer 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_2O (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 Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A 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 AlphaLISA Immunoassay Buffer for serum and/or plasma samples.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an ***example*** for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols 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 protocol 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 and biotinylated antibody**	**SA-Donor beads**	**Plate recommendation**
AL355HV	100	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL355C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	20 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	8 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	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL355F	5 000	50 µL	5 µL	20 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	8 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	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

2 Step Protocol 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 (for 100 mL):
   a. Add 10 mL of 10X AlphaLISA Immunoassay Buffer to 90 mL H_2O.

2) Preparation of hPDL-1 analyte standard dilutions:
   a. Reconstitute lyophilized hPDL-1 (0.3 µg) in 100 µL H_2O.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of hPD-L1 (µL)	Vol. of diluent (µL) *	[hPD-L1] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted hPD-L1	90	3.00E-07	300 000
B	60 µL of tube A	120	1.00E-07	100 000
C	60 µL of tube B	140	3.00E-08	30 000
D	60 µL of tube C	120	1.00E-08	10 000
E	60 µL of tube D	140	3.00E-09	3 000
F	60 µL of tube E	120	1.00E-09	1 000
G	60 µL of tube F	140	3.00E-10	300
H	60 µL of tube G	120	1.00E-10	100
I	60 µL of tube H	140	3.00E-11	30
J	60 µL of tube I	120	1.00E-11	10
K	60 µL of tube J	140	3.00E-12	3
L	60 µL of tube K	120	1.00E-12	1
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

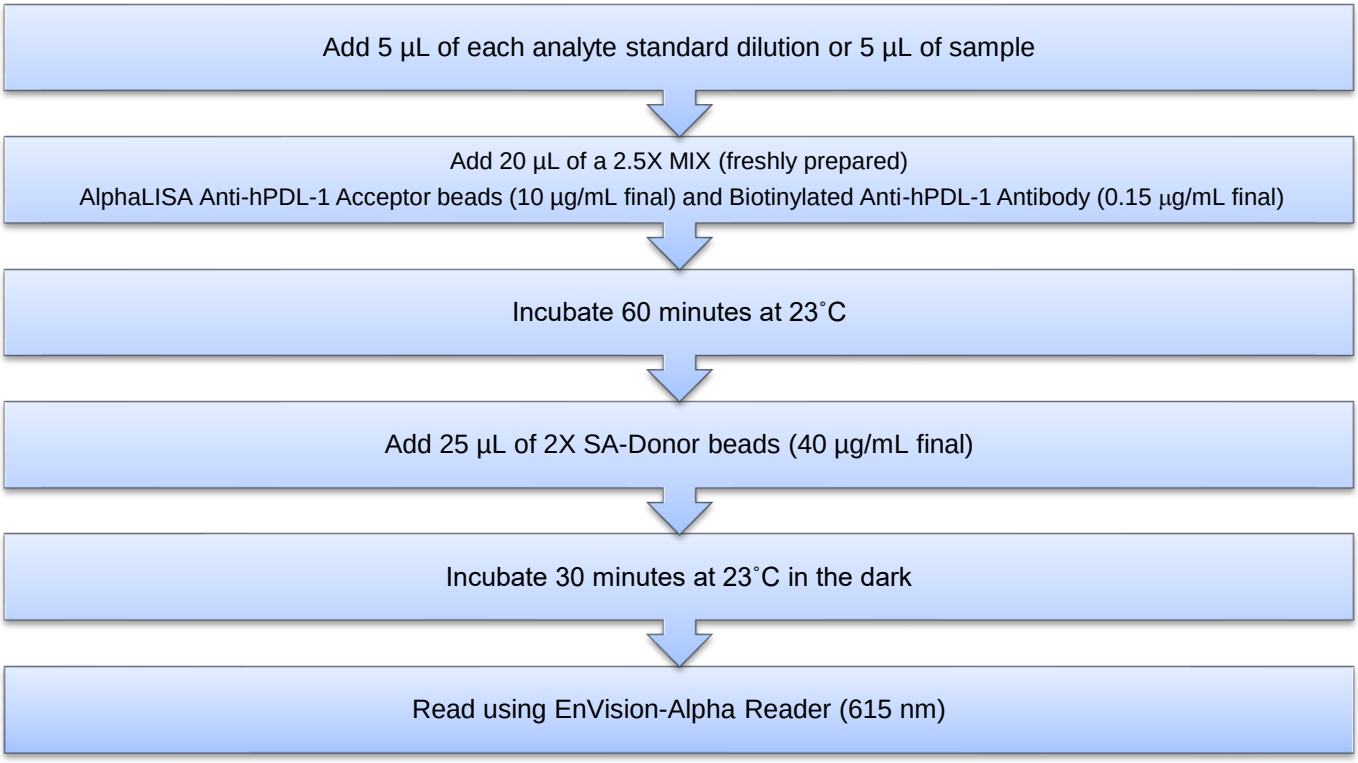
* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** 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 the mix of 2.5X AlphaLISA Anti-hPDL-1 Antibody Acceptor beads (25 µg/mL) and 2.5X Biotinylated Anti-hPDL-1 Antibody (0.375 µg/mL):
   a. Add 50 µL of 5 mg/mL AlphaLISA Anti-hPDL-1 antibody Acceptor beads and 75 µL of 50 µg/mL Biotinylated anti-hPDL-1 antibody to 9875 µL of 1X AlphaLISA Immunoassay Buffer.
   b. Prepare just before use.

4) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):
   a. Keep the beads under subdued laboratory lighting.
   b. Add 200 µL of 5 mg/mL SA-Donor beads to 12 300 µL of 1X AlphaLISA Immunoassay Buffer.
   c. Prepare just before use.

5) In a white Optiplate (384 wells):



Read Settings: AlphaLISA signal is detected using an EnVision Multilabel Reader equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser: 680 nm, Excitation Time: 180 ms, Mirror: 640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).

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 2 step protocol using AlphaLISA Immunoassay Buffer.

- Assay Sensitivity:

The LDL and LLOQ were calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions.

LDL (pg/mL)	Buffer/Serum/Medium*	# of experiments
2	IAB	6
4	DMEM+ 10% FBS	6
7	RPMI + 10% FBS	6

* The standard was prepared in these diluents. Note that LDL/ LLOQ can be decreased (i.e. sensitivity increased) by preparing standards in different matrixes.

- 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. 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%.

hPD-L1	IAB	DMEM	RPMI
CV (%)	4	6	7

- Inter-assay precision:

The inter-assay precision was determined using a total of 4 independent determinations in triplicate for 3 ng/mL sample. Shown as CV%.

hPD-L1 (3 ng/ml)	IAB
CV (%)	6

- Spike Recovery:

Three known concentrations of analyte were spiked in IAB, or in cell culture media containing 10% FBS. All samples, including non-spiked buffer or media were measured in the assay. The average recovery from three independent measurements is reported. Note that the standard curves were prepared in IAB, DMEM, and RPMI.

Spiked hPDL-1 (ng/mL)	% Recovery*		
	IAB	DMEM	RPMI
100	98	108	109
10	105	101	98
1	97	98	102

- Specificity:

Cross-reactivity of the hPD-L1 Kit was tested using the following proteins at 100 ng/mL in IAB. Reactivity to hPDL-1 is 100%.

Protein	% Cross-reactivity
Human B7-H2	0
Human B7-H3	0
Human B7-1	0
Human B7-2	0
Human PDL-2	0
Mouse PDL-1	0

Human Serum Experiments

To validate the assay kit, commercially available human serum sample with unknown concentration of hPDL-1 was used to examine dilution linearity and spike recovery.

PDL-1 is not detected in the serum tested. Therefore, the dilution linearity and spike recovery are determined by human serum spiked with hPDL-1 (100 ng/mL after spiking). The spiked serum is then further diluted and recovery of PDL-1 is shown in the table below. A good recovery is observed when a sample is diluted 256 fold or greater.

Dilution Factor	% Recovery
1	**9**
2	**21**
4	**32**
8	**45**
16	**58**
32	**69**
64	**73**
128	**75**
256	**81**
512	**81**
1024	**87**
2048	**90**

Troubleshooting Guide

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

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

This product is not for resale or distribution except by authorized distributors.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs