MANUAL

Technology: AlphaLISA®

AlphaLISA Human IL15 Detection Kit

Part number:	**AL3196HV**	**AL3196C**	**AL3196F**
Assay points:	100	500	5,000

Storage: Store kit in the dark at 4 °C. For reconstituted analyte, aliquot and store at -20 °C. Avoid freeze-thaw cycles.

Version: 1 **Date:** January 2024

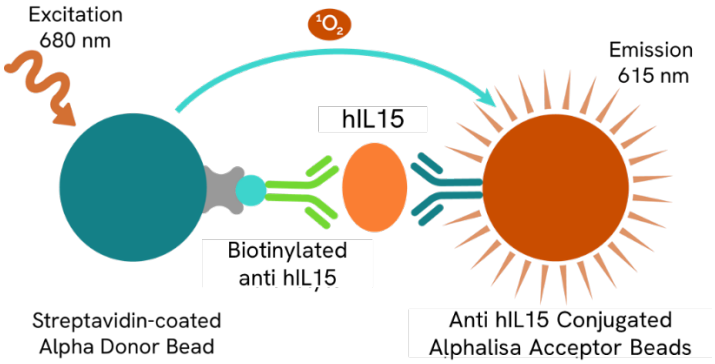
ANALYTE OF INTEREST

Human Interleukin 15 (IL15) is a cytokine produced as a precursor maturing into a 114 amino acid glycoprotein. IL15 is generated by multiple tissues (placenta, skeletal muscle, kidney, lung, heart, and monocytes/macrophages). IL15 acts mainly on T and B cells and it is well known as a key cytokine in natural killer cell development, proliferation, and survival.

IL15 binds with high affinity to the IL15 receptor α and forms a complex with the α and γ chains of the IL2 receptor for signal transduction. It can regulate inflammatory response and NK cell activation during immune responses. IL15 signalling has become a focus of interest for therapeutic intervention especially in autoimmune diseases like inflammatory bowel disease, systemic lupus erythematosus, inflammatory synovitis, psoriasis, diabetes mellitus, asthma bronchiale, rheumatoid arthritis, and T-cell leukemia.

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 this AlphaLISA assay, a biotinylated anti-IL15 antibody binds to the streptavidin coated AlphaLISA Donor beads, while an anti-IL15 antibody is conjugated to AlphaLISA Acceptor beads. In the presence of IL15, 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.



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) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated anti-IL15 antibody contains sodium azide. Contact with skin or inhalation should be avoided.

KIT CONTENT: REAGENTS AND MATERIALS

Kit components	AL3196HV***	AL3196C****	AL3196F****
AlphaLISA Anti-IL15 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP II, pH 7.2	25 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 CG/ICP II, pH 7.4	100 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 (x brown tubes, black caps)
Biotinylated *Anti-IL15* Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	25 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
Lyophilized Recombinant Human IL15*	0.01 µg (1 tube, clear cap)	0.01 µg (1 tube, clear cap)	0.01 µg (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X)	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H_2O. **IMPORTANT: do not vortex the analyte**. 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 is stable up to 6 weeks. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL3196S).

** 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.

**** The number of assay points is based on an assay volume of 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. Note that sodium azide from the biotinylated anti-IL15 antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Additional reagents and materials:

The following materials are recommended but not provided in the kit:

Item	Suggested source
Light gray AlphaPlate™-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.

RECOMMENDATONS

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 the Donor and Acceptor beads by vortexing before use.
- Use Milli-Q® grade H_2O to dilute buffers and to reconstitute the lyophilized analyte. Do not vortex the 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 Film to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multimode 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. It is recommended to avoid multiple reads of the same well of the assay plate.
- The standard curves shown in this manual are provided for information only. A standard curve must be generated for each experiment.

ASSAY PROCEDURE

- 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 different amounts of samples are tested, the volumes of all reagents must 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.

Format	# Of data points	Volumes for 2-Step Protocol				Plate recommendation
		Final	Sample	AlphaLISA Acceptor Beads + biotinylated Ab Mix	SA-Donor beads	
AL3196HV	**100**	**100 µL**	**10 µL**	**40 µL**	**50 µL**	**White OptiPlate-96 White ½ AreaPlate-96**
AL3196C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96
	500	**50 µL**	**5 µL**	**20 µL**	**25 µL**	**½ Area AlphaPlate-96 White OptiPlate-384 Light gray AlphaPlate™-384**
	1 250	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 ProxiPlate™-384 Plus White OptiPlate-384
	2 500	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536
AL3196F	**5 000**	**50 µL**	**5 µL**	**20 µL**	**25 µL**	**½ Area AlphaPlate-96 White OptiPlate-384 Light gray AlphaPlate-384**
	12 500	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 ProxiPlate-384 Plus White OptiPlate-384
	25 000	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536

The 2-Step protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amounts of samples are tested, the volumes of all reagents must be adjusted accordingly.*

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

2) Preparation of IL15 analyte standard dilutions:
   a. Reconstitute lyophilized human IL15 (0.01 µg) in 100 µL Milli-Q® grade H_2O. Important- Do not vortex the analyte. The remaining reconstituted analyte should be aliquoted immediately and stored at -20 °C for future assays (see page 2 for more details).
   b. A standard curve must be generated for each experiment. The standard curve should be performed in a similar matrix diluent as the samples (e.g. cell culture media for cell supernatant samples, FBS for serum samples). Use of the 1X AlphaLISA Immunoassay Buffer is recommended as a diluent to confirm assay performance.
   c. Prepare standard dilutions as follows (change tip between each standard dilution).

Tube	Vol. of human IL15 (µL)	Vol. of diluent (µL)*	[IL15] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted human IL15	90	1E-8	10 000
B	60 µL of tube A	140	3E-9	3 000
C	60 µL of tube B	120	1E-9	1 000
D	60 µL of tube C	140	3E-10	300
E	60 µL of tube D	120	1E-10	100
F	60 µL of tube E	140	3E-11	30
G	60 µL of tube F	120	1E-11	10
H	60 µL of tube G	140	3E-12	3
I	60 µL of tube H	120	1E-12	1
J	60 µL of tube I	140	3E-13	0.3
K	60 µL of tube J	120	1E-13	0.1
L	60 µL of tube K	140	3E-14	0.03
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 appropriate 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).

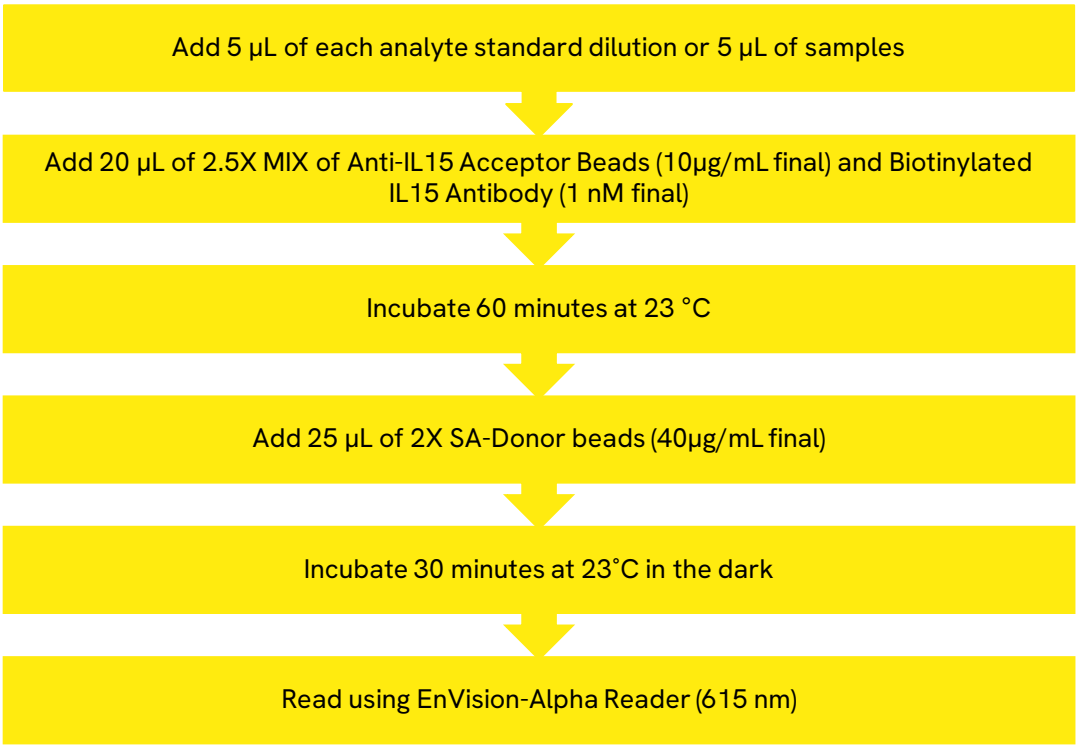
3) Preparation of 2.5X MIX AlphaLISA Anti-Il15 Acceptor beads (25 µg/mL) + Biotinylated Anti-Il15 Antibody (2.5 nM):
   a. Prepare just before use.
   b. Add 50 µL of 5 mg/mL AlphaLISA Anti-Il15 Acceptor Bead and 50 µL of 500 nM Biotinylated Anti-Il15 Antibody to 9900 µL of 1X AlphaLISA Immunoassay Buffer.

4) 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 12 300 µL of 1X AlphaLISA immunoassay Buffer

5) 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

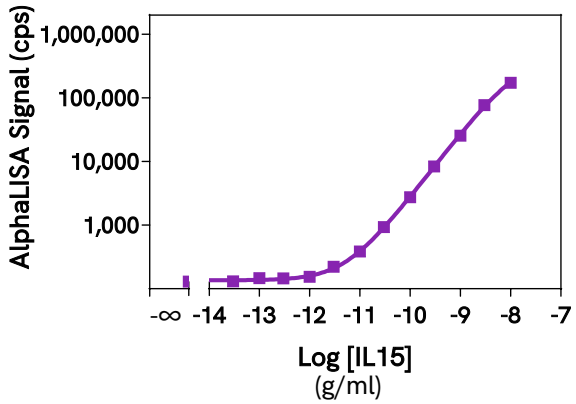
AlphaLISA assay performance described below was determined by using a 2-step protocol using 1X AlphaLISA Immunoassay Buffer as an assay buffer. The analytes (standards) were prepared in different matrix diluents depending on sample type. All other components were prepared in 1X AlphaLISA Immunoassay Buffer.

Standard curve:

A typical sensitivity curve is shown below, using the 2-step protocol described on page 4, using 1X AlphaLISA Immunoassay Buffer to dilute the standard.

Assay sensitivity:

The LDL was 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 values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL of sample using the recommended assay conditions.



Analyte diluent	*LDL (pg/mL)*	LLOQ (pg/mL)
AlphaLISA Immunoassay Buffer 1X	0.43	1.3
DMEM+10% FBS	0.76	2.9
RPMI+10% FBS	0.30	2.8
100% FBS	0.55	2.4
Human Serum	0.51	2.8

Assay precision:

The following assay precision data were calculated from three independent assays using two different kit lots. In each lot, the analytes were prepared in AlphaLISA Immunoassay Buffer, DMEM + 10%FBS, RPMI +10% FBS, and 100% FBS. All other components were prepared in AlphaLISA Immunoassay Buffer. 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 a 384-well plate format.

Intra-assay precision:

The intra-assay precision was determined using a total of 3 independent determinations in triplicate. Shown as CV% of measured concentration.

IL15 CONCENTRATION	IAB	RPMI +10%FBS	DMEM + 10%FBS	100% FBS
250 pg/mL	2%	3%	2%	1%
500 pg/mL	2%	2%	1%	2%
1000 pg/mL	2%	2%	3%	1%

Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 12 measurements for 3 samples at 250, 500 and 1000 pg/mL. Shown as CV% as measured concentration.

IL15	IAB	RPMI + 10% FBS	DMEM + 10%M FBS	100% FBS
250 pg/mL	8%	5%	3%	2%
500 pg/mL	4%	3%	5%	2%
1000pg/mL	4%	4%	5%	5%

Spike and recovery:

Three known concentrations of analyte were spiked into AlphaLISA Immunoassay Buffer, RPMI + 10% FBS, DMEM + 10% FBS and 100% FBS. All samples, including non-spiked diluents, were measured in the assay. Note that the analytes for the respective standard curves were prepared in AlphaLISA Immunoassay Buffer, RPMI + 10% FBS, DMEM + 10% FBS and 100% FBS. All other assay components were diluted in AlphaLISA Immunoassay Buffer.

Spiked IL15 (pg/mL)	% Recovery			
	IAB	RPMI + 10% FBS	DMEM + 10% FBS	100%FBS
250	92%	99%	103%	103%
500	105%	102%	104%	101%
1000	108%	100%	106%	108%

Specificity:

Cross-reactivity of the AlphaLISA Human IL15 Detection Kit was tested using recombinant IL15 from mouse and human IL2 or human IL21 as analytes in an assay using the protocol as described above. The cross reactivities were established using the related proteins below at a test concentration of 100 ng/mL. Percentage recovery was computed by comparing the measured Interpolated concentration versus the theoretical one.

Proteins	Cross reactivity (%)
Mouse IL15	0.00
Human IL2	0.00
Human Il21	0.00

The possible interference from human Interleukin 15 Receptor alpha / Fc Chimera (IL15 Rα) was investigated. No interference was observed up to 200 ng/mL for IL15 Rα.

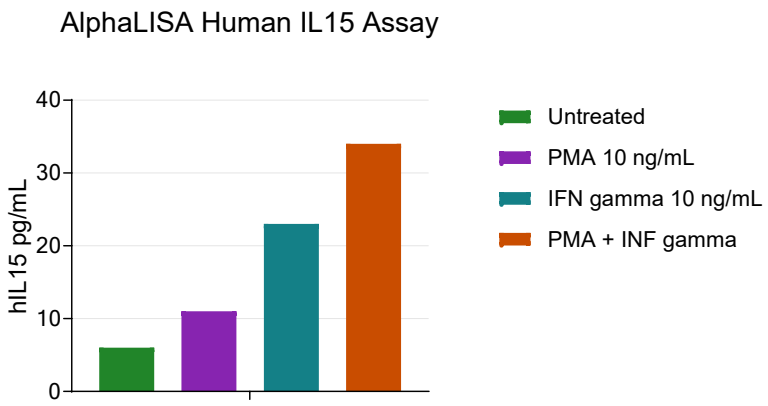
Calibration:

Target NIBSC/WHO International Standard (code 95/554) was tested using the AlphaLISA Human IL15 Detection Kit: 1 unit of Standard NIBSC 95/554 corresponds to **127** pg/mL of AlphaLISA human IL15, respectively.

Cell experiments:

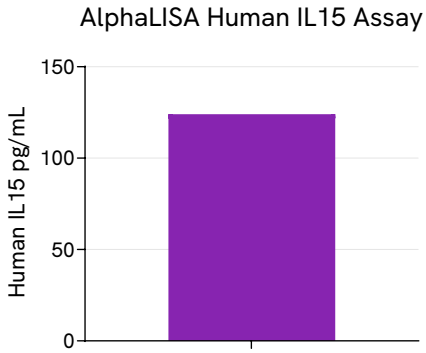
Cell culture conditions and Pharmacological treatment

- NCI-H2172 cells plated at 50 kcells/well were stimulated for 48h with PMA 10 ng/mL, IFNɣ 10 ng/mL or PMA + IFNɣ. 5 µL of supernatants were transferred into a Light gray AlphaPlate-384 to be analyzed by the AlphaLISA Human IL15 Assay.



As expected treatment with PMA and IFNɣ induce IL15 production and secretion (*IFN-**γ** Induces IL-15 Trans-Presentation by Epithelial Cells via IRF1 J Immunol (2022) 208 (2) : 338-346.)*

- HUT-102 human cutaneous T cell lymphocyte cells were cultured in RPMI medium with 10% FBS for 24H at 37°C for supernatant generation to be analyzed by the AlphaLISA Human IL15 Assay. IL15 concentration in HUT-102 cell supernatant is **124** pg/mL. These results are in accordance with the literature (Blood (1998) 91 (11): 4265–4272).



TROUBLESHOOTING

To find detailed recommendations for common situations you might encounter with your AlphaLISA assay kit, please visit our website at www.revvity.com.

www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com