TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Caution: For Laboratory Use. A research chemical for research purposes only.

Human Interleukin 6 (IL6) Kit

Product No.: AL223 C/F

Lot No.: 679-865-A

Material Provided

Format: AL223C: 500 assay points AL223F: 5 000 assay points
The number of assay points is based on an assay volume of 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

Manufacturing date: **July 18, 2011**

Product Information

Kit content: The kit contains 5 components: AlphaLISA Acceptor beads coated with an Anti-Analyte Antibody, Streptavidin-coated Donor beads, Biotinylated Anti-Analyte Antibody, lyophilized analyte and 10X AlphaLISA Immunoassay Buffer.
Assay microplates (96-, 384- or 1536-well plates) must be purchased separately (see page 3 for more details).

Storage: Store kit in the dark at +4˚C.

Stability: This product is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Application: This kit is designed for the quantitative determination of human IL6 in serum, buffered solution or cell culture medium using a homogenous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 1.3 pg/mL (see page 8: Assay Performance Characteristics).

Dynamic range: 1.3 – 30 000 pg/mL (see page 8: Assay Performance Characteristics).

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Precautions

- Only the AlphaScreen® 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 (Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous. Some analytes are from 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.

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Quality Control

The LDL for this lot was verified using the AlphaLISA assay described in this technical data sheet. We certify that these results meet our quality release criteria.

Reagents and Materials

The reagents provided in the AlphaLISA kit are listed in the table below:

Kit components	AL223C (500 assay points)	AL223F (5 000 assay points)
AlphaLISA Anti-IL6 Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	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% Proclin-300, pH 7.4	200 µL @ 5 mg/mL (1 brown tube, blue cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, blue caps)
Biotinylated Antibody Anti-IL6 stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
AlphaLISA human IL6 (0.1 µg), lyophilized analyte *	1 tube, clear cap	1 tube, clear cap
AlphaLISA Immunoassay Buffer (10X) **	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute human IL6 in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes, if possible, or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. It has been demonstrated that reconstituted human IL6 is stable for at least 60 days at -20°C. One vial contains an amount of human IL6 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL223S).

** Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100 and 0.5% Proclin-300. Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).
Note: 10X buffer might be slightly yellow. However, this does not affect the assay results.

Once diluted, 1X AlphaLISA Immunoassay Buffer contains 25 mM HEPES, pH 7.4, 0.1% Casein, 1 mg/mL Dextran-500, 0.5% Triton X-100 and 0.05% Proclin-300.

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.	6005185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

Protocols have been optimized for 50 µL assays in white OptiPlate™-384 microplates. Other assay volumes can be used with similar protocols and identical final AlphaLISA reagent concentrations:

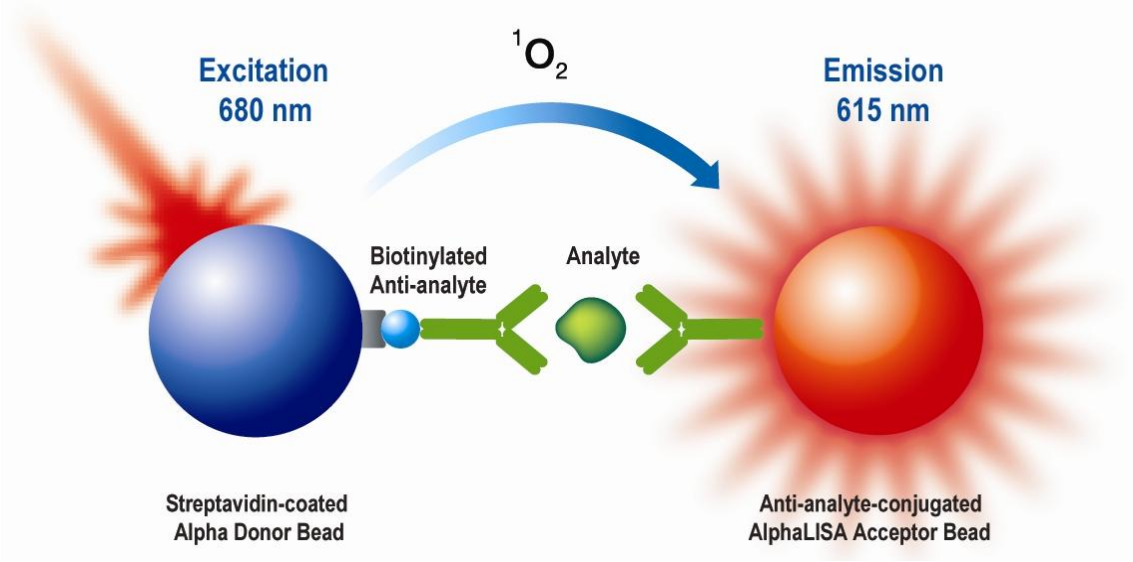
Format	# of data points	Total assay volume	Sample volume	AlphaLISA beads / Biotin Antibody MIX volume	SA-Donor beads volume	Plate recommendation
AL223C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	20 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (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)
AL223F	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)

Analyte of Interest

Interleukin 6 (IL6) is a ~22 kDa pleiotropic cytokine that acts not only on the immune system, but also affects many physiological events in various organs. IL6 exerts pro- or anti-inflammatory effects, depending on the target cell analyzed and the in vivo environmental circumstances. IL6 is a differentiation and proliferation factor for B and T cells, and acts as a migration factor on monocytic cells. It is the major activator of acute-phase protein expression in the liver, a hematopoietic factor, and acts as a survival factor on neuronal cells. IL6 signals through binding to the gp130/ IL-6R receptor complex, leading to the activation of JAK/STAT, MAPK and PI3K cascades.

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 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 (see figure below).



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Recommendations

General 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 prewet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2 000 g, 10-15 sec). Resuspend 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 in 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 a similar matrix as the samples (e.g. FBS for serum samples).

Specific recommendations:

- AlphaLISA assays can be performed in cell culture medium with or without phenol red, with the following recommendations: If possible, avoid biotin-containing medium (e.g. RPMI medium) as lower counts and lower sensitivity are expected. Add at least 1% FBS or 0.1% BSA to cell culture medium.
- When analyzing serum samples, perform the standard curve in analyte-depleted serum. Serum should not exceed 10% of final assay volume (i.e. 5 µL serum sample in 50 µL final assay volume).

Protocol

High sensitivity protocol (2 incubation steps) – Dilution of standards in 1X AlphaLISA Immunoassay Buffer, cell culture medium or analyte-depleted serum *

The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocol also includes testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly. 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 (Lower Detection Limit) is calculated. One background point in triplicate (3 wells) can be used when LDL is not calculated.

* See the analyte-depleted serum preparation protocol in the “AlphaLISA Assay Development Guide” (page 20) at **www.perkinelmer.com/nowashelisa**

IMPORTANT: PLEASE READ THE RECOMMENDATIONS ABOVE BEFORE USE

Steps for Preparing Reagents

The protocol described below is for one standard curve (48 wells) and samples (452 wells). Dilution of standards can be done in 1X AlphaLISA Immunoassay Buffer, cell culture medium or analyte-depleted serum.

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 2.5 mL of 10X AlphaLISA Immunoassay Buffer to 22.5 mL H_2O.

2) Preparation of human IL6 analyte standard dilutions:
Reconstitute lyophilized human IL6 (0.1 µg) in 100 µL H_2O.
Prepare standard dilutions as follows (change tip between each standard dilution):

Tube	Vol. of human IL6 (µL)	Vol. of diluent (µL) *	[human IL6] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted human IL6	90	1E-07	100 000
B	60 µL of tube A	140	3E-08	30 000
C	60 µL of tube B	120	1E-08	10 000
D	60 µL of tube C	140	3E-09	3 000
E	60 µL of tube D	120	1E-09	1 000
F	60 µL of tube E	140	3E-10	300
G	60 µL of tube F	120	1E-10	100
H	60 µL of tube G	140	3E-11	30
I	60 µL of tube H	120	1E-11	10
J	60 µL of tube I	140	3E-12	3
K	60 µL of tube J	120	1E-12	1
L	60 µL of tube K	140	3E-13	0.3
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, cell culture medium or analyte-depleted serum). 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 AlphaLISA Anti-IL6 Acceptor beads + Biotinylated Antibody Anti-IL6 MIX (25 µg/mL / 2.5 nM):
Add 50 µL of 5 mg/mL AlphaLISA Anti-IL6 Acceptor beads and 50 µL of 500 nM Biotinylated Antibody Anti-IL6 to 9 900 µL of 1X AlphaLISA Immunoassay Buffer. Prepare just before use.

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

5) Samples: If applicable, dilute samples to be tested in diluent (e.g. 1X AlphaLISA Immunoassay Buffer, cell culture medium or analyte-depleted serum).

6) In a 96- or 384-well microplate:

Add 5 µL of each analyte standard dilution or 5 µL of sample

⇩

Add 20 µL of a 2.5X MIX (freshly prepared)
AlphaLISA Anti-Analyte Acceptor beads (10 µg/mL final) and Biotinylated Antibody Anti-Analyte (1 nM final)

⇩

Incubate 60 minutes at 23˚C

⇩

Add 25 µL of 2X SA-Donor beads (40 µg/mL final)

⇩

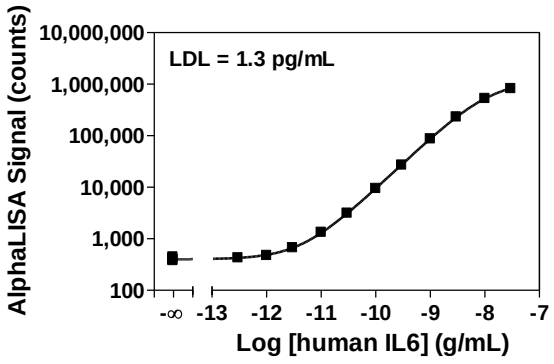
Incubate 30 minutes at 23˚C in the dark

⇩

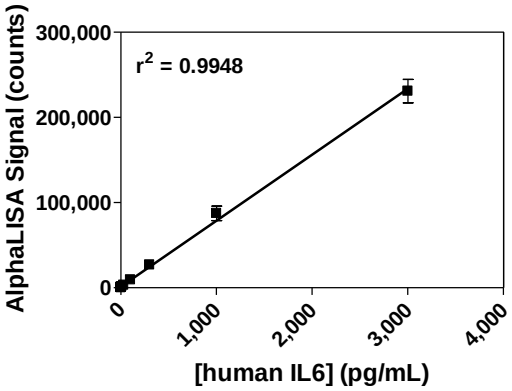
Read using EnVision-Alpha Reader

Typical results in 1X AlphaLISA Immunoassay Buffer

Log-Log scale:



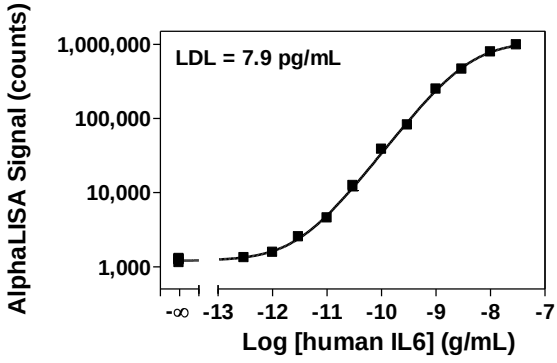
Linear-Linear scale (linear range):



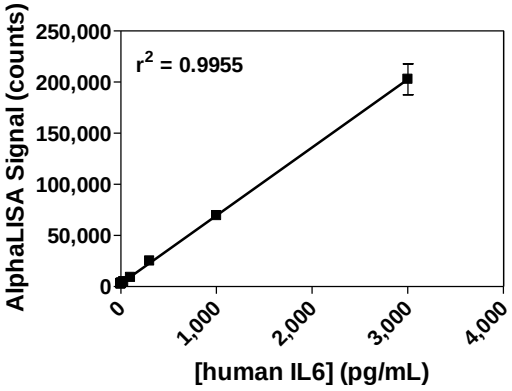
The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102.

Typical results in analyte-depleted serum

Log-Log scale:



Linear-Linear scale (linear range):



The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2101.

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Interpreting the Data

- 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.
- 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

Sensitivity:

The LDL was calculated as described above. This value corresponds to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions.

- Average LDL is 1.3 pg/mL * (using 5 µL of analyte in AlphaLISA Immunoassay Buffer) (mean of 18 independent experiments).
- Average LDL is 8.6 pg/mL (using 5 µL of analyte in analyte-depleted serum) (mean of 9 independent experiments).

* Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 µL of analyte in a final assay volume of 50 µL).

Dynamic range: 1.3 – 30 000 pg/mL (in AlphaLISA Immunoassay Buffer)

Assay precision:

The following assay precision data were calculated from a total of 18 assays. Two operators performed three independent assays using three different kit lots. Each assay consisted of one standard curve and three control samples of high (A), medium (B) and low (C) concentration, assayed in triplicate. The assays were performed in 384-well format using AlphaLISA Immunoassay Buffer.

- Intra-assay precision:

The intra-assay precision was determined using a total of 18 independent determinations in triplicate for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 18)
A	1 079	115	10.7
B	101	7.6	7.5
C	11	0.88	8.3

- Inter-assay precision:

The inter-assay precision was determined using a total of 6 independent determinations with 9 measurements for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 6)
A	1 079	99.1	9.2
B	101	8.8	8.8
C	11	1.2	11.8

- Recovery:

Three known concentrations of analyte were spiked in AlphaLISA Immunoassay Buffer or analyte-depleted serum. The % of measured versus theoretical amount was calculated for each concentration in 18 independent experiments (recovery in AlphaLISA Immunoassay Buffer) or 6 independent experiments (recovery in serum).

Spike (ng/mL)	% Recovery (in Buffer)	% Recovery (in Serum)
1	108	93
0.1	101	97
0.01	106	97

Calibration:

Human IL6 (NIBSC/WHO First International Standard (code 89/548)) was tested using this kit: 1 unit of Standard corresponds to 22.0 pg of AlphaLISA IL6.

Specificity:

Cross-reactivity of the AlphaLISA IL6 Kit was tested using the following proteins at 0.1 µg/mL in AlphaLISA Immunoassay Buffer.

Protein	% Cross-reactivity
Mouse IL6	0
Rat IL6	0

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