TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures

Mouse IL-3 AlphaLISA Detection Kit

Product number: AL587HV/C/F

Contents

Product Information

Application: This kit is designed for the quantitative determination of mouse mouse IL-3 in cultume medias, cell lysates, cell supernatents and serum samples using a homogeneous AlphaLISA assay (no wash steps). Specifications described below are based on assay performed in HiBlock buffer 1X.

Sensitivity: Lower Detection Limit (LDL): 0.75 pg/mL

Lower Limit of Quantification (LLOQ): 2.2 pg/mL

EC_{50}: 10 ng/mL

Dynamic range: 0.75 – 100 000 pg/mL

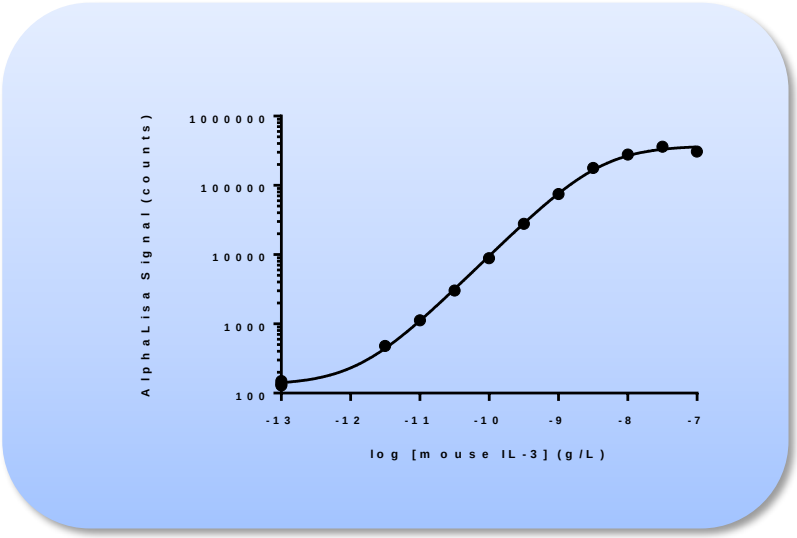


Figure 1. Typical sensitivity curve in HiBlock buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

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

Analyte of Interest

Mouse IL-3 is a 152 amino acid protein with significant glycosylation in vivo. It is secreted by activated T-cells after response to infection or inflammation and act as a growth factor for T-cells and members of the myeloid cell linage. It is also a key mediator for survival of hematopoietic cell progenitor stem cells. It acts by binding to an high affinity receptor, IL3R.

The mouse IL-3 AlphaLISA detection kit allows for the detection of mouse IL-3 in cell lysates and supernatents, along with serum and plasma.

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).

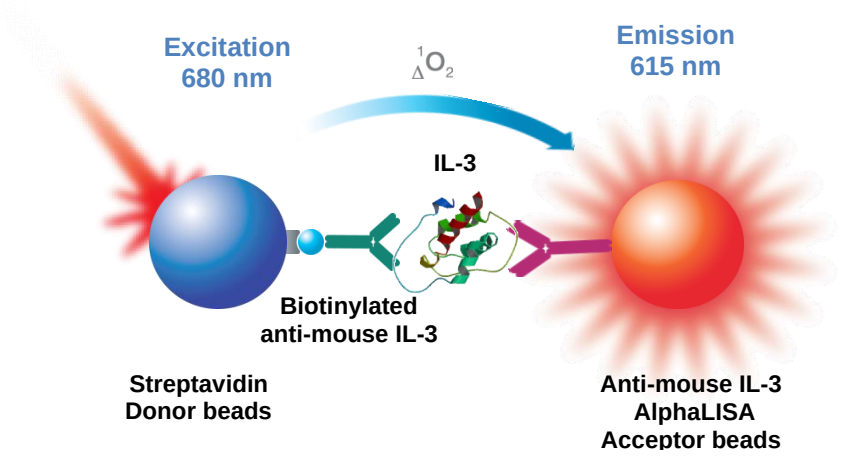


Figure 2. AlphaLISA Assay Principle.

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.
- 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	AL587HV (100 assay points)**	AL587C (500 assay points)	AL587F (5000 assay points)
AlphaLISA Anti-IL-3 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, 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 CG/ICP, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	100 µL @ 5 mg/mL (1 brown tube, black cap)	1 000 µL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated Anti-IL-3 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	20 µL @ 500 nM (1 tube, black cap)	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
Lyophilized Glycosylated IL-3 from *E. coli* ***	1 µg (1 tube, clear cap)	1 µg (1 tube, clear cap)	1 µg (1 tube, clear cap)
HiBlock buffer (10X) *	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Extra buffer can be ordered separately (cat # AL001C: 10 mL, cat # AL004F: 100 mL).

** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

*** 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 stored at -20 °C is stable up to 30 days. The reconstituted material can tolerate up to two freeze-thaw cycles. However, freeze-thaw cycles should be avoided if possible. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL587S).

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 Plus 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 HiBlock buffer.
- 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.

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					
For mat	**# of data points**	**Final**	**Sample**	**AlphaLISA Acceptor beads**	**Biotinylated Antibody**	**SA-Donor beads**	**Plate recommendation**
AL HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL C	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)
AL F	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	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)

3 Step Hi Concentration Protocol (3 incubation steps) – Dilution of standards can be done in 1X AlphaLISA Hiblock Buffer. 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 have to be adjusted accordingly*

1) Preparation of 1X AlphaLISA Hiblock Buffer:
Add 5 mL of 10X AlphaLISA Hiblock Buffer to 50 mL Milli-Q® grade H_2O.

2) Preparation of mouse IL-3 analyte standard dilutions:

   a. Reconstitute lyophilized IL-3 (1 µg) in 100 µL Milli-Q® grade H_2O.
   b. Prepare standard dilutions as follows in 1X AlphaLISA Hiblock Buffer (change tip between each standard dilution):

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

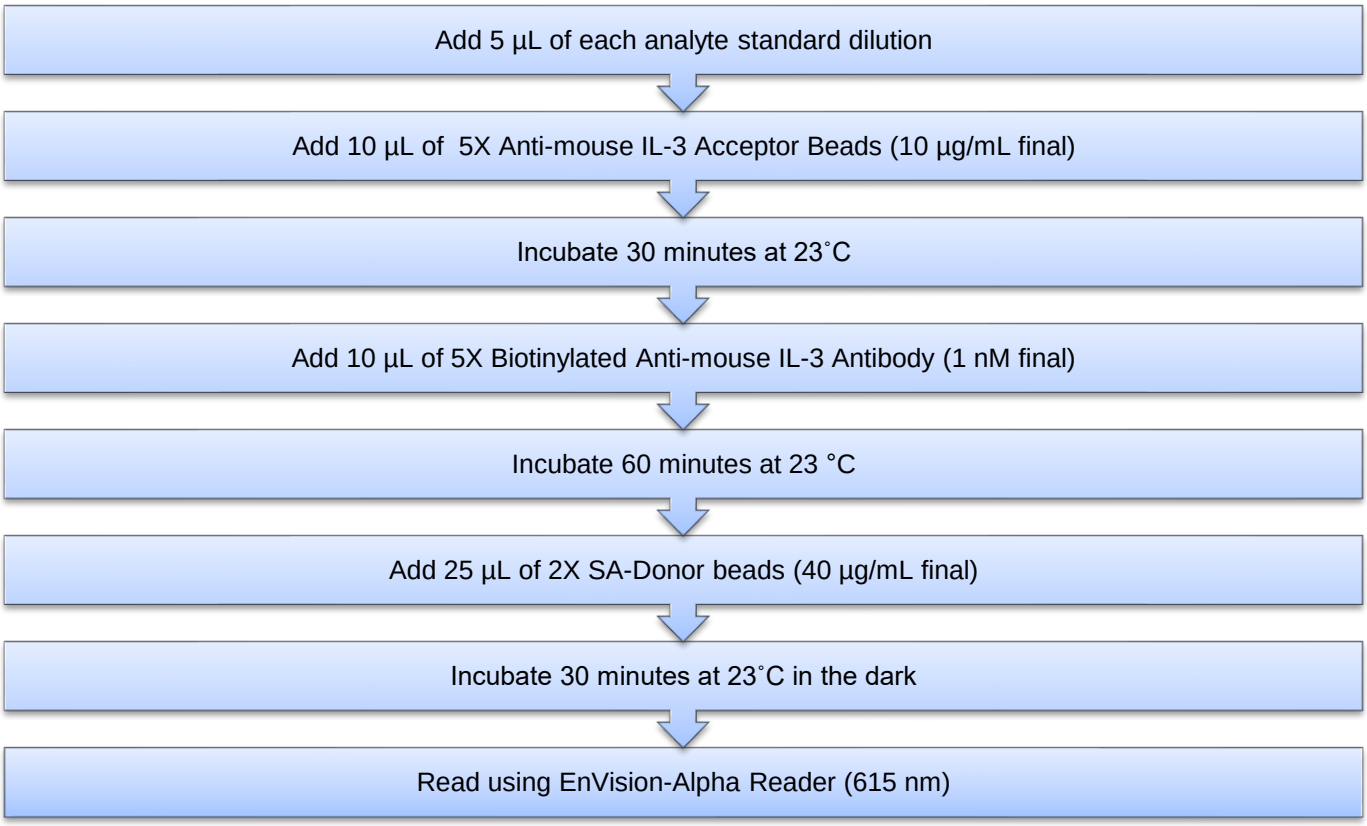
** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-mouse IL-3 Antibody Acceptor beads (50 µg/mL):
   a. Prepare just before use.
   b. Add 50 µL of 5 mg/mL AlphaLISA Anti-mouse IL-3 Antibody Acceptor to 4950 µL of 1X AlphaLISA Hiblock Buffer.

4) Preparation of 5X Biotinylated Anti-mouse IL-3 Antibody (5 nM):
   a. Prepare just before use.
   b. Add 50 µL of 500 nM Biotinylated Anti-mouse IL-3 Antibody to 4950 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 12 300 µL of 1X AlphaLISA Hiblock 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 varHiBlock bufferle 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 steps protocol using HiBlock buffer.

- 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 µL using the recommended assay conditions.

LDL (pg/mL)	Buffer *	# of experiments
0.75	HiBlock Buffer	7
1.8	AlphaLISA Lysis Buffer (ALB)	7
0.85	DMEM + 10% FBS	7
1.15	RPMI + 10% FBS	7
1.52	Iscove media + 10% FBS	7
1.45	FBS	7

* The standard was prepared in these diluents and all other components were diluted in HiBlock buffer. 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).

- 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 HiBlock buffer, AlphaLISA Lysis Buffer, DMEM, RPMI or Iscove modified Dubelcco's medium. Cell culture media was supplemented with 10% FBS. All other components were prepared in HiBlock 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 384-well format.

Intra-assay precision:

- The intra-assay precision was determined by averaging 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

mouse IL-3	HiBlock buffer	Lysis Buffer	DMEM + 10% FBS	RPMI + 10% FBS	Iscove medium + 10% FBS	FBS
CV (%)	5.8	3.5	5.0	9.8	5.4	6.4

- Inter-assay precision:

The inter-assay precision was determined comparing 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

mouse IL-3	HiBlock buffer	Lysis Buffer	DMEM + 10% FBS	RPMI + 10% FBS	Iscove medium + 10% FBS	FBS
CV (%)	12	12.2	14.5	14.3	11.5	12.8

- Spike Recovery:

Known concentrations of analyte were spiked into HiBlock buffer, DMEM + 10% FBS, RPMI + 10% FBS, 100% FBS or lysis buffer. All samples, including non-spiked buffer were measured in the assay. Note that the standard curves were prepared in the same matrix as the samples. All other components were diluted in HiBlock buffer.

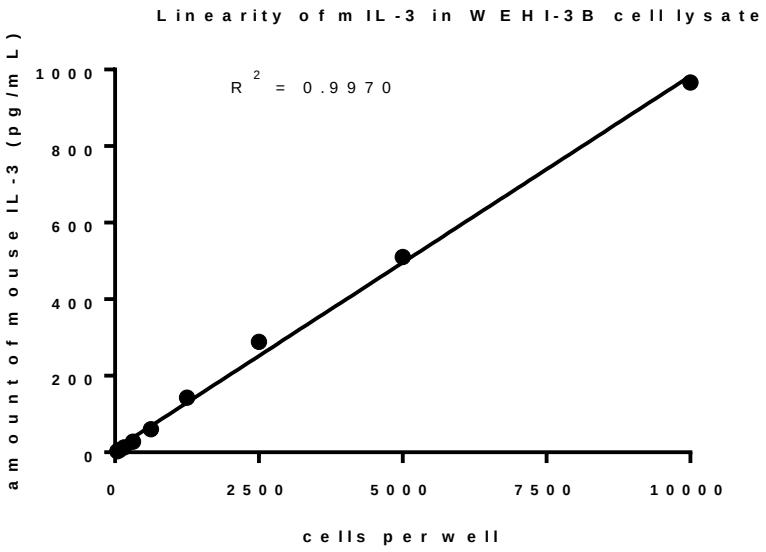
Spiked mouse IL-3 (ng/mL)	Media tested					
	HiBlock buffer	Lysis Buffer	DMEM + 10% FBS	RPMI + 10% FBS	Iscove medium + 10% FBS	FBS
10	110	121	114	111	110	121
1	104	109	104	90	103	129
0.1	98	99	91	110	98	102

Cell lysates Experiments

To validate the assay kit, the mouse cell line WEHI-3B (mouse leukemia) was used. The standard curve should be performed in AlphaLISA Lysis Buffer.

WEHI-3B were grown in Iscove modified Dubelcco's medium + 10% FBS to confluence and then plated into wells of a 96 well culture plate with the following amounts:
10 000; 5 000; 2 500; 1 250; 625; 313; 157; 79; 39 and 0 cells in Iscove modified Dubelcco's medium + 10% FBS.
The cells were left overnight.
The plate was centrifuged for 5 minutes at 1500 g, and the supernatant was carefully taken out and deposited in wells of a 96 well polypropylene plate.
To the cell pellet was added 100 uL of AlphaLISA Lysis buffer 1X and the plate was incubated for 30 minutes at 37ºC.
The lysate was tested against a standard curve of analyte in Lysis Buffer.

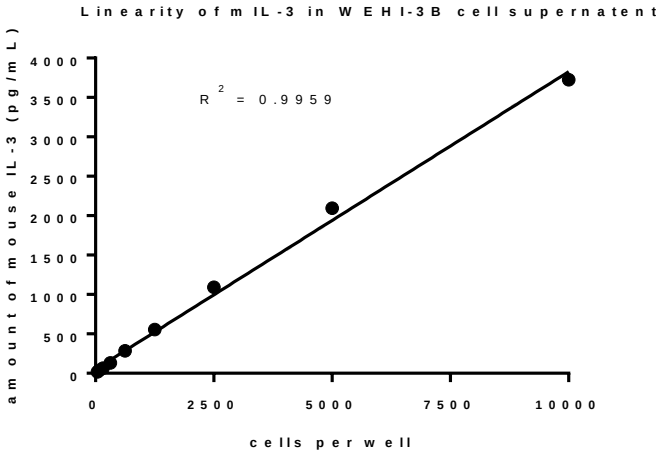
Extrapolation on the standard curve show that the expression of IL-3 is linear to the number of cells present.



Cell supernatant Experiments

The cell supernatants collected above were also tested using a standard curve of mouse IL-3 diluted in Iscove modified Dubelcco's medium + 10% FBS

Extrapolations of the results for treated cells on the standard curve show linear expression.



Mouse Serum and Plasma Experiments

Known concentrations of analyte were spiked into mouse serum or mouse plasma. Standard curves were prepared in the same matrix as the samples. All other components were diluted in HiBlock buffer.

Spiked mouse IL-3 (ng/mL)	Media tested	
	Mouse serum	Mouse plasma
10	88	94
1	79	89
0.1	92	81

Specificity Experiments

Cross-reactivity of the mouse IL-3 kit was tested using the following proteins up to 1 µg/mL in HiBlock buffer.

Protein	% Cross-reactivity
Human IL-3	0
Rat IL-3	100*

* Mouse and rat IL-3 sequences are identical.

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

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