

AlphaLISA IL-17A (Mouse / Rat) Biotin-Free Detection Kit

Product number: AL590 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 mouse and rat Interleukin 17A (IL-17A) in cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The kit utilizes a Digoxigenin (DIG) / Anti-DIG interaction as opposed to the traditional Streptavidin/Biotin interaction. This enables optimal performance when working with biotin-rich media (e.g. RPMI) or samples containing endogenous biotin (e.g. milk, brain extracts).
- Sensitivity:** Lower Detection Limit (LDL): 4.1 pg/mL
Lower Limit of Quantification (LLOQ): 13.4 pg/mL
EC₅₀: 31.5 ng/mL
- Dynamic range:** 4.1 – 100 000 pg/mL (Figure 1).

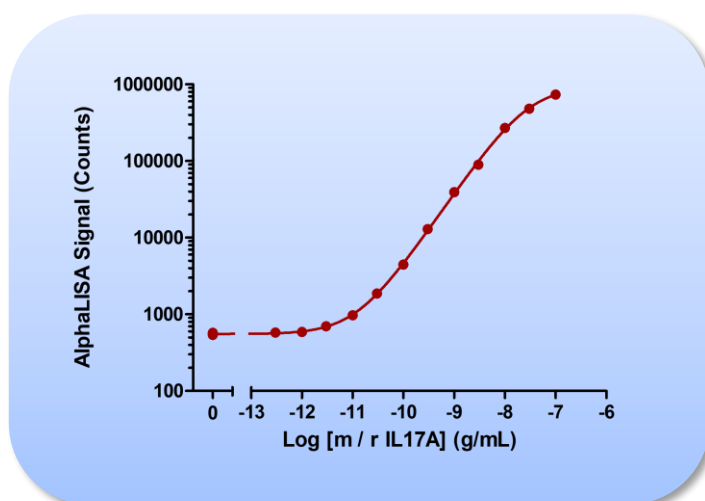


Figure 1. Typical sensitivity curves in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2103.

- Storage:** Store kit in the dark at +4°C. Store reconstituted analyte at -20°C.
- Stability:** This product is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions. Note: Once reconstituted, the mouse IL17A analyte is stable for at least 75 days at -20°C.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC₅₀ 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

Interleukin 17A (IL-17A) is the founding member of a group of cytokines called the IL-17 family. The other members are IL-17B, C, D, E, and F. Mature mouse or rat IL17A is a glycosylated homodimer formed of two 133 amino acid subunits. IL-17A is produced by a subset of T helper cells. It is a proinflammatory cytokine that enhances T cell priming and stimulates macrophages, fibroblasts, endothelial and epithelial cells to produce mediators of inflammation like IL1, IL-6, and TNF α . It is also critical for neutrophil activation and migration, and induces IL8, a key chemokine for neutrophils. IL-17 has been involved in the proinflammatory patterns associated with joint inflammation and rheumatoid arthritis in mouse and human models and may be a novel therapeutic target for the treatment of these diseases.

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 DIG-labeled Anti-Analyte Antibody binds to the anti-DIG 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).

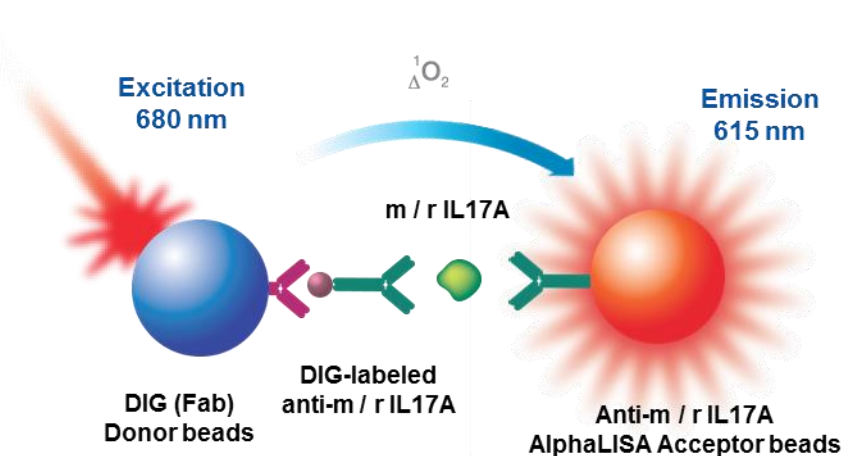


Figure 2. AlphaLISA Assay principle.

Precautions

- Anti-Digoxigenin Fab Fragment 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 Mouse source.
- Some analytes are present in saliva. Take precautionary measures to avoid contamination of the reagent solutions.
- The DIG labeled anti-analyte antibody is toxic. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

| Kit components | AL590HV (100 assay points ^{***}) | AL590C (500 assay points ^{***}) | AL590F (5000 assay points ^{***}) |
|--|---|--|--|
| AlphaLISA Anti-m / r IL17A 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) |
| Anti-Digoxigenin Fab Fragment Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4 | 80 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap) | 200 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap) | 2 x 1 mL @ 5 mg/mL (2 brown tubes, <u>black</u> caps) |
| DIG labeled Anti-m / r IL17A stored in PBS, 0.1% Tween-20, 0.05% NaN ₃ , pH 7.4 | 20 µL @ 500 nM (1 tube, <u>black</u> cap) | 50 µL @ 500 nM (1 tube, <u>black</u> cap) | 500 µL @ 500 nM (1 tube, <u>black</u> cap) |
| Lyophilized mouse IL17A* | 0.1 µg 1 tube, <u>clear</u> cap | 0.1 µg 1 tube, <u>clear</u> cap | 0.1 µg 1 tube, <u>clear</u> cap |
| AlphaLISA Immunoassay Buffer (10X)** | 2 mL, 1 small bottle | 10 mL, 1 medium bottle | 100 mL, 1 large bottle |

* Reconstitute mouse IL17A in 100 µL Milli-Q® grade H₂O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped polypropylene vials and stored at 4°C for further experiments. Avoid multiple freeze-thaw cycles. It has been demonstrated that reconstituted mouse IL17A is stable for at least 75 days at -20°C. One vial contains an amount of mouse IL17A sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL588S).

** 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 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.

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 H₂O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer 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 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.

| Format | # of data points | Volume | | | | Plate recommendation |
|---------|------------------|--------|--------|---|-------------|---|
| | | Final | Sample | AlphaLISA beads / Anti-DIG Antibody MIX | Donor beads | |
| AL590HV | 100 | 100 µL | 10 µL | 10 µL | 80 µL | White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560) |
| AL590C | 250 | 100 µL | 10 µL | 10 µL | 80 µL | White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560) |
| | 500 | 50 µL | 5 µL | 5 µL | 40 µL | White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350) |
| | 1 250 | 20 µL | 2 µL | 2 µL | 16 µL | Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) |
| | 2 500 | 10 µL | 1 µL | 1 µL | 8 µL | Light gray AlphaPlate-1536 (cat # 6004350) |
| AL590F | 5 000 | 50 µL | 5 µL | 5 µL | 40 µL | White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350) |
| | 12 500 | 20 µL | 2 µL | 2 µL | 16 µL | Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) |
| | 25 000 | 10 µL | 1 µL | 1 µL | 8 µL | Light gray AlphaPlate-1536 (cat # 6004350) |

High Concentration 2 step protocol - The 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:
Add 3 mL of 10X AlphaLISA Immunoassay Buffer to 27 mL H₂O.
- 2) Preparation of mouse IL17A analyte standard dilutions:
 - a) Reconstitute lyophilized mouse IL17A (0.1 µg) in 100 µL of H₂O.
 - b) Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

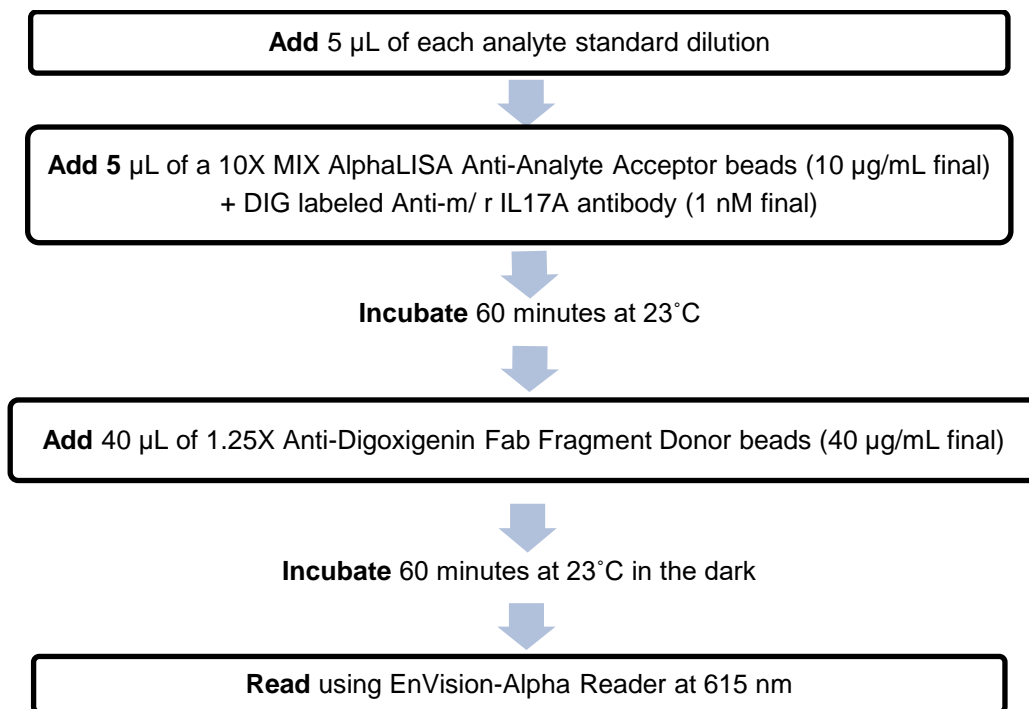
| Tube | Vol. of mouse IL17A (µL) | Vol. of diluent (µL) * | [mouse IL17A] in standard curve | |
|----------------------|------------------------------------|------------------------|---------------------------------|-----------------|
| | | | (g/mL in 5 µL) | (pg/mL in 5 µL) |
| A | 10 µL of reconstituted mouse IL17A | 90 | 1.00E-07 | 100 000 |
| B | 60 µL of tube A | 140 | 3.00E-08 | 30 000 |
| C | 60 µL of tube B | 120 | 1.00E-08 | 10 000 |
| D | 60 µL of tube C | 140 | 3.00E-09 | 3 000 |
| E | 60 µL of tube D | 120 | 1.00E-09 | 1 000 |
| F | 60 µL of tube E | 140 | 3.00E-10 | 300 |
| G | 60 µL of tube F | 120 | 1.00E-10 | 100 |
| H | 60 µL of tube G | 140 | 3.00E-11 | 30 |
| I | 60 µL of tube H | 120 | 1.00E-11 | 10 |
| J | 60 µL of tube I | 140 | 3.00E-12 | 3 |
| K | 60 µL of tube J | 120 | 1.00E-12 | 1 |
| L | 60 µL of tube K | 140 | 3.00E-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).
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 10X AlphaLISA Anti-m/ r IL17A Acceptor beads (100 µg/mL) + DIG labeled Anti-m/ r IL17A Antibody (10 nM) MIX:
 - a. Add 50 µL of 5 mg/mL AlphaLISA Anti-m/ r IL17A Acceptor beads and 50 µL of 500 nM DIG labeled Anti-m/ r IL17A Antibody to 2400 µL of 1X AlphaLISA Immunoassay Buffer.
 - b. Prepare just before use.

- 4) Preparation of 1.25X Anti-Digoxigenin Fab Fragment Donor beads (50 µg/mL):
- Keep the beads under subdued laboratory lighting.
 - Add 200 µL of 5 mg/mL Anti-Digoxigenin Fab Fragment Donor beads to 19800 µL of 1X AlphaLISA Immunoassay Buffer.
 - Prepare just before use.
- 5) 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) + 3x 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.

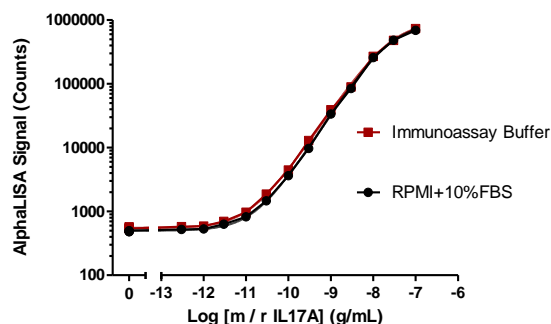
Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the High Concentration 2 step protocol.

- Assay Sensitivity:

The LDL was calculated as described in the data analysis section. The values correspond to the lowest concentration of analyte that can be detected using the recommended assay conditions.

| LDL (pg/mL) | Buffer/Media | # of experiments |
|-------------|--------------------|------------------|
| 4.1 | Immunoassay Buffer | 9 |
| 4.1 | RPMI with 10% FBS | 9 |



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

Intra-assay precision:

The intra-assay precision was determined using 3 independent experiments for a total of 16 independent determinations in triplicate. CV% were calculated for each individual experiment then averaged. Shown is the average intra-experimental CV%.

| m / r IL17A | Immunoassay Buffer | RPMI |
|-------------|--------------------|------|
| CV% | 5% | 4% |

- Inter-assay precision:

The inter-assay precision was determined using the data across 3 independent experiments with 16 measurements in triplicate. CV% was calculated by comparing the same measurement in each experiment. The CV% for all 16 measurements was then averaged. Shown is the inter-experimental CV%.

| m / r IL17A | Immunoassay Buffer | RPMI |
|-------------|--------------------|------|
| CV% | 11% | 11% |

- Spike Recovery:

Four known concentrations of analyte were spiked in AlphaLISA Immunoassay Buffer and cell culture media supplemented with 10% FBS. The spiked samples were referenced to the analyte curve produced in AlphaLISA Immunoassay Buffer and cell culture media.

| Spiked m IL17A (ng/mL) | % Recovery | |
|---------------------------|--------------------|------|
| | Immunoassay Buffer | RPMI |
| 1 | 103 | 99 |
| 0.3 | 103 | 98 |
| 0.1 | 98 | 100 |
| 0.03 | 103 | 102 |

- Specificity:

Cross-reactivity of the AlphaLISA m / r IL17A Kit was tested using the following proteins at 0.1 µg/mL in AlphaLISA Immunoassay Buffer.

| Protein | % Cross-reactivity |
|---------------|--------------------|
| Rat IL17A | 83 |
| Human IL17A | 0 |
| Rat IL17 AF | 80 |
| Mouse IL17 AF | 74 |
| Rat IL17F | 0 |
| Mouse IL17F | 0 |

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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