**TECHNICAL DATA SHEET**

AlphaPlex™-645 Research Reagents

# IL-17A AlphaPlex™-645 Immunoassay Kit

Product number: AP219SM-HV/C/F

Research Use Only. Not for use in diagnostic procedures.

## Contents

## Product Information

**Application:** This kit is designed for the quantitative determination of interleukin-17 in cell culture media, sera and plasma, using a homogeneous AlphaPlex assay (no wash steps).

**Sensitivity:** Lower Detection Limit (LDL): 18 pg/mL

Lower Limit of Quantification (LLOQ): 50 pg/mL

$EC_{50}$: 28 ng/mL

Min/Max counts: 340/ 155000 counts

**Dynamic range:** 55 - 100 000 pg/mL (Figure 1).

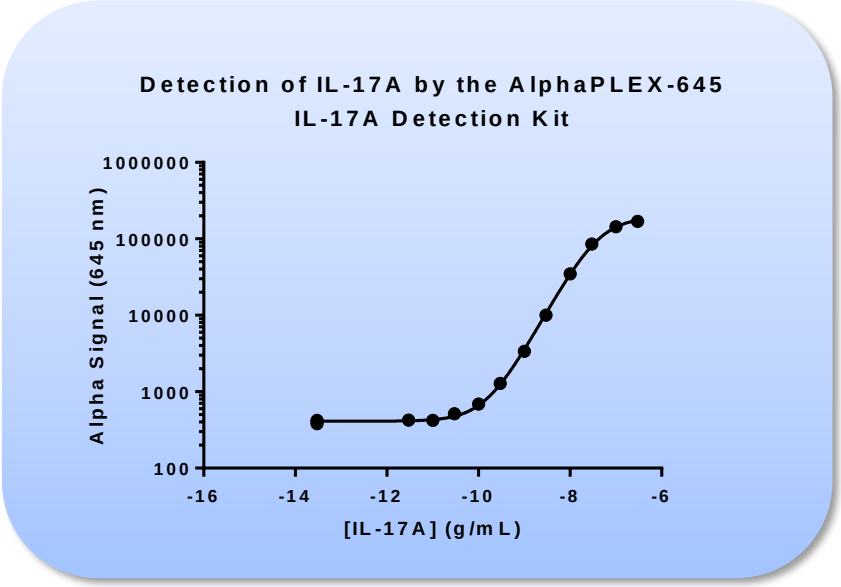


Figure. 1. Typical sensitivity curve in AlphaLISA Immunoassay buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2102.

**Storage:** The kit components must be stored dark at +4˚C.

**Stability:** This kit is stable for at least 12 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 AlphaPlex 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

Human Interleukin 17 (IL17 or IL17A) is a homodimer formed of two ~15 kDa subunits produced by a subset of T helper cells named Th17. It is a proinflammatory cytokine that enhances T cell priming and stimulates macrophages, fibroblasts, endothelial and epithelial cells to produce multiple mediators of inflammation like IL1, IL6, TNF-α, NOS-2, metalloproteases, and chemokines. IL17 has been implicated in the proinflammatory patterns associated with joint inflammation and rheumatoid arthritis (RA) in mouse and human models. It is also critical for neutrophil activation and migration, and induces IL8, a key chemokine for neutrophils. IL17 signals through IL-17R, which in mice has at least two members, IL-17RA, and IL-17RC. Recent studies suggest that the IL17 pathway may be a novel therapeutic target for the treatment of chronic inflammatory diseases like asthma and RA.

## Description of the AlphaPLEX Assay

AlphaPLEX 645 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 AlphaPLEX 645 assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-Analyte Antibody is conjugated to AlphaPLEX 645 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 645 nm (Figure 2).

Combining this assay with an AlphaLISA 615- or AlphaPLEX 545 - based kit will allow the quantification of 2 (or more) analytes in the same well.Indeed, the presence of two acceptor beads allow for the following assays:

- Two unrelated analyte measurements.
- Total versus modified analyte.
- Two different modifications on same analyte.
- Cascade effects.
- Protein-molecule interactions

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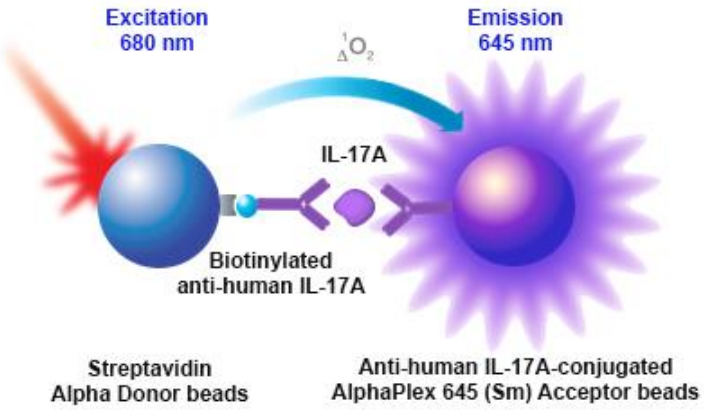



Figure 2. AlphaPLEX Assay principle.

## 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.
- 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 | AP219Sm HV (100 assay points***) | AP219Sm C (500 assay points***) | AP219Sm F (5 000 assay points***) |
|---|---|---|---|
| AlphaPLEX 645 Anti-IL-17A Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2 | 25 µL @ 5 mg/mL (1 brown tube, purple cap) | 50 µL @ 5 mg/mL (1 brown tube, purple cap) | 500 µL @ 5 mg/mL (1 brown tubes, purple caps) |
| Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4 | 100 µL @ 500 nM (1 tube, black cap) | 200 µL @ 500 nM (1 tube, black cap) | 2 mL @ 500 nM (1 tube, black cap) |
| Biotinylated Antibody Anti-IL-17A 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) |
| AlphaLISA IL-17A lyophilyzed standard | 1 tube, clear cap | 1 tube, clear cap | 1 tube, clear cap |
| AlphaLISA Immunoassay Buffer (10X) ** | 2 mL, 1 small bottle | 10 mL, 1 small bottle | 100 mL, 1 large bottle |

* The analyte should be resuspended in 100µL of distilled water and used within 60 minutes 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 the IL-17A analyte solution is stable for at least 6 months at -20°C. One vial contains an amount of IL-17A sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AP219S).

** Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100, 5% BSA and 0.5% Proclin-300. Extra buffer can be ordered separately (cat # AL004C: 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.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaPLEX 645 signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaPLEX 645 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. | 6050195 |
| 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 AlphaPLEX signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaPLEX 645 settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: 535/40, Center Wavelength 535 nm, Bandwidth 40 nm, Transmittance 75%) for sequential readings (645 then 615 reads).
- AlphaPLEX 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).
- AlphaPLEX assays should be performed in cell culture medium without phenol red, as phenol red will lower counts and sensitivity. The following recommendations should also be followed: 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.

## 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** | **AlphaPLEX 645 beads / Biotin Antibody MIX** | **SA-Donor beads** | **Plate recommendation** |
| **AP219Sm- HV** | 100 | 100 µL | 10 µL | 40 µL | 50 µL | White OptiPlate-96 (cat # 6005290)<br>White ½ AreaPlate-96 (cat # 6005560) |
| **AP219Sm- C** | 250 | 100 µL | 10 µL | 40 µL | 50 µL | White OptiPlate-96 (cat # 6005290)<br>White ½ AreaPlate-96 (cat # 6005560) |
| | 500 | 50 µL | 5 µL | 20 µL | 25 µL | White ½ AreaPlate-96 (cat # 6005560)<br>White OptiPlate-384 (cat # 6007290)<br>Light gray AlphaPlate™-384 (cat # 6005350) |
| | 1 250 | 20 µL | 2 µL | 8 µL | 10 µL | Light gray AlphaPlate-384 (cat # 6005350)<br>ProxiPlate™-384 Plus (cat # 6008280)<br>White OptiPlate-384 (cat # 6007290) |
| | 2 500 | 10 µL | 1 µL | 4 µL | 5 µL | Light gray AlphaPlate-1536 (cat # 6004350) |
| **AP219Sm- F** | 5 000 | 50 µL | 5 µL | 20 µL | 25 µL | White ½ AreaPlate-96 (cat # 6005560)<br>White OptiPlate-384 (cat # 6007290)<br>Light gray AlphaPlate-384 (cat # 6005350) |
| | 12 500 | 20 µL | 2 µL | 8 µL | 10 µL | Light gray AlphaPlate-384 (cat # 6005350)<br>ProxiPlate-384 Plus (cat # 6008280)<br>White OptiPlate-384 (cat # 6007290) |
| | 25 000 | 10 µL | 1 µL | 4 µL | 5 µL | Light gray AlphaPlate-1536 (cat # 6004350) |

**High sensitivity protocol (3 incubation steps) – Dilution of standards in 1X AlphaLISA Immunoassay Buffer**

The protocol described below is recommended when generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations with manual pipetting). *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 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL $H_2O$.

2) Preparation of IL-17A analyte standard dilutions:

- The provided IL-17A standard is provided at as 0.1µg lyophilized stock. The analyte must be resuspended in 100µL of MilliQ grade water before use.
- Prepare standard dilutions as follows (change tip between each standard dilution) in 1X AlphaLISA Immunoassay Buffer or the matrix used for the analysis.

| Tube | **Vol. of Human IL-17A (µL)** | **Vol. of diluent (µL) *** | **[hIL-17A] in standard curve (pg/mL in 5 µL)** |
|---|---|---|---|
| A | 10 µL of resuspended IL-17A | 90 | 1 000 000 |
| B | 60 µL of tube A | 140 | 300 000 |
| C | 60 µL of tube B | 120 | 100 000 |
| D | 60 µL of tube C | 140 | 30 000 |
| E | 60 µL of tube D | 120 | 10 000 |
| F | 60 µL of tube E | 140 | 3 000 |
| G | 60 µL of tube F | 120 | 1 000 |
| H | 60 µL of tube G | 140 | 300 |
| I | 60 µL of tube H | 120 | 100 |
| J | 60 µL of tube I | 140 | 30 |
| K | 60 µL of tube J | 120 | 10 |
| L | 60 µL of tube K | 140 | 3 |
| M ** (background) | 0 | 100 | 0 |
| N ** (background) | 0 | 100 | 0 |
| O ** (background) | 0 | 100 | 0 |
| P ** (background) | 0 | 100 | 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/LLOQ is calculated. If LDL/LLOQ does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 2.5X AlphaPLEX-645 Anti-IL-17A Acceptor beads and biotinylated anti-IL-17A antibody (25 µg/mL / 2.5 nM):
Add 50 µL of 5 mg/mL AlphaPLEX 645 Anti-IL17A Acceptor beads and 50 µL of 500 nM Biotinylated Antibody Anti-IL17A to 9 900 µL of 1X AlphaLISA Immunoassay Buffer. Prepare just before use.

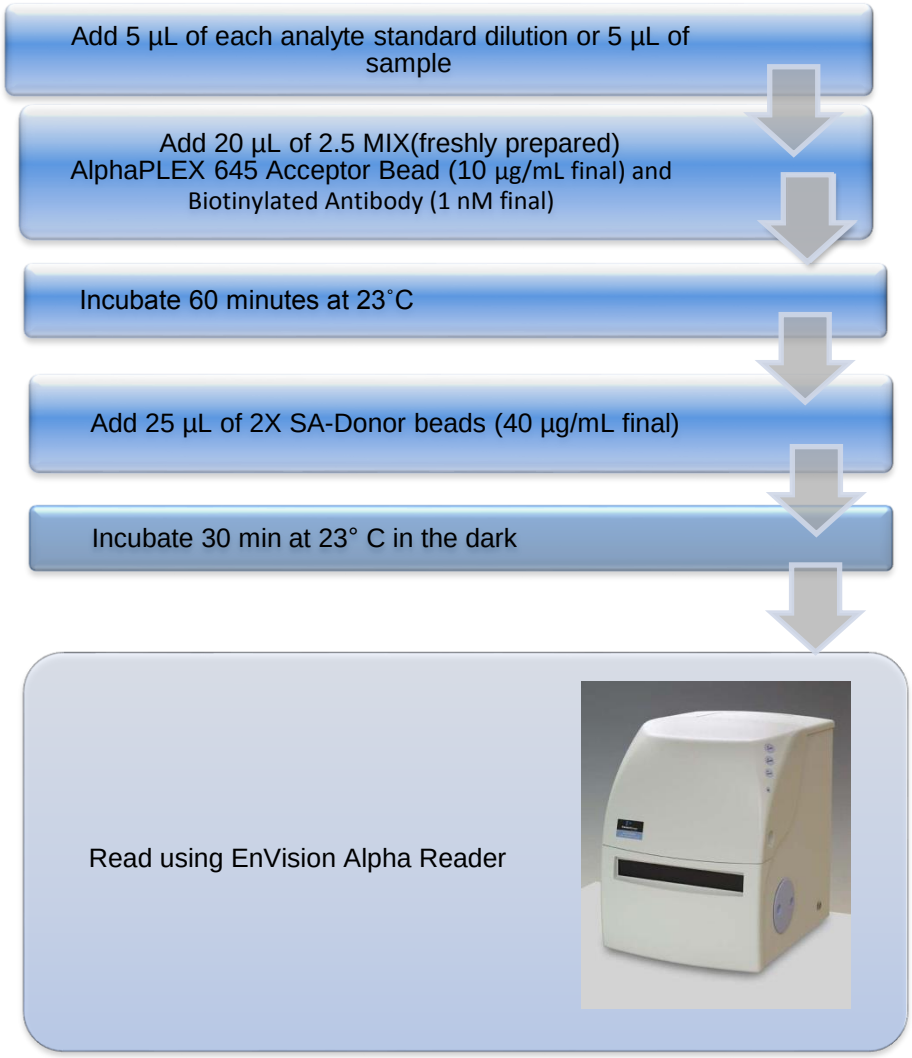
4) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL): Keep the beads under subdued laboratory lighting.

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:



## Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaPLEX 645 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

*AlphaPLEX assay performance described below was determined using the quick protocol.*

**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) | LLOQ (pg/ml) | Buffer/Media used | # of experiments |
|---|---|---|---|
| 13 | 55 | AlphaLISA Immunoassay Buffer | 10 |

* Note that LDL/ LLOQ 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 five independent assays using three different kit lots. In each lot, the analytes were prepared in AlphaLISA Immunoassay Buffer (IAB). 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 a total of 6 independent determinations in triplicate. Shown CV%.

| IL-17A (ng/ml) | IAB | Depleted Serum |
|---|---|---|
| **30** | 3% | 5% |

- Inter-assay precision:

The inter-assay precision was determined using a total of 6 independent determinations.

| IL-17A (ng/ml) | IAB | Depleted Serum |
|---|---|---|
| **30** | 5% | 12% |

- Recovery:

Three known concentrations of analyte were spiked into DMEM cell culture media containing 10% FBS,RPMI cell culture media containing 10% FBS, HAT cell culture media containing 10% FBS and AlphaLISA Immunoassay Buffer (IAB). All samples were run alongside a standard curve diluted in AlphaLISA immnunoassay Buffer, this standard curve was used to interpolate the concentrations of the samples. The percent recovery is defined as assay measured concentration with respect to the spiked concentration. The average recovery from two independent measurements is reported.

| Spike (IL-17A ng/mL) | %Recovery | |
|---|---|---|
| | AlphaLISA Immunoassay Buffer | Depleted Serum |
| **30** | 81% | 88% |
| **10** | 90% | 66% |
| **3** | 75% | 73% |

## Troubleshooting Guide

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

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

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