APPLICATION NOTE

An evaluation of the EnVision Nexus multimode plate reader for AlphaLISA cytokine assays.

For research purposes only.
Not for use in diagnostic procedures.

Abstract

This application note will evaluate the performance of the newly released EnVision® Nexus™ multimode plate reader using Alpha technology. A total of 4 different AlphaLISA® cytokine detection assays (IFNγ, IL-17A, IL-5, IL-2) were run in parallel on the EnVision® in 'Standard Alpha' mode and EnVision Nexus plate readers in 'Enhanced Alpha' mode. In contrast to Standard Alpha, Enhanced Alpha works with a special aperture to block straylight from neighboring wells & reduces crosstalk. These two read modes are treated as equivalent as they are both the most basic Alpha read mode available on the reader. The results demonstrated that the EnVision Nexus had consistently higher AlphaLISA signal than the EnVision. The EC50 for each AlphaLISA cytokine assay was similar across the plate readers and detection modes. The Lower Limit of Quantification (LLOQ) range for the AlphaLISA cytokine assays was either slightly better on the EnVision Nexus or the same for both plate readers.

Introduction

The EnVision line of multimode plate readers has been the proven leader in high-throughput screening for over 20 years, and the EnVision Nexus (#HH36000002) is the next-generation plate reader with enhanced speed and sensitivity. The EnVision Nexus works with all standard microplates, including 24-, 48-, 96-, 384- and

1536-well formats. This state-of-the-art plate reading platform supports both standard and advanced detection technologies with excellent dynamic range, kinetic and scanning measurements, and bottom reading. The EnVision Nexus comes standard with the capability of measuring absorbance, fluorescence intensity (including FRET), luminescence (including BRET), time-resolved fluorescence (TRF) and TR-FRET, as well as fluorescence polarization. Optional advanced technologies include Alpha (with both 'Enhanced' and 'HTS' modes), TRF Laser, and Ultrasensitive Luminescence. This application note evaluates the EnVision Nexus plate reader using Enhanced Alpha technology.

Materials and methods

AlphaLISA immunoassays offer a homogenous (no-wash) alternative to ELISAs, with a simple, streamlined workflow that can be used to detect and quantitate biomolecules in both simple and complex sample types. AlphaLISA is a bead-based luminescent amplification assay, offering greater sensitivity, a wider dynamic range, and smaller sample sizes over traditional technologies. In a conventional AlphaLISA assay, a biotinylated antibody and an antibody-conjugated AlphaLISA Acceptor bead are used to capture the target analyte. The biotinylated antibody associates with an Alpha streptavidin-coated Donor bead. When the analyte is present in the sample, the Donor and Acceptor beads are brought together. Upon excitation, a photosensitizer inside the Donor bead converts ambient oxygen to an excited singlet state. Singlet oxygen diffuses up to 200 nm to produce a chemiluminescent reaction in the Acceptor bead, leading to light emission. The amount of light is proportional to the amount of analyte present in the sample. An illustration depicting AlphaLISA immunoassay technology is provided in Figure 1. Common applications for AlphaLISA are the detection of biomarkers, characterization of protein kinases, cytokines, and GPCRs, as well as epigenetics and protein-protein interactions.

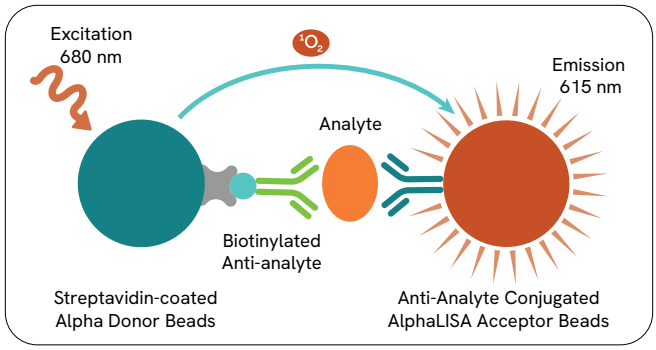

Figure 1: Detection of cytokine using AlphaLISA immunoassay technology.

The product information for the AlphaLISA cytokine detection assays used for this work is listed in Table 1. The analyte standards provided with each kit were prepared and serially diluted following the instructions found in the respective AlphaLISA kit manual. The diluted standards were transferred in triplicate to a 384-well AlphaPlate, gray (6005350). The AlphaLISA kit reagents were prepared and combined with analyte standards according to the protocol listed in each cytokine detection kit manual. The cytokine analyte standard curves were plated on triplicate AlphaPlates (3 plates for each AlphaLISA cytokine detection assay). The AlphaLISA signal was read on EnVision and EnVision Nexus plate readers. The raw data from the analyte standard curves were fit using nonlinear regression and a 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) with a 1/Y2 data weighting using GraphPad Prism 9.5.1. Data values past the hook point of the curve were removed to ensure a proper fit. The EC50 for each curve (Figure 2) was calculated using GraphPad Prism software. The cytokine analyte standard curves for each AlphaLISA Cytokine Detection Kit are provided in Figure 2. The LLOQ (average AlphaLISA signal (counts) + (10 x the standard deviation)) was calculated for each kit from 12 background wells containing only detection reagents (no analyte) prepared in assay buffer (provided with each kit). The LLOQ is given as a range representing the data acquired from triplicate AlphaPlates (Figure 2).

Alpha technology is optimal for detecting proteins in cell culture, supernatant, cell lysate, serum, plasma, urine, saliva, and CSF - making it the ideal homogenous immunoassay for cytokine research.

Table 1: AlphaLISA Cytokine Detection Assays were used to evaluate the performance of the EnVision Nexus multimode plate reader.

Description	AlphaLISA Cytokine Detection Kit	Product #
IFN gamma is a pro-inflammatory cytokine secreted by T and NK cells in response to viral infection and other inflammatory conditions. IFN gamma plays a major role in innate and adaptive immunity.	High-Performance Human IFNγ Detection Kit	AL3153
IL-17A is a proinflammatory cytokine that enhances T-cell priming and stimulates macrophages, fibroblasts, endothelial, and epithelial cells to produce multiple mediators of inflammation such as IL-1, IL-6, TNF-α, NOS-2, metalloproteases, and chemokines. IL-17A has been implicated in the proinflammatory patterns associated with joint inflammation and rheumatoid arthritis (RA) in mouse and human models.	High-Performance Human IL-17A Detection Kit	AL3161
IL-2's major role is to modulate cell-mediated immunity through its effect on T cells. IL-2 promotes the differentiation of T cells into effector or memory T cells, thus helping the body fight off infection. IL-2 is also a key player in immuno-oncology since it is the main promoter of the differentiation of T cells into Treg (regulatory T cells).	High-Performance Human IL-2 Detection Kit	AL3155
IL-6 is a pro-inflammatory cytokine involved in acute-phase reaction, inflammation, and cancer progression. Secreted by T cells, macrophages, and fibroblasts, it induces B and T cell proliferation. It is being studied in a wide variety of research areas including diabetes, Alzheimer's disease, depression, and several cancers. Along with TGF beta, IL-6 is the main promoter of T cell differentiation into TH17, a new component of immuno-oncology.	Human IL-6 Detection Kit	AL223

A table summarizing the plate read time for the relative read speeds for each plate reader is provided in Table 2.

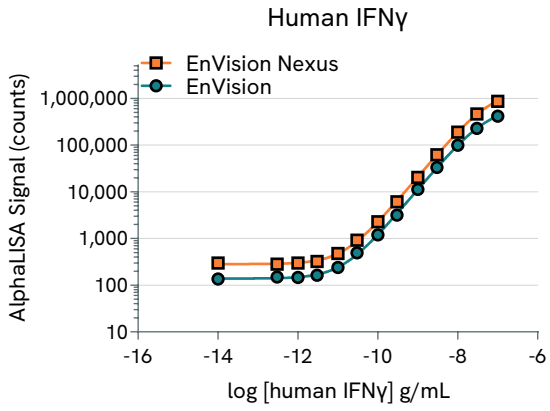

Plate Reader	EC50 (ng/mL)	LLOQ (pg/mL)
EnVision Nexus	64.6	2.2 - 5.0
EnVision	53.0	5.0 - 10.0

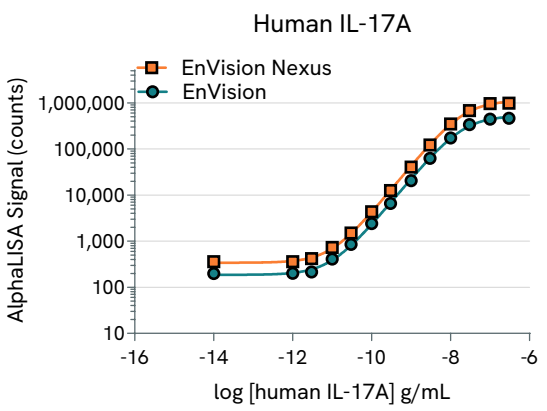

Plate Reader	EC50 (ng/mL)	LLOQ (pg/mL)
EnVision Nexus	22.1	4.0 - 10.0
EnVision	20.9	5.0 - 21.0

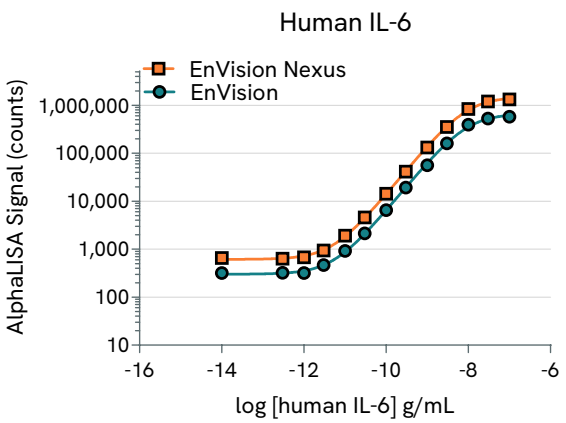

Plate Reader	EC50 (ng/mL)	LLOQ (pg/mL)
EnVision Nexus	9.2	5.0 - 10.0
EnVision	9.2	5.0 - 10.0

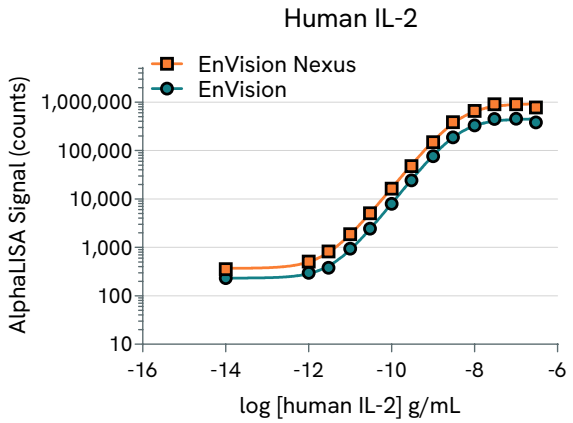

Plate Reader	EC50 (ng/mL)	LLOQ (pg/mL)
EnVision Nexus	4.6	0.1 - 0.25
EnVision	4.6	0.1 - 0.25

Figure 2: Analyte standard curves are shown for each of the AlphaLISA Cytokine Detection Kits measured by both EnVision plate readers using the 'Enhanced' Alpha modeThe EC50 for each curve is provided in the tables directly below each standard curve in addition to the LLOQ calculated from 12 background wells from each triplicate AlphaPlate given as a range.

Table 2: The relative plate read times for EnVision Nexus and EnVision plate readers using a gray, 384-well AlphaPlate.

Reader	Mode	Read Time (mm:ss)
EnVision Nexus	Enhanced Alpha	4:12
EnVision	Standard Alpha	4:32

Results and discussion

AlphaLISA Cytokine Detection Assays were run on both the EnVision Nexus and EnVision plate readers The EnVision Nexus consistently produced AlphaLISA signal slightly higher than the EnVision. As expected, the range of EC50 values calculated for the cytokine standard curves from triplicate plates was similar between the EnVision plate readers and detection modes (Figure 2) demonstrating that the AlphaLISA Cytokine Detection Kit results are consistent regardless of the plate reader used. These results highlight how the next-generation EnVision Nexus multimode plate reader delivers the performance needed to support AlphaLISA applications.

For high-throughput AlphaLISA applications, the EnVision Nexus can also be equipped with a High-Throughput Alpha mode, though not covered in this application note. High-Throughput (HTS) Alpha has a separate detector and beam path, and is optimized for speed in high-throughput applications. HTS-Alpha also utilizes a special aperture to block straylight for best performance. In contrast with previous EnVision readers, the HTS Alpha mode can be configured alongside the Enhanced Alpha mode – providing the flexibility to perform a variety of AlphaLISA applications in a single instrument.

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