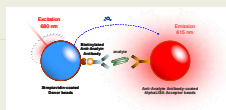


## 1 Introduction

The new EnSpire™ is a user-friendly reader providing short assay start-up times for occasional users. There are two EnSpire models available: EnSpire™ and EnSpire™ AlphaPLUS. EnSpire™ is a monochromator based plate reader for running any absorbance or fluorescence quantification assay. EnSpire™ AlphaPLUS model is specifically designed for AlphaLISA® and absorbance based ELISA detection retaining all the high quality features of PerkinElmer's AlphaScreen® microplate readers, including a highly sensitive laser-based detection system.

AlphaLISA® /AlphaScreen® technology is a state of the art immunoassay and biomarker detection method. In addition to the homogenous (no-wash) nature, outstanding assay sensitivity and dynamic range it is very versatile and straightforward. Quantitation methods like protein and nucleic acids (DNA/RNA) quantitation or estimation assays are important in life science research laboratories.



## 2 Material and Methods

The AlphaLISA IL17 (Cat. No. AL219) and Aβ40 (Cat. No. AL202) detection kits were prepared according to kit inserts. 96-well Optiplate® (Cat. No. 6005290) and 384-well Optiplate (Cat. No. 6007299) microplates used in assays were from PerkinElmer.

DNA dilution series was prepared by dissolving the purified and lyophilized DNA (Salmon testes DNA; #D1626, Sigma) in water.

The Quant-iT™ PicoGreen® dsDNA Assay was performed according to kit insert (# P11496, Molecular Probes) in a black opaque microplate (OptiPlate-96; #6005279, PerkinElmer).

Bradford assay was performed according to Bio-Rad's microassay procedure for microplates (#500-0006, Bio-Rad).

## 2 EnSpire AlphaPLUS Results

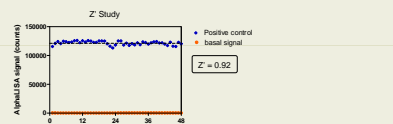
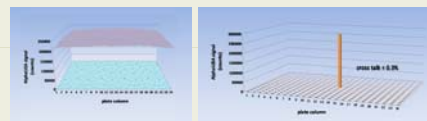
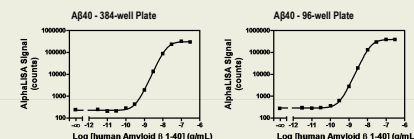
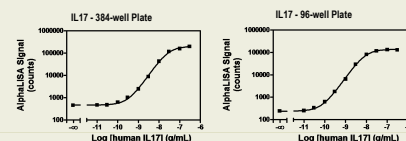


Plate reading consistency and crosstalk behavior were studied with IL17 AlphaLISA assay to assess the robustness of the reader in AlphaLISA® detection.

Full plate coefficient of variation (CV) was 3 %. The cross-talk was less than 0.3% in the system without utilizing the cross-talk correction feature of the EnSpire. Z'-factor value of 0.92 was obtained with the IL17 assay.



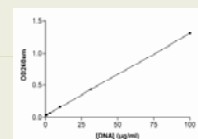
The EnSpire™ AlphaScreen performance was evaluated with two AlphaLISA assays: human Interleukin-17 (IL17) and Amyloid beta 40 (Aβ40).

Lowest detection limits (LDL) in IL17 assays were 32 pg/mL and 16 pg/mL in 384- and 96-well plates, respectively. In Aβ40 assays the obtained LDLs were 126 pg/mL and 131 pg/mL for 384- and 96-well plates, respectively.

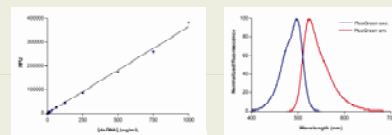
These sensitivities are in agreement with reported LDLs using the EnVision Multilabel Plate Reader.

## 4 EnSpire Results

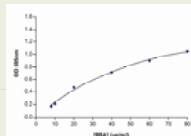
The flexibility of the EnSpire™ monochromator enables wavelength scanning assays (FI/Abs) in addition to single wavelength measurements. This was evaluated by quantitating nucleic acid and protein concentrations using direct UV-absorbance method at 260/280 nm and by using more sensitive fluorescent dyes.



Direct absorbance-based DNA quantitation assay. A dilution series from 0.1 to 100 μg/ml was prepared in triplicates and transferred into a UV-absorbance compatible microplate (96-well UV-Star® plate; #655801, Greiner) and read absorbance at 260 nm.



The fluorescence based dsDNA quantitation assay. Linear range covers four decades of DNA concentrations (approx. 0.1 – 1000 ng/ml) highlighting the benefits of using fluorescence based approach over an UV-absorbance methods for DNA quantitation applications. The used wavelengths for excitation and emission (496/525 nm) were chosen based on excitation and emission spectra of PicoGreen dye.



Bradford Protein Assay. Albumin from bovine serum (BSA; #05479, BioChemika) was dissolved in water to 2 mg/ml and a dilution series was prepared following Bio-Rad's microassay procedure for microtiter plates (0-80 μg/ml, 4 replicates).

Assay was performed in clear bottom 96-well SpectraPlate MB (#6005279, PerkinElmer). The Protein Dye Concentrate was mixed with the protein dilution samples and added to wells. Plate was shaken and incubated at room temperature prior to reading the absorbance at 595 nm with the EnSpire Multilabel Plate Reader. All data was analyzed with GraphPad Prism®.

## 6 Summary

The EnSpire Multilabel Plate Reader combines an easy-to-use and high performance instrument for fluorescence and absorbance assays. Bottom reading in fluorescence technology enables optimal detection for cellular assays. As demonstrated by these three common quantification assays and touch screen-based software interface with built-in data analysis capabilities make it an excellent choice to many types of laboratories.

The EnSpire AlphaPLUS Multilabel reader is a high performance and easy to use AlphaScreen/AlphaLISA reader that will satisfy the most demanding biochemical detection assays.

Results demonstrate an excellent assay performance for two AlphaLISA products, IL17 and Aβ40 kits, developed earlier using the EnVision Multilabel reader.

A full range of AlphaLISA reagents is available for use with the EnSpire. These include fully optimized detection kits and standalone reagents for easy plug-and-play assay development.

