Evaluating a TNF-α immunoassay using EnSpire AlphaPLUS: ELISA and AlphaLISA technologies

Introduction

Immunoassays are used for detection and quantification of low analyte concentrations. Enzyme Linked-Immuno-Sorbent Assay (ELISA) technology is the most widely adopted assays method for performing sandwich or competition-based immunoassays. ELISA is a heterogeneous (non-homogeneous) technology offering great selectivity, sensitivity and assay versatility, but it requires multiple assay steps, including plate washing.

The AlphaLISA® assay platform (based on AlphaScreen® technology) offers sensitive and homogeneous "mix-and-measure" technology for performing immunoassays. Since it does not require any wash steps, assay development is simple and fast. Additionally, hands-on time, as well as total assay time, is greatly reduced. AlphaLISA provides sensitive results with a broad dynamic assay range. It is also an enabling technology for applications that require measuring low affinity interactions between two or more binding partners. Most of the ELISA-based immunoassays can be converted to AlphaLISA technology.

The EnSpire™ Multilabel Plate Reader with AlphaPLUS supports measurement of both traditional ELISA and homogeneous AlphaLISA immunoassays. A flexible, easy-to-use instrument, EnSpire includes intuitive user software for running any absorbance or AlphaScreen applications. Setting up measurement protocols with the EnSpire reader has been made simple and easy. The software can be controlled by using the *touch screen*. New or existing measurement protocols are easily started without any prior instrument knowledge or training. EnSpire measures plates very quickly and therefore works in a screening environment as well.

This application note describes running Tumor Necrosis Factor Alpha (TNF-α) ELISA and AlphaLISA assay kits using the EnSpire AlphaPLUS Plate Reader. TNF-α is a multi-functional pro-inflammatory cytokine synthesized mainly by nucleated blood cells. TNF-α plays a role in lipid metabolism, coagulation and endothelial function and has been associated with cancer, infection and inflammation, ischemia/reperfusion injury and heart failure, and insulin resistance. TNF-α assays require high sensitivity and a broad dynamic range.

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This assay is well suited for evaluating both the AlphaLISA assay technology and the EnSpire reader performance.

Materials and Methods

Materials required

- EnSpire AlphaPLUS Multilabel Plate Reader
- AlphaLISA TNF-α Kit (Cat. No. AL208C)
- Invitrogen® Human TNF-α Immunoassay kit (Cat. No. KHC3012)
- AlphaLISA human TNF-α (0,1 µg), lyophilized analyte (Cat. No. AL208S)
- TopSeal-A™ Adhesive Sealing Film (Cat. No. 6005185)
- OptiPlate® microplates, white 384-well (Cat.No. 6007290) and 96-well (Cat.No. 6005500)
- Pipettes, Eppendorf®and Falcon tubes

Preparing the assay reagents

Both AlphaLISA TNF-α and Invitrogen ELISA TNF-α assays were prepared according to the kit instructions. For testing the dynamic range in the ELISA assay, additional dilutions of TNF-α analyte (Cat. No. AL208S) were prepared (2,000, 10,000 and 50,000 pg/mL). Samples (dilutions) were pipetted into plates in replicate (ELISA) or triplicate (AlphaLISA). The ELISA assay was performed on 96-well plates and AlphaLISA on 96- and 384-well plates.

Pipetting of donor beads (AlphaLISA) was performed in low light laboratory conditions (<100 lux) to protect light-sensitive reagents. For the same reason, the final incubation step was performed in the dark. Plates were incubated in a covered box next to the instrument to maintain the same temperature during the incubation and measurement.

ELISA and AlphaLISA measurements

Starting a measurement is initiated by clicking the *Run protocol* button and selecting a factory-set (validated) protocol. Measurement starts by clicking the same button (*Run protocol*) again (Figure 1). The TNF-α ELISA assay was measured using the *Photometric 96-well plate* protocol. The *AlphaScreen 384-well OptiPlate* and *AlphaScreen 96-well OptiPlate* protocols were used for running AlphaLISA TNF-α assay.

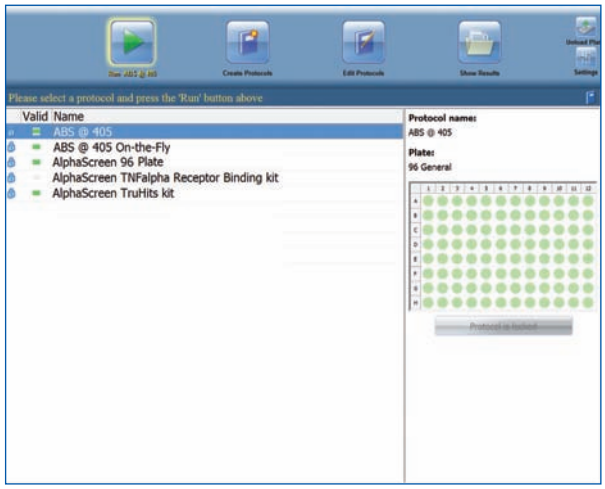

Figure 1. **Starting measurement protocols in EnSpire software**. The software can be controlled by using the *touch screen*.

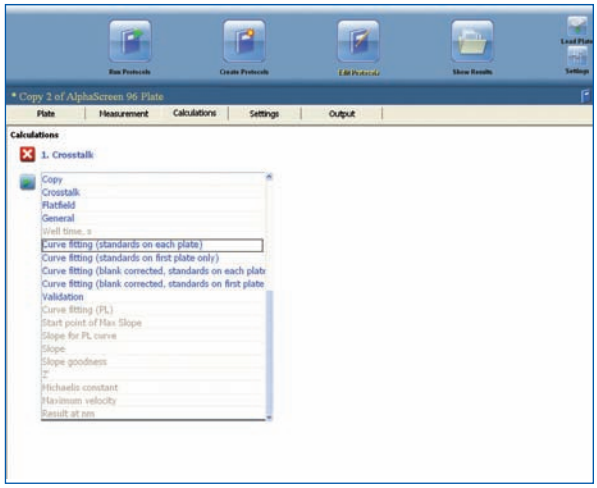

Figure 2. **Adding curve fitting calculation** in the EnSpire software.

Analyzing results

EnSpire software includes data analysis functions such as standard curve fitting and calculations of unknown samples. These are attached to a measurement protocol by defining the sample types in a plate map (std., unknowns etc.).

This will activate a standard curve calculation following entry of the concentrations of standards (Figure 2). After an assay run, EnSpire reports the standard curve and concentrations for unknown samples.

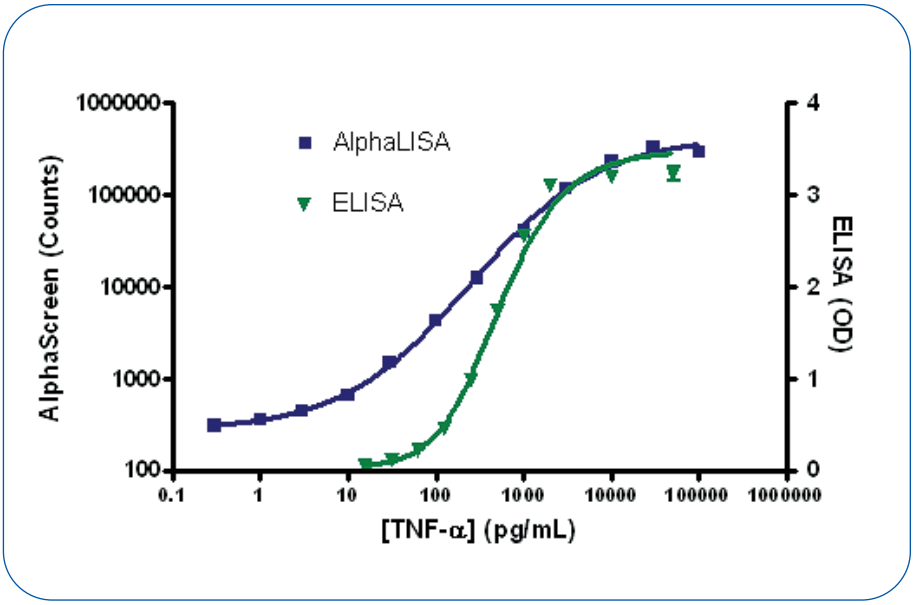

Figure 3. **Dilution curves of TNF-α ELISA** and AlphaLISA assays (96-well plate).

Results from these TNF-α assays were copied to MS Excel and followed by PRISM (GraphPad Software Inc) analysis for standard curves. Lowest detection limits (LDL) were calculated using the equation:

LDL = Average (blank) + 3SD (blank) (equation 1)

Results and Discussion

Results obtained from ELISA TNF-α assay show high data consistency and good sensitivity (Figure 3). The lowest detection limit obtained, 16 pg/mL, was equal to typical values reported in the kit insert. Signal saturation occurred at concentrations >2,000 pg/mL. Linear and dynamic ranges were limited to 1 and 2 orders of magnitude, respectively.

The AlphaLISA TNF-a assay generates a broader concentration range and offers better sensitivity (Figure 3). The highest detectable concentration level was at 50,000 pg/mL. Linear and dynamic ranges were 2.5 and 4 orders of magnitude, respectively. Lowest detection limit for in AlphaLISA TNF-α assay was 2.2 pg/mL.

Homogeneous AlphaLISA is automation friendly technology and works equally in 96-well and 384-well plates. Changing plate density does not require any volume adjustment; both format had total volume of 50 µL/well (Figure 3).

Traditional ELISA represents a commonly used immunoassay method. The enzyme and substrate reaction in ELISA produces a colored reaction product and requires photometric measurement of absorbance. Detection wise, absorbance technology is limited to a narrow measurement range, typically 0 – 3 OD. As a result the assay linear and dynamic range in ELISA is limited to max imum of 3 orders of magnitude. For this reason there are ELISA kits targeted to different concentration ranges.

Luminescence based, "mix-and-measure" AlphaLISA technology takes advantage of broad dynamic range and high sensitivity signal detection. Luminescence counting has a dynamic range of 6 orders of magnitude. High counting sensitivity, together with a broad measurement range, is guaranteed by using a single-photon counting method. For a researcher, this means the same assay set-up is valid for measuring high and low analyte concentrations; no additional dilutions are required. Use of this sensitive homogeneous technology results in fewer assay steps and the ability to use lower sample volumes with high density plates (e.g. 384-well plate).

EnSpire AlphaPLUS Multilabel Plate Reader provides high quality data for both measurement technologies. Absorbance performance is enhanced by using improved detection electronics and top-to-bottom direction for the light path. This is especially important when using clear plates. AlphaLISA technology measured with EnSpire continues to demonstrate the throughput and data quality standards inherited from the EnVision® Multilabel Plate Reader, the gold standard for measuring AlphaScreen.

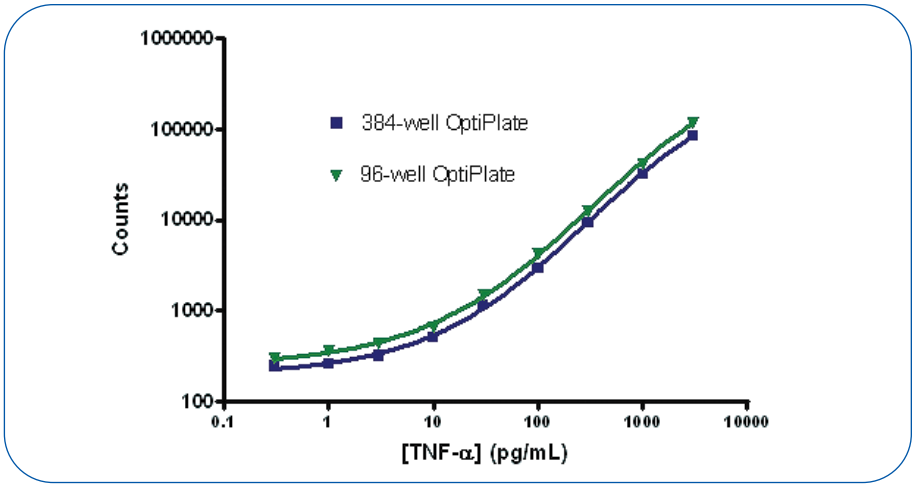

Figure 4. **Dilution curves of TNF-α AlphaLISA assays** (96- and 384-well plates).

Summary

Immunoassays, like traditional ELISA, are frequently used methods for analysis and quantification of low analyte concentrations. Utilizing a proprietary bead-based technology, AlphaLISA is the premier alternative to traditional ELISA. The narrow and bright emission spectra of the unique AlphaScreen technology makes AlphaLISA the most sensitive no-wash, high throughput assay for small molecules, large proteins and complex samples such as serum and plasma. Conversion from ELISA to AlphaLISA has been made easy and straightforward.

Evaluation of ELISA and AlphaLISA TNF-α assay platforms was performed using EnSpire AlphaPLUS Multilabel Plate Reader. The EnSpire reader with AlphaPLUS capability is a high performance and easy-to-use tool for running any absorbance-based ELISA assays and AlphaLISA assays. EnSpire produced high quality TNF-α assay data with both measurement technologies. Results highlight the benefits of AlphaLISA technology over ELISA, such as broad dynamic range (no dilutions required), high sensitivity and fewer assay steps. Total time for performing AlphaLISA TNF-α assay was only half of the time required for ELISA.

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