Performing a Quantitative ELISA Assay to Detect Human TNF-a

Keywords: Human Tumor Necrosis Factor Alpha (TNF-α), ELISA, Multiskan FC, microplate photometer, Wellwash, Wellwash Versa, microplate washer, strip washer

Goal

This application note describes a method for measuring Human TNF-α using a quantitative Enzyme-Linked Immunosorbent Assay (ELISA) with the Thermo Scientific Multiskan FC microplate photometer. To demonstrate the instrument performance we utilized the Invitrogen Human TNF-α ELISA kit along with the Thermo Scientific SkanIt software™ for microplate reader control and data analysis. The Thermo Scientific Wellwash Versa microplate washer was incorporated into the ELISA procedure to facilitate the microplate washing steps.

Note: Most Invitrogen ELISA kits are compatible to work with Thermo Scientific Multiskan FC microplate photometer and Wellwash Versa microplate washer.

Introduction

Multiskan FC microplate photometer and Wellwash Versa microplate washer make a great pair for wide variety of ELISA assays. The experiments are easy to set up, flexible for modifications and have simplified data analysis.

TNF-α is a cytokine mainly produced by activated macrophages. It is involved in various biological activities including, but not limited to, the regulation of immune cells. The protein plays an important role in the inflammatory response, metabolic activities, and cell necrosis or apoptosis. The Invitrogen Human TNF-α ELISA kit is a solid-phase sandwich ELISA designed to detect and quantify the level of Human TNF-α in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Human TNF-α.

In this kit the enzyme, horseradish peroxidase (HRP), catalyzes the conversion of the chromagen, tetramethylbenzidine (TMB), to produce a colored product. The color produced is directly proportional to the amount of Human TNF-α present in the solution.

The assay consists of several steps.

1. Human TNF-α in the sample is allowed to bind with monoclonal Human TNF-α antibody immobilized on the surface of the microplate well.

2. After washing, a biotinylated monoclonal antibody specific for Human TNF-α is added and binds the immobilized Human TNF-α captured from the sample during the first incubation.

3. After removal of the excess antibody, the Streptavidin-HRP enzyme is then added and binds to the complex. An additional wash is performed after incubation.

The color formation is initiated by adding a substrate (chromagen) that reacts with the enzyme.

After substrate addition, the reaction is terminated by the addition of a stop solution and absorbance is then measured.

This application note describes the use of the Multiskan FC and Wellwash Versa for the determination of Human TNF-α via ELISA.

Materials and Methods

Instruments

- Multiskan FC microplate photometer #51119000 or #51119100
  - – Photometric filter 450nm
  - – SkanIt software
- Wellwash Versa microplate washer #5165010 (or Wellwash #5165000)
  - – 1x8 wash head

Reagents

- Invitrogen Human TNF-α ELISA Kit #KHC301

Test Setup:

The plate was prepared according to the kit instructions Three unknown samples were prepared by spiking the standard diluent buffer with reconstituted Human TNF-α standard (2000pg/ml) at varying concentrations throughout the assay range. All samples and standards where run in duplicate.

Wash procedures were performed with the Wellwash Versa. The washer was configured with a 1x8 head to accommodate the 8-well strip format required for the assay.

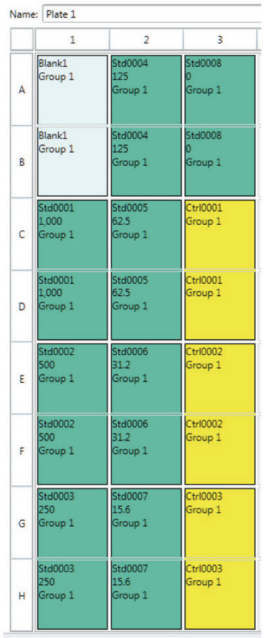

Figure 1. Example of a SkanIt software layout for the Human TNF-α assay.

Wells were washed 4x with 400µl of wash buffer at each wash step. The plates were processed one column at a time to allow for flexibility in the number of strips needed for the run. During each wash step the liquid was aspirated from the wells and 400µl of wash buffer was dispensed before moving to the next column. A 30 second soak was programmed between each of the four washes. A final aspiration was performed after the last wash to prepare the wells for further reagent additions.

The plate was measured with the Multiskan FC controlled by SkanIt software. A 5 second shake was performed prior to reading at 450nm in *Fast* mode.

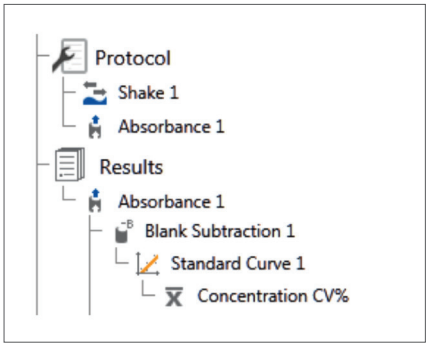

Figure 2. SkanIt software measurement protocol and calculation steps for the determination of Human IL-6 concentrations.

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Results and Discussion:

Results were calculated according to the kit instructions. Background absorbance for the 0pg/ml standard was subtracted from all data points prior to plotting the standard curve using a four parameter logistic algorithm. The standard curve and unknown sample results are shown in Figure 3.

The data demonstrates that the Multiskan FC along with the Wellwash Versa provides accurate and reliable results for determining Human TNF-α via ELISA. The data generated in this study returned a R2 value for the standard curve of 0.999 with the average % recovery for the spiked samples of 110%.

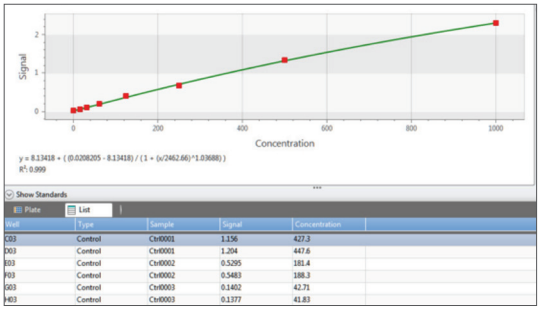

Figure 3. Standard Curve created in SkanIt software with calculated concentration of unknowns displayed in the table below the graph.

Conclusion:

- The Thermo Scientific Multiskan FC microplate photometer is ideally suited for performing ELISA. On board shaking, optional incubation, and fast read times provide a complementary platform for your ELISA experiments
- The SkanIt software facilitates ELISA workflow by providing the user with simplified data acquisition and analysis.
- The Thermo Scientific Wellwash microplate washers allow automation of critical wash steps necessary during ELISA. Incomplete washing will adversely affect the test outcome of most ELISA.

Table 1: % Recovery of Spiked Human TNF-α samples

Sample Concentration (pg/ml)	Average Concentration	Target Concentration	% Recovery
427.3 447.6	437.5	400	109.3
181.4 188.3	184.9	160	115.6
42.71 41.83	42.27	40.0	105.7

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