

Application Note

Performing a Quantitative ELISA Assay to Detect Human IL-6 with Thermo Scientific™ Multiskan™ FC Microplate Photometer and Thermo Scientific™ Wellwash™ Versa Microplate Washer

Key Words

Human Interleukin-6 (IL-6), ELISA, Multiskan FC, microplate photometer, Wellwash, Wellwash Versa, microplate washer, strip washer

Goal

This application note describes a method for measuring Human IL-6 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 IL-6 ELISA kit along with the Thermo Scientific Skant 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.



IL-6 is a cytokine that affects the activity of multiple cell types. It is involved in several cellular processes including inflammation, formation of blood cells, and immune response. The Invitrogen Human IL-6 ELISA kit is a solid-phase sandwich ELISA designed to detect and quantify the level of Human IL-6 in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Human IL-6.

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 IL-6 present in the solution.



The assay consists of several steps.

1. Human IL-6 in the sample is allowed to bind with Anti-Human IL-6 antibody immobilized on the surface of the microplate well. A secondary antibody is also added in this first step and binds to the human IL-6 in the sample at a different epitope forming the sandwich complex. This secondary antibody is coupled to a biotin molecule.
2. The Streptavidin-HRP enzyme is then added and binds to the complex
3. 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 IL-6 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 IL-6 ELISA Kit #KHC0061

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 IL-6 standard (2500pg/ml) at varying concentrations throughout the assay range. All samples and standards were 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. Wells were washed 3x with 300µl 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 300µl of wash buffer was dispensed before moving to the next column. A final aspiration was performed after the last wash to prepare the wells for further reagent additions.

Name: Plate 1

| | 1 | 2 | 3 |
|---|---------------------------------|------------------------------------|--------------------------|
| A | Blank1 Group 1 | Std0004 62.5 pg/mL Group 1 | Un0001 1:1 Group 1 |
| B | Blank1 Group 1 | Std0004 62.5 pg/mL Group 1 | Un0001 1:1 Group 1 |
| C | Std0001 500 pg/mL Group 1 | Std0005 31.25 pg/mL Group 1 | Un0002 1:1 Group 1 |
| D | Std0001 500 pg/mL Group 1 | Std0005 31.25 pg/mL Group 1 | Un0002 1:1 Group 1 |
| E | Std0002 250 pg/mL Group 1 | Std0006 15.625 pg/mL Group 1 | Un0003 1:1 Group 1 |
| F | Std0002 250 pg/mL Group 1 | Std0006 15.625 pg/mL Group 1 | Un0003 1:1 Group 1 |
| G | Std0003 125 pg/mL Group 1 | Std0007 7.8125 pg/mL Group 1 | |
| H | Std0003 125 pg/mL Group 1 | Std0007 7.8125 pg/mL Group 1 | |

Figure 1. Example of a SkanIt software layout for the Human IL-6 assay.

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.

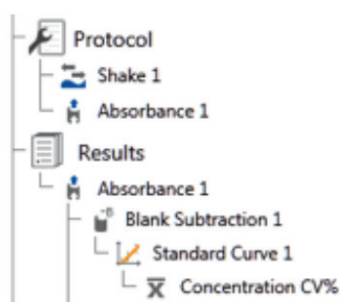


Figure 2. The SkanIt software measurement protocol and calculation steps for the determination of Human IL-6 concentrations.

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 Fig 3.

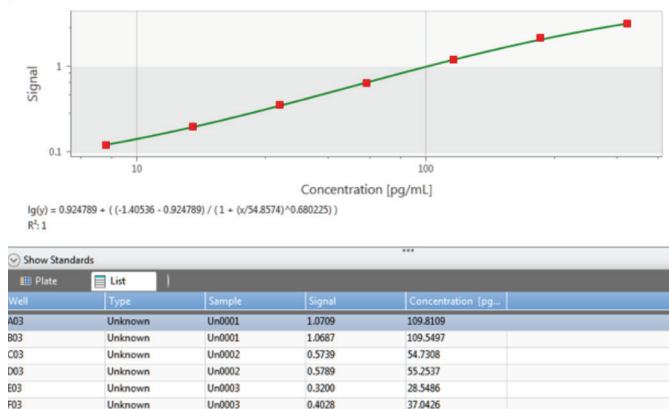


Figure 3. Standard Curve created in the SkanIt software with calculated concentration of unknowns displayed in the table below the graph.

Table 1: % Recovery of Spiked Human IL-6 samples.

| Sample Concentration (pg/ml) | Average Concentration | Target Concentration | % Recovery |
|------------------------------|-----------------------|----------------------|------------|
| 109.8 | 109.7 | 107.0 | 102.5 |
| 109.5 | | | |
| 54.7 | 55.0 | 53.5 | 102.8 |
| 55.3 | | | |
| 28.5 | 32.8 | 26.8 | 122.6 |
| 37.0 | | | |

The data demonstrates that the Multiskan FC along with the Wellwash Versa provides accurate and reliable results for determining Human IL-6 via ELISA. The data generated in this study returned a R² value for the standard curve of 1 with the average % recovery for the spiked samples of 109%.

Conclusions

- 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.

Find out more at

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