

How to Perform a Quantitative ELISA Assay to Detect TNF α with Thermo Scientific Multiskan FC Microplate Photometers

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Key words

- Tumor necrosis factor alpha (TNF α)
- Horseradish peroxidase (HRP)
- Thermo Scientific Multiskan FC
- Microplate Photometer
- Thermo Scientific SkanIt Software

Abstract

This application note describes how to easily and reliably perform a quantitative ELISA assay with a Thermo Scientific Multiskan FC. The kit used in this study was a Thermo Scientific Tumor Necrosis Factor-alpha (TNF α) kit.

Introduction

The Thermo Scientific TNF α kit is an enzyme-linked immunosorbent assay for measuring human TNF α in serum: EDTA, heparin and sodium citrate plasma, and culture supernatants.

The principle of the assay is based on a solid-phase enzyme immuno assay with horseradish peroxidase as the marker enzyme (Figure 1).

Horseradish peroxidase (HRP) is a protein that catalyzes the oxidation of substrates by hydrogen peroxide and a colored or fluorescent product will be created if TNF α is present. In this assay TMB (3,3',5,5'-tetramethylbenzidine) is used as the substrate.

The assay consists of three incubation and wash cycles:

1. The first antibody is immobilized on the bottom of the microplate well to capture the TNF α if present in the sample.
2. The second (detection) antibody recognizes a different epitope on the TNF α molecule. This antibody is coupled to a biotin molecule.
3. The HRP reagent is then bound by streptavidin to the complex.

After substrate addition, the color formation reaction is terminated by the addition of a stop solution. Absorbance is then measured.

The measurement can be performed with the Multiskan[®] FC microplate photometer using either of the software options provided with the system: the internal software or Thermo Scientific SkanIt Software for Multiskan FC (PC software).

Materials and Methods

Thermo Scientific Human TNF α ELISA Kit (Product no. EH3TNFA)

- Pyloriset[®] EIA-A III, Helicobacter pylori assay kit, Orion Diagnostica (Product no. 68107)
- Thermo Scientific Multiskan FC (Product no. 51119000 or 51119100)
- Photometric filters 450 nm (factory installed in Multiskan FC) and 550 nm (Thermo Scientific Product no.1425505)

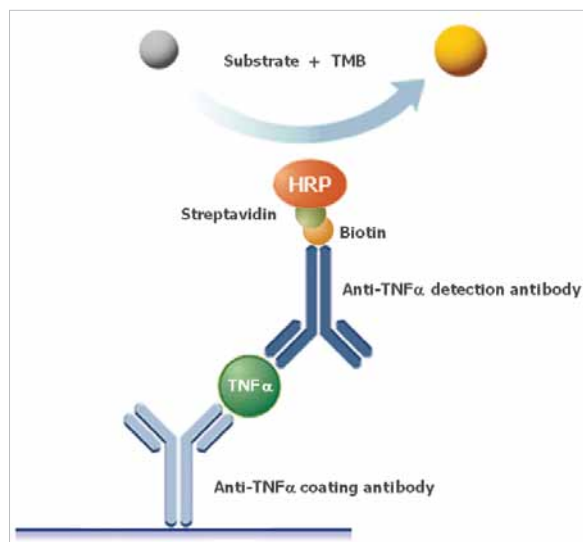


Figure 1. Principle of the assay

- Thermo Scientific Finnpiquette F1 (Product no. 4641070 Single Channel, 4661030 Multichannel)
- Thermo Scientific Wellwash 4 Mk 2 (Product no. 5160770)

The assay was performed using both the internal software and SkanIt® Software for Multiskan FC separately.

The plate was prepared according to the kit instructions. Eleven unknown samples (n=11) were prepared by spiking the assay buffer with the TNF standard provided with the kit. One of the samples was diluted 1:10.

The plate was measured with the Multiskan FC microplate photometer at 450 nm and 550 nm.

The results were calculated according to the kit instructions by plotting the average absorbance (450 nm minus 550 nm) obtained for each standard concentration vs. the corresponding TNF α concentration.

According to the kit instructions, any duplicate values that differ from the mean by greater than 10% should be carefully considered.

The following section provides instructions on how to set up a protocol for this assay on the Multiskan FC photometer with both pieces of software. Data processing parameters can be added before or after the assay is run using either type of software. Protocols can also be downloaded from the Thermo Scientific Reading Room (www.thermo.com/readingroom). If downloaded, the layout must be modified according to the current layout and the filter information checked and/or changed according to the instrument setup.

Thermo Scientific SkanIt Software for the Multiskan FC

A new protocol can be created by selecting *New session* from the tool bar or from the Home view.

Layout

Create the layout according to the setup on the assay plate, for example, as in Figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal_0001 Assay 12.00ug/ml	Cal_0002 Assay 12.00ug/ml	Un_0001 Assay	Un_0008 Assay								
B	Cal_0001 Assay 12.00ug/ml	Cal_0002 Assay 12.00ug/ml	Un_0002 Assay	Un_0009 Assay								
C	Cal_0001 Assay 13.325 ug/ml	Cal_0003 Assay 250 ug/ml	Un_0003 Assay	Un_0010 Assay								
D	Cal_0002 Assay 14.650 ug/ml	Cal_0004 Assay 250 ug/ml	Un_0004 Assay	Un_0011 Assay								
E	Cal_0003 Assay 16.975 ug/ml	Cal_0005 Assay 500 ug/ml	Un_0005 Assay	Un_0012 Assay								
F	Cal_0004 Assay 19.300 ug/ml	Cal_0006 Assay 500 ug/ml	Un_0006 Assay	Un_0013 Assay								
G	Cal_0005 Assay 21.625 ug/ml	Cal_0007 Assay 1000 ug/ml	Un_0007 Assay	Un_0014 Assay								
H	Cal_0006 Assay 23.950 ug/ml	Cal_0008 Assay 1000 ug/ml	Un_0008 Assay	Un_0015 Assay								

Figure 2. Example of a SkanIt Software layout for the TNF α assay. In the example the unknown 11 is diluted 1:10.

Protocol

Add two photometric measurement steps with the measurement mode *Normal*. Select filter *450 nm* for the first step and *550 nm* for the second step.

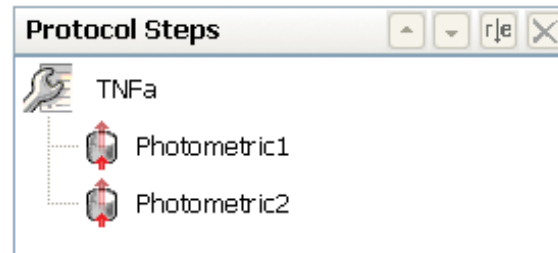


Figure 3. Protocol step tree in SkanIt Software

Results

Add a Pre-calculation step and subtract the 550 nm values from the 450 nm values. The curve fit for the standard curve is made with the pre-calculated data. Add a Quantitative Curve Fit step to the Pre-calculation step. Select *Four parameter logistics* as the curve fit method.

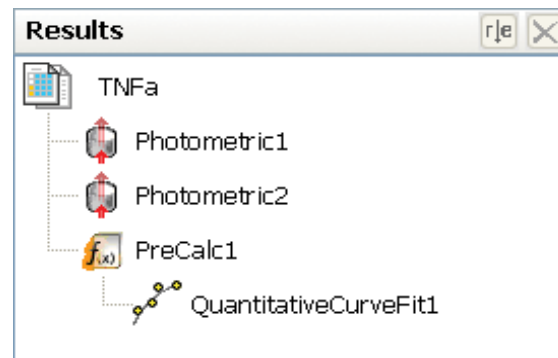


Figure 4. Result step tree in SkanIt Software

Internal software protocol

A new protocol can be created by selecting *File/New* or changing the parameters of the current protocol and selecting *Save as*.

Main view

In the measurement parameters, select *450 nm* as filter 1, *550 nm* as filter 2 and the measurement mode *Normal*.

Processing

Create the layout according to the setup on the assay plate, for example, as in Figure 5.

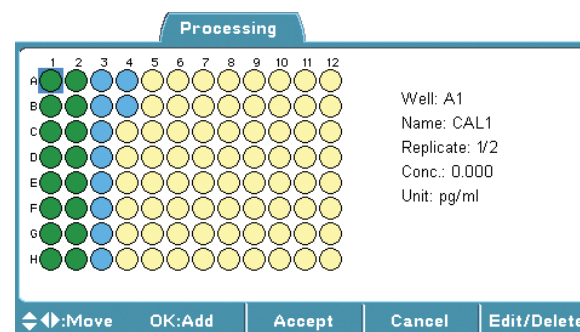


Figure 5. Example of an internal software layout for the TNF α assay

The reference wavelength is subtracted by adding the Pre-processing step, *Pre-processing: Mea1-Mea2*.

The quantitative calculation is enabled by enabling *Calculation* and selecting *Four parameter logistics* as the calculation type.

Results

The results of the assay were calculated as instructed in the kit insert. A four parameter logistics curve was drawn with both pieces of software. The SkanIt Software curve is shown below (Figure 6). The concentrations of the samples are automatically calculated from the curve and reported in list and table formats using both pieces of Multiskan FC software.

Both pieces of software also report the concentrations of the samples with mean, standard deviation and CV% data.

The dilution factor can also be added to the samples in SkanIt Software, and the result is then automatically corrected with the dilution factor.

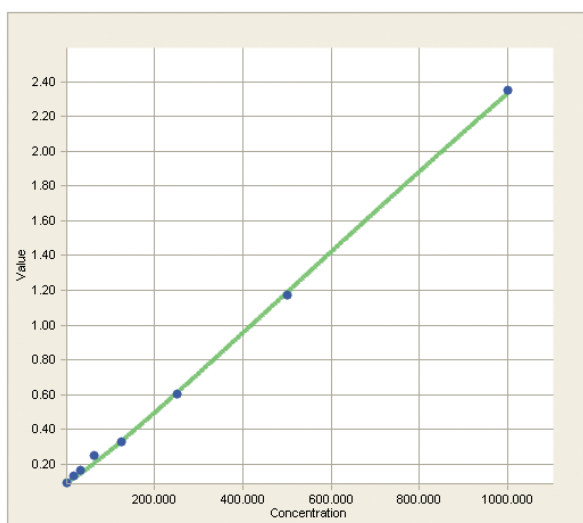


Figure 6. Example of a SkanIt Software curve fit for the TNF α assay

Conclusion

The Thermo Scientific Multiskan FC microplate photometer is a good tool for performing ELISA assays, including those used for TNF α studies. For both the PC software and internal software, the system offers the user all of the data processing parameters needed for easy and reliable result reporting.

Further Information

For further information about Multiskan FC, please refer to the following Web pages:

- www.thermo.com/readingroom
- www.thermo.com/mpi

For further information about Thermo Scientific Human TNF α ELISA, please refer to the following Web page:

- TNF alpha Assay Kits and Reagents

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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