Simplified workflow for Instant ELISA kits using the Multiskan Sky Microplate Spectrophotometer and Wellwash Versa Microplate Washer

Abstract

This application note demonstrates the benefits of using the Invitrogen™ Human TNFα Instant ELISA™ Kit with the Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotomer and Wellwash™ Versa™ Microplate Washer. In addition, we show how to streamline the workflow by incorporating a preprogrammed pipetting protocol developed with the My Pipette™ Creator app and a Thermo Scientific™ E1-ClipTip™ Bluetooth™ electronic pipette.

Introduction

TNFα is a cytokine mainly produced by activated macrophages. It is involved in various biological activities, including the regulation of immune cells. The protein plays an important role in the inflammatory response, metabolic activities, and cell necrosis or apoptosis.

The Human TNFα Instant ELISA Kit is designed for the quantitative detection of human TNFα in cell culture supernatant, serum, plasma, urine, synovial fluid, amniotic fluid, or other body fluids. This assay utilizes an Instant ELISA format.

In contrast to conventional ELISA kits in which precoated plates include only the capture antibody when the sample is added, plates in Instant ELISA kits contain all of the necessary components, including capture antibody, lyophilized detection antibody, streptavidin–HRP conjugate, and sample diluent (Figure 1). In addition, strip wells containing the standard for the standard curve are provided separately—they are ready to use, saving both time and wells.

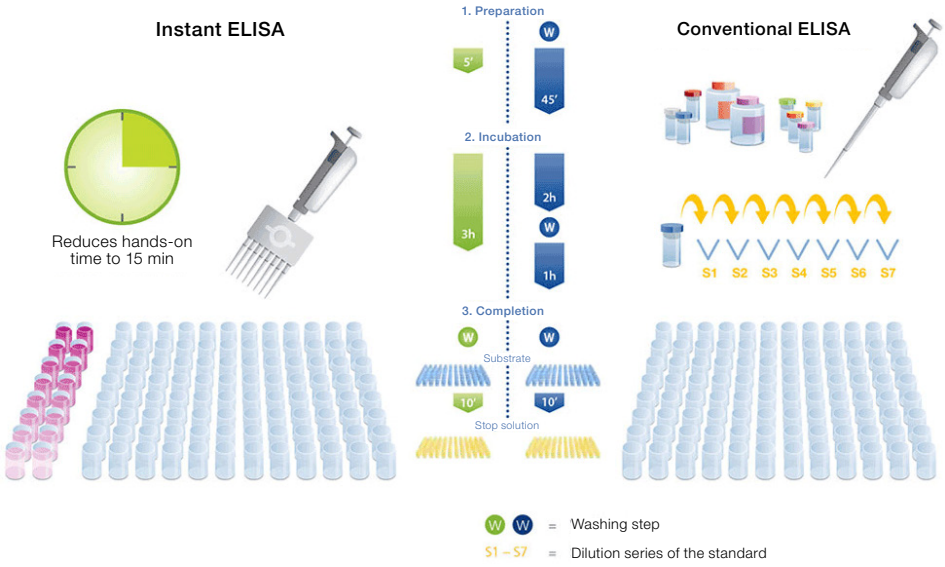

Figure 1. Instant ELISA kit vs. conventional ELISA.

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Rehydrating your standards and adding sample and reagents can be simplified using a preprogrammed pipetting protocol that can be easily transferred to an E1-ClipTip electronic pipette. This helps save valuable time and minimize potential errors. The pipette will automatically guide you through the additions with concise and clear instructions, even prompting you when it is time for incubation.

Pairing the Wellwash Versa Microplate Washer with the Multiskan Sky Microplate Spectrophotometer and Thermo Scientific™ SkanIt™ Software make washing, detection, and analysis during the ELISA workflow easier and more efficient. The experiments are straightforward to set up and flexible for modifications, and offer simplified data analysis. The real-time data monitoring feature of SkanIt Software is especially helpful in determining the ideal time period for stopping the reaction.

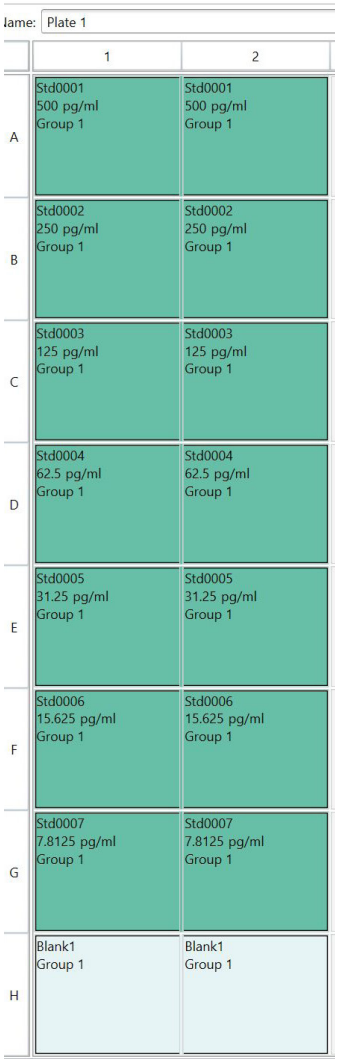

Figure 2. Example of a standard curve layout in SkanIt Software for the Human TNFα Instant ELISA Kit.

Materials and methods

Materials

- Multiskan Sky Microplate Spectrophotometer (Cat. No. 51119700)
- SkanIt Software (Cat. No. 5187139)
- Wellwash Versa Microplate Washer, 2 x 8 wash head (Cat. No. 5165010)
- E1-ClipTip Bluetooth Electronic Single-Channel Pipette (Cat. No. 4670040BT) or Multichannel Pipette (Cat. No. 4671100BT)
- Human TNFα Instant ELISA Kit (Cat. No. BMS223INST)

Test setup

The plate was prepared according to the kit instructions (Figure 2) using an E1-ClipTip electronic pipette and a preprogrammed pipetting protocol downloaded from the My Pipette Creator app.

Pipetting protocol for the Human TNFα Instant ELISA Kit, using 3 different samples:

1. Add 150 µL of distilled water to the standard and blank wells.
2. Add 100 µL of distilled water to the sample wells.
3. Add 50 µL of each sample to the sample wells in duplicate and mix, repeating for the three different samples.
4. Cover the plate and incubate it for 3 hours on a microplate shaker at 400 rpm.
5. Remove the plate cover and empty the wells. Wash the microwell strips 6 times with approximately 400 µL of wash buffer per well, with thorough aspiration of the microwells' contents.
6. Pipette 100 µL of the TMB substrate solution into each well, including the blank.
7. Incubate the microwell strips at room temperature (18–25°C) for 10–30 minutes. Avoid direct exposure to intense light.
8. Add 100 µL of the stop solution to each well.

The wash procedure was performed with the Wellwash Versa Microplate Washer. The washer was configured with a 2 x 8 head to accommodate the 2 x 8-well strip format for the standard curve. Wells were washed 6 times with 400 µL of the wash buffer. During each wash step, the liquid was aspirated from the wells and 400 µL of the wash buffer was dispensed before moving to the next column. A 3-second soak was programmed between each of the 6 washes. A final aspiration was performed after the last wash to prepare the wells for addition of the TMB substrate solution.

After the substrate was added, the optical density (OD) at 620 nm was monitored for standard 1 on the Multiskan Sky Microplate Spectrophotometer using the kinetic loop function in SkanIt Software (Figure 3). The standard was read every 3 minutes. Once the OD reached 0.9 (21 minutes), the run was stopped by addition of the stop solution to the wells. Final measurements were then performed on all wells using 450 nm as the primary wavelength and 620 nm as the reference wavelength, in fast mode (Figure 4).

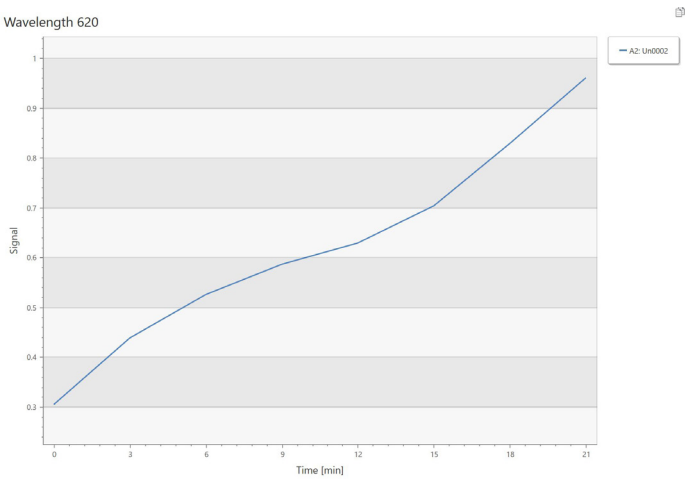

Figure 3. Kinetic graph produced during the run to monitor OD for standard 1. The reaction was stopped once the OD reached 0.9.

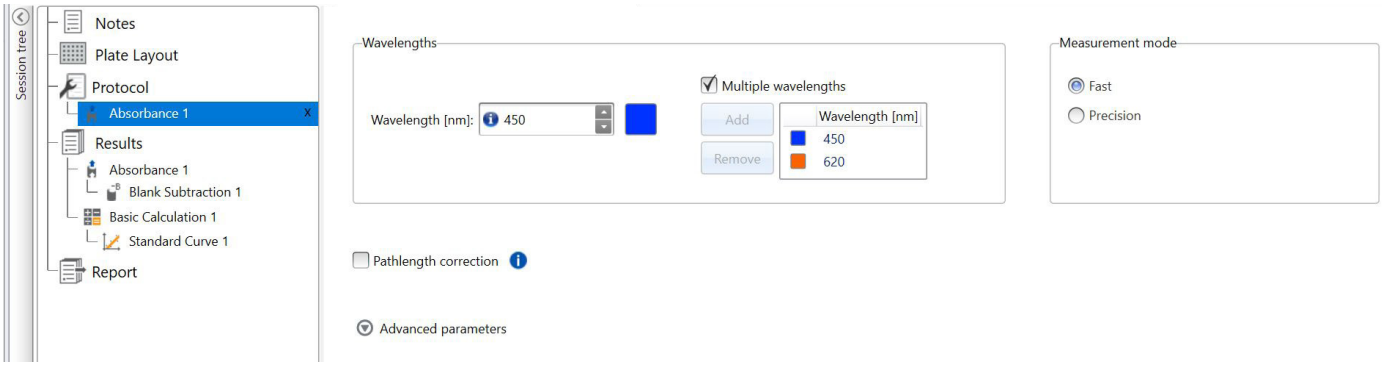

Figure 4. Protocol in SkanIt Software for determination of human TNFα concentrations.

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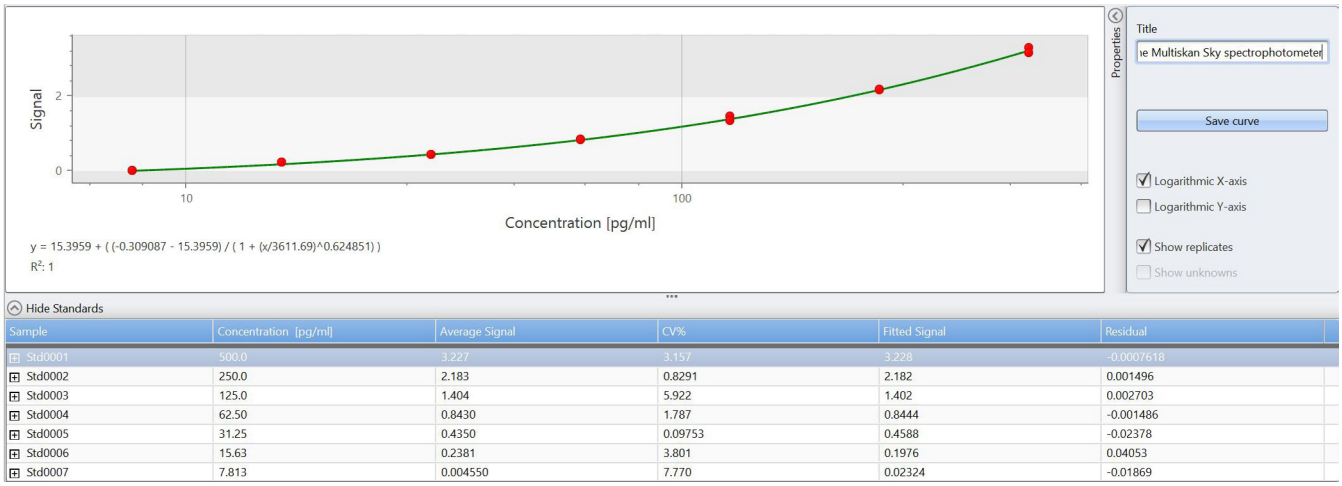

Sample	Concentration [pg/ml]	Average Signal	CV%	Fitted Signal	Residual
Std0001	500.0	3.227	3.157	3.228	-0.0007618
Std0002	250.0	2.183	0.8291	2.182	0.001496
Std0003	125.0	1.404	5.922	1.402	0.002703
Std0004	62.50	0.8430	1.787	0.8444	-0.001486
Std0005	31.25	0.4350	0.09753	0.4588	-0.02378
Std0006	15.63	0.2381	3.801	0.1976	0.04053
Std0007	7.813	0.004550	7.770	0.02324	-0.01869

Figure 5. Standard curve for the TNFα assay using the Multiskan Sky Microplate Spectrophotometer.

Results and discussion

The reference values at 620 nm were subtracted from their respective 450 nm primary wavelength readings for each well. The average signal and coefficient of variation (CV) were calculated for each standard. The data were used to plot the standard curve using a 4-parameter logistic algorithm (Figure 5).

The data demonstrate that the Multiskan Sky Microplate Spectrophotometer, used together with the Wellwash Versa Microplate Washer, produces a reliable standard curve for determining TNFα concentrations using the Human TNFα Instant ELISA kit. The data generated in this study returned $R^2 = 1$ for the standard curve, with average CV = 3.3% for the standards.

Conclusions

- The Multiskan Sky Microplate Spectrophotometer is ideally suited for performing ELISAs. Onboard shaking, incubation, and fast read times provide a complementary platform for your ELISA experiments. In addition, the ability to freely select wavelengths from 200 to 1,000 nm helps ensure that the instrument can accommodate a wide range of photometric assays to meet the demand of changing workflows in the laboratory.
- SkanIt Software facilitates ELISA workflows with simplified data acquisition and analysis.
- The Wellwash Versa Microplate Washer automates the critical wash steps necessary during ELISAs. Incomplete washing will adversely affect the results of most ELISAs.
- The My Pipette Creator app and E1-ClipTip electronic pipette help streamline pipetting in ELISA workflows with easy-to-use technology. Using the app, custom pipetting programs can be quickly created, edited, shared, and transferred to the E1-ClipTip electronic pipette. Additionally, preprogrammed protocols for a variety of applications are available from the ever-expanding protocol library that can be found within the My Pipette Creator app.

Find out more at
thermofisher.com/elisainstrumentsandequipment

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