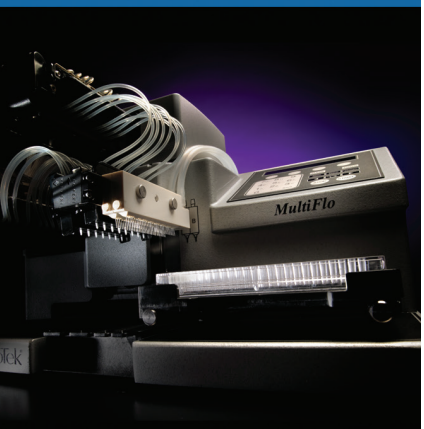
Automation of Optimiser™ Microplate Technology for High Throughput Applications

Peter J. Brescia, Peter Banks, BioTek Instruments, Inc., Winooski, VT
Junhai Kai, Aniruddha Puntambekar, and David Sehy, Siloam Biosciences, Inc., Cincinnati, OH



Automation of the workflow for a microfluidics-based 96-well microplate technology allows for a low-volume, high-throughput ultra-sensitive fluorescent–based ELISA to be completed within approximately 2 hours. The inherent characteristics of the microfluidics contained within the vessel allow for flexible incubation times amenable to scalability when used in combination with automated liquid handling, dispensing and robotic instrumentation.

Key Words:

Fluorescence

Microfluidics

IL-6

ELISA

Automation

Biomarker

Introduction

Enzyme-linked immunosorbent assays (ELISAs) provide an inexpensive, sensitive method to quantify analyte from a variety of sources suitable for use in a high-throughput microplate format. A previously described novel microplate (Optimiser™) has been recently developed that combines the typical SBS footprint and layout of a 96-well microplate with a dedicated microfluidic channel for each well (see Figure 1)[1]. The conventional SBS footprint and newly integrated absorbent pad and holder in the OptiMax™ Automation Plates result in seamless integration with microplate instrumentation such as automated pipetting stations, dispensing units and microplate readers. The ELISA assay workflow is similar when using OptiMax plates with the exception of a significant reduction in reagent and sample usage and absence of typical washing steps. Thus, the microfluidics workflow significantly reduces both the number of steps required when compared to conventional ELISA assays as well as overall time to completion.

In this application note we demonstrate automated high throughput assay performance using the OptiMax Evaluation Kit using IL-6 cytokine as analyte in conjunction with an automated liquid handling workstation, multi-channel dispenser and multi-mode microplate reader.

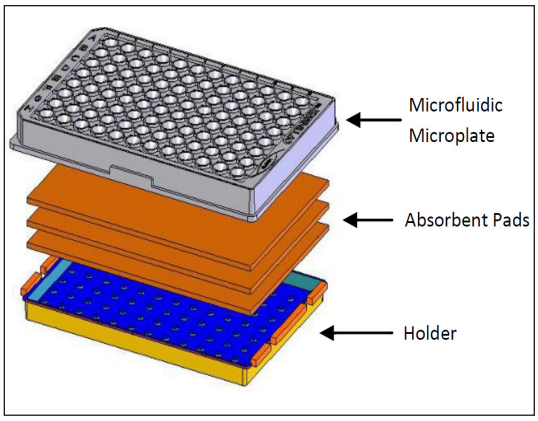


Figure 1. **OptimMax Microplate.** The OptiMax microplate has integrated absorbent pads, 96-well density and SBS footprint. Each well contains a microchannel which draws in reagent and analyte by capillary action while subsequent fluid additions expel fluid from the microchannel into the absorbent pad. The large surface area of the microchannel increases binding efficiency compared to a standard microplate well surface. The detection volume is defined by the microchannel and is read using a conventional microplate reader without disassembly.

Materials and Methods

Materials

OptiMax Evaluation Kit (human IL-6) (OPV-IL6) including OptiMax Plate and additional reagents volumes as required for automation were provided by Siloam Biosciences (Cincinnati, OH).

BioTek Instruments, Inc.
P.O. Box 998, Highland Park,
Winooski, Vermont 05404-0998 USA
Phone: 888-451-5171
Outside the USA: 802-655-4740
Email: **customercare@biotek.com**
www.biotek.com

Instrumentation

The Precision™ Microplate Pipetting System (BioTek Instruments, Winooski, VT) was used for serial dilution and loading of analyte (IL-6) for determination of assay precision and sensitivity. MultiFlo™ Reagent Dispenser was used for all reagent additions (Figure 2). Detection of the fluorescent signal from the Optimiser plate was performed with a Synergy™ Neo HTS Multi-Mode Microplate Reader using the settings in table 1.

Synergy Neo Read Parameters	
Mode	Fluorescence
Gain	<auto>
Excitation	544 nm
Emission	590 nm
Optics position	Top
Read Speed	Normal
Delay after plate movement	100 msec
Measurements per data point	10
Lamp Energy	Low (faster)
Read Height	8.00 mm

Table 1. Reader Settings.

Figure 2. Automated Workflow.

OptiMax Automated Workflow

The OptiMax automated workflow steps were performed as previously described with the following modifications[1]. A MultiFlo™ Microplate Dispenser was used to rapidly deliver substantially smaller volumes during the reagent addition steps. Briefly, each step consisted of the sequential addition of either 5 µL of reagent or 30 µL of OptiWash buffer using a microplate dispenser as outlined in figure 2. All dispense steps used a 1 µL dispense cassette and peristaltic pump using the high flow rate with a x-axis offset of -50 steps (2.29 mm) left of the center of the well. The analyte of interest, IL-6, was serial diluted 1:3 into OptiBlock buffer and added to the OptiMax microplate using the Precision automated pipettor following the addition of capture antibody and subsequent blocking steps. Replicate measurements were added row-wise to the OptiMax Plate resulting in 12 replicates of each concentration.

Results and Discussion

The analysis of automated methods was determined by calculating the precision of replicate measurements. Precision is expressed as a % CV across the entire range of IL-6 concentrations tested (Figure 3). These determinations correlate well with manual methods (data not shown).

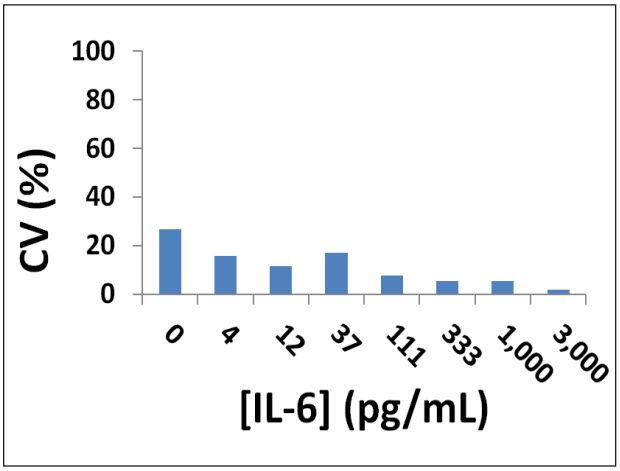


Figure 3. Precision of automated methods by computation of the CVs across a range of IL-6 concentrations. Data is representative of twelve replicates at each concentration tested.

The data can be fit using a five parameter logistic curve fit and shows excellent correlation as depicted in Figure 4. Increased signal is seen when the assay is performed using automated methods. Completion of the assay was achieved in ~ 2 hours.

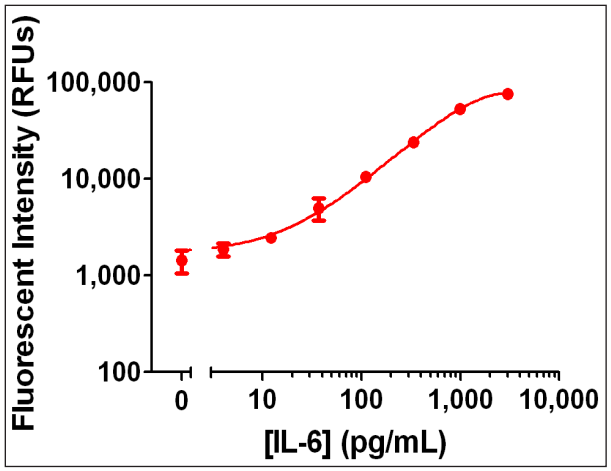


Figure 4. **IL-6 Standard Curve.** Representative data of IL-6 standard curve generated using automated methods (error bars indicate SEM of twelve replicate data point). Data fit with five parameter logistic curve fit using Graph Pad Prism.

Conclusion

This study provides methods for automation of the steps required for successful biomarker quantification with improved sensitivity compared to typical microplate based ELISAs using the OptiMax microplate. While automation generally improves assay performance with the Optimizer, use of the MultiFlo™ can result in higher sample throughput relative to using the Precision™.

References

1. Kai J., et al. (2011) "Amplifying Immunoassay Sensitivity with the Optimiser™ Microplate Technology-Repetitive Sample Loading of the Optimiser Microplate allows for Amplification of Assay Sensitivity from Picogram/mL Levels to Femtogram/mL Levels". Application Note. BioTek Instruments, INC., Winooski, Vermont, 05404.