THE NEXT GENERATION MICROPLATE USING POWER OF MICROFLUIDICS FOR FEMTOGRAM/ML LEVEL SENSITIVITY

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ABSTRACT

In this work we describe the development and characterization of the Optimiser™ microplate as the Next Generation Microplate for unparalleled sensitivity. A combination of microfluidics technology with standard SBS-configured 96-well microplate architecture, in the form of Optimiser™ microplate technology, allows for the improvement of ELISA workflows, conservation of samples and reagents, improved reaction kinetics, and the ability to improve the sensitivity of the assay by multiple analyte loading. This work demonstrates the "standard" and "high sensitivity" results for Optimiser™ with human IL-4 ELISA assay, showing Limit of Detection (LOD) at 2 femtogram/mL, representing ~ 100 fold improvement over conventional ELISA assay.

KEYWORDS: microfluidics, immunoassay, high sensitivity, 96-well microplate

INTRODUCTION

A novel Optimiser™ microplate has been recently developed that combines the typical SBS footprint and layout of a 96-well microplate with a dedicated microfluidic channel connected to each well where binding events occur (see Figure 1). The conventional 96-well layout allows for the use of conventional peripheral microplate instrumentation such as automated pipetting stations and microplate readers; the microchannel dimensions (200 x 200 μm) promotes efficient binding reactions by a large increase in surface area to volume (SAV) ratio for capture antibody binding compared to standard ELISA techniques where capture antibodies are immobilized on the microplate well bottom. The ELISA assay workflow is similar when using Optimiser™ except addition volumes is significantly less and there is no need to use microplate washers. As each microchannel has a volume of only ~ 5 μL, the addition of excess wash buffer to the well flushes out the microchannel contents to an absorbent pad beneath the microplate, replacing the conventional labor-intensive wash step. The microchannel is arranged in a spiral pattern directly below the well of the microplate and with the final addition of substrate, defines the detection volume. As the area of the microchannel spiral is similar to a microplate well, a conventional fluorescence microplate reader can be used for detection.

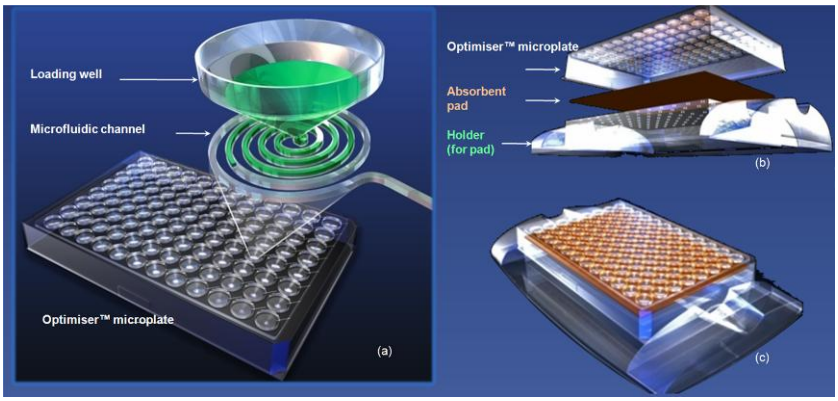

Figure 1. Optimiser™ microplate illustration: *(a) Optimiser™ microplate with magnified view of one cell; (b) exploded and (c) assembled view of Optimiser™ microplate with absorbent pad and holder*

In this paper we demonstrate Optimiser™'s assay performance using human IL-4 as an analyte and demonstrate the ability to tune assay sensitivity by repeating analyte loading/incubation cycles.

OPTIMISER™ PLATE OPERATION

Figure 2 shows the operation sequence. Reagents are sequentially added to the loading well, drawn into the channel by capillary forces and excesses are drawn out by absorbent pad. The microfluidic design ensures that the channel is not emptied by absorbent pad allowing static incubation step. Subsequent liquid addition breaks capillary barrier at the inlet and flow resumes. All assay reactions occur in the microfluidic channel at the bottom surface under each well. Optimiser™

Microfluidic ELISA Plates are compatible with existing pipetting systems (manual or automated) and fluorescent ELISA plate readers. The SAV ratio of each microfluidic reaction chamber represents a 50-fold increase when compared to the well of a conventional 96-well immunoassay plate.

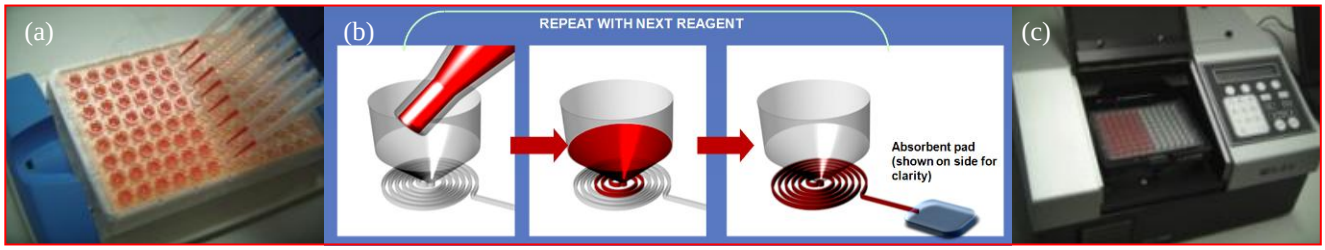

Figure 2. Operation of Optimiser™ microplate: *(a) dispense 5 µL of reagent into each well of Optimiser™ microplate with standard pipette; (b) capillary forces transport the reagent to the channel and further to the absorbent pad. As a key design feature, the reagent will drain from well but flow stops (due to capillary forces) when the liquid exits the well but is still filling the channel and this step serves as an incubation step. The next reagent dispensed into the well will break the capillary barrier at the inlet and flow resumes; (c) Optimiser™ microplate is read with a standard fluorescence microplate reader after assay flow sequencing.*

The improvement in SAV ratio increases binding kinetics dramatically and allow rapid reactions (5 to 10 minutes incubation/step for 30 to 90 minutes immunoassays) as shown in Figure 3(a) with 95% of protein adsorption completed within 5 minutes incubation time.

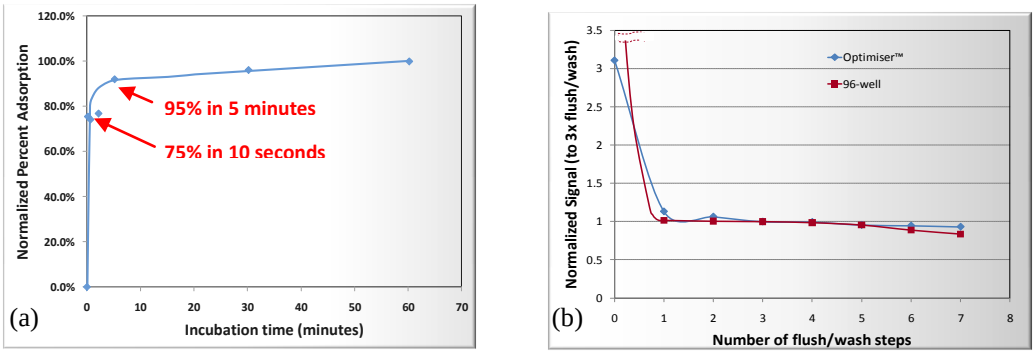

Figure 3.Microscale assay principles: (a) Incubation times for Optimiser™: *7 µL of 10 µg/mL FITC labeled antibody added, incubated and flushed out at varying intervals using 30 µL wash buffer, Signal measured with Biotek Flx 800 fluorescence reader. ~4% change from 5 minutes to 30 minutes. So slight timing variations after incubating 5 minutes do not impact assay results and (b)* ***Efficiency of "flushing" action to replace the conventional wash step.*** ***5*** *µL of 100 µg/mL FITC labeled antibody added, incubated for 5 minutes and then washed with successive loads of 7 µL buffer. For 96-well 100 µL of 3 µg/mL antibody used (to ensure same # of fluorescence labels in each case).*

Figure 3(b) shows that the Optimiser flush action is equally effective as traditional wash step, but where the flush only requires a simple pipetting step. The Optimiser provides significant reagent savings, time savings and elimination of traditional wash requirements.

HIGH SENSITIVE IL-4 ELISA ASSAY WITH OPTIMISER™ PLATE

The ELISA assay with Optimiser™ plate mirrors the steps involved in conducting a typical sandwich ELISA. The entire assay is performed in room temperature. As illustrated in figure 5, after assembling the Optimiser™ Plate (with absorbent pad and holder) and priming with 5 µL of priming buffer, 5 µL OptiBind™ coating solution containing 2 µg/mL IL-4 capture antibody was dispensed into wells of Optimiser™ plate and incubated for 10 minutes. Then 5 µL OptiBlock™ blocking buffer was dispensed into wells and incubated for 10 minutes. Subsequently, for a single-loading assay, 5 µL solution containing human recombinant IL-4 was dispensed into the wells and incubated for 10 minutes. For multiple-loading assay, 5 µL solution containing human recombinant IL-4 was dispensed into the wells and incubated for 5 minutes and then repeated for multiple cycles. Then 5 µL OptiWash™ wash buffer was dispensed in to the wells and soaked for 10 minutes to "flush" the microchannel. After "flushing", 5 µL OptiBlock™ blocking solution containing 2 µg/mL IL-4 detection antibody was added and incubated for 10 min. Following another flush step with 5 µL wash buffer, the 5 µL OptiBlock™ solution containing HRP-linked streptavidin was added and incubated for 10min. Following two times of "flushes" with 30 µL wash

buffer, the plates were loaded with 10 µL of OptiGlow™ chemifluorescence substrate working solution into each well. After 5 minutes, the plates were detached from the holder and the residues on the bottom of plate were wiped off with Kimwipe. The fluorescence signal was read with the Flx800™ fluorescence reader in 15 minutes after addition of substrate solution. Repeat loading of antigen solution allows the analyte to be captured and concentrated on the microchannel surface [1]. Hence, the assay sensitivity will be enhanced proportion to number of repeat loads.

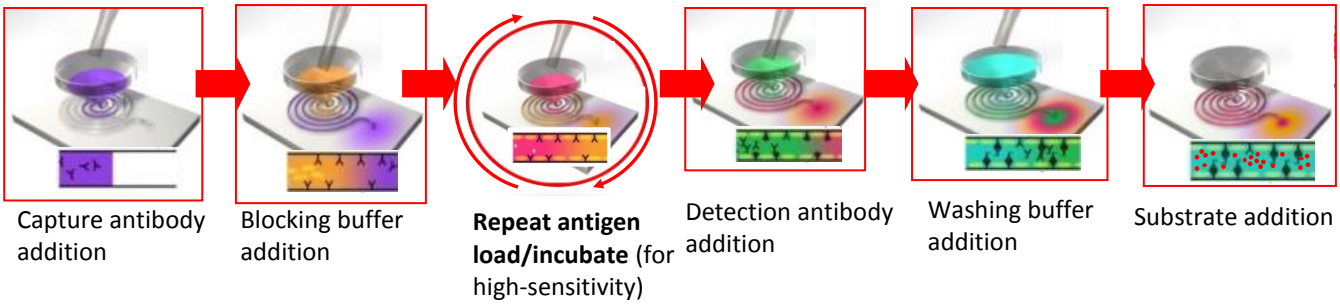

Figure 5. Assay procedure with Optimiser™ microplate. *The "repeat loading" sequence for high sensitivity mode was completed on a BioTek Precision liquid handler.*

As results, figure 6 shows a IL-4 cytokine assay [2] where the "conventional assay" has a operating range of ~ 125-1 picogram/ml and with repeat loads on the Optimiser the SAME assay can achieve a ~ 100x improved detectable range of ~1000-8 femtogram/ml (the LoD is an astounding ~ 2 femtogram/ml or ~ 65 attoMolar). To the best of our knowledge this is the highest sensitivity ever reported for this assay. Analytical sensitivity can be represented as the slope of a linear calibration curve as this defines the change in detection signal induced by a change in analyte concentration. Figure 6(b) shows the gain in sensitivity as a function of repeat loads; where sensitivity is defined as the slope of the curve shown in Figure 6(a).

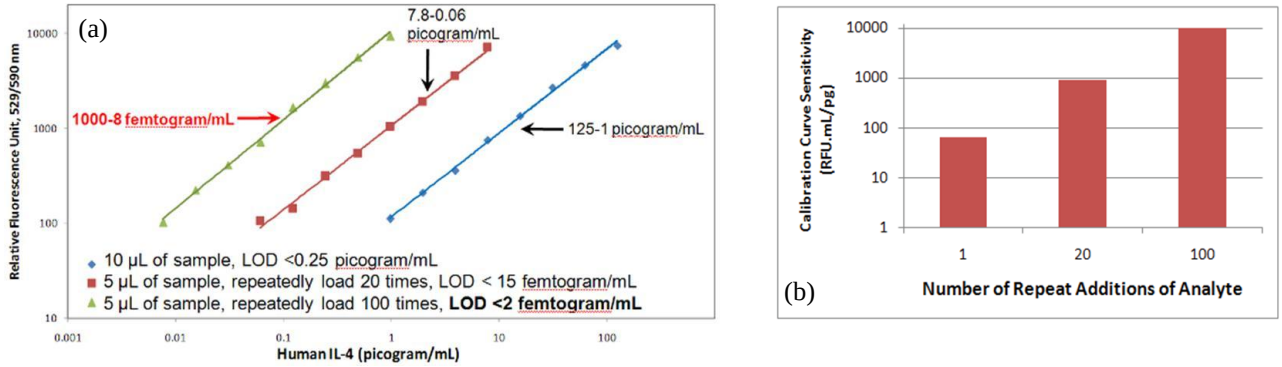

Figure 6. Human IL-4 sandwich immunoassay in Optimiser™ microplate: *(a)* ***Standard curve using three protocols for antigen step*** *1) load 10 µl of sample volume once, 2) repeatedly load 5 µl sample 20 times and 3) repeatedly load 5 µl sample 100 times. And* ***(b) Sensitivity gain is proportional to #of repeat loads and can be extended further.***

In conclusion, Optimiser™ can revolutionize biotech research by allowing researchers to reach sensitivity levels never possible to-date. For example, it enables investigators to quantify cytokines level using harvested supernatants, rather than fresh ex vivo activation of cells, which cannot be detected with conventional ELISA technology. Optimiser™ assays can also revolutionaize medical research and IVD applications; for example where ultra-low concentration detection of certain biomarkers may allow for early diagnosis of cancer. Furthermore, ~ 90%+ ELISA users [3] still use the 96-well plate and Optimiser™ can serve as the ideal vehicle to deliver the Power of Microfluidics to a global user-base.

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