OPTOFLUIDIC BIOASSAY

White paper

MICROFLUERE®

for

Faster, Less Expensive, and More Efficient ELISA

MicroFluere® plate uses 20% of sample and reagents of that used for a traditional plate and achieves a larger dynamic range in 5-10X shorter time.

Background

Since the early 1970's, Enzyme Linked Immunosorbent Assay (ELISA) has become a powerful and standard method for detecting and quantifying specific analytes in complex liquid mixtures. It is widely used in clinics and industrial/research labs. However, ELISA using a traditional microplate (the most common format used in ELISA is a 96-well plate having 12 columns x 8 rows) suffers from a few major drawbacks: (1) long assay time (3-6 hours); (2) large sample and reagent consumption (50-100 μL per well); and (3) low dynamic range.

To address the shortcomings mentioned above, Optofluidic Bioassay, LLC has developed a novel microfluidic well plate, called MicroFluere®, which greatly improves ELISA performance. The plate is made of plastic (i.e., clear or black polystyrene) and consists of 96 flow-through microfluidic units arranged in the standard ELISA 96-well format so that MicroFluere® is fully compatible with existing plate readers. The plate significantly improves the conventional ELISA test workflow by eliminating many time-consuming steps and reducing incubation times. More importantly, it enables the researcher to use only 20% of the sample and reagent volumes as compared to those used for a traditional 96-well plate and achieves a larger dynamic range with the same lower detection limit. MicroFluere® also allows the assay to be completed in 5-10X shorter time.

Technology

A MicroFluere® plate comprises 96 microfluidic units arranged in the same format as in a traditional 96-well plate. The plate's footprint, height, and bottom and outside flange are in compliance with the ANSI (American National Standards Institute) and SLAS (Society for Laboratory Automation and Screening) 96-well plate standard. Therefore, it can be read with existing plate readers without any modifications to the readers.

As shown in **Figures 1** and **2**, each microfluidic unit has a liquid inlet, an optically clear detection channel containing micro-posts, and a liquid outlet. In the microfluidic unit, samples and reagents flow from a funnel-shaped structure through a micro-post array embedded microfluidic channel. The liquid can be withdrawn from an opening outlet using a wicking method or pressure differential. The optical signal is acquired at the center of the microfluidic loop located at the standard plate reader optical excitation/collection position. Since the inlet and outlet are offset from the detection area, there is no interference to the optical signal caused by sample/reagent residuals at the inlet (*i.e.*, funnel-shaped structure) or the outlet.

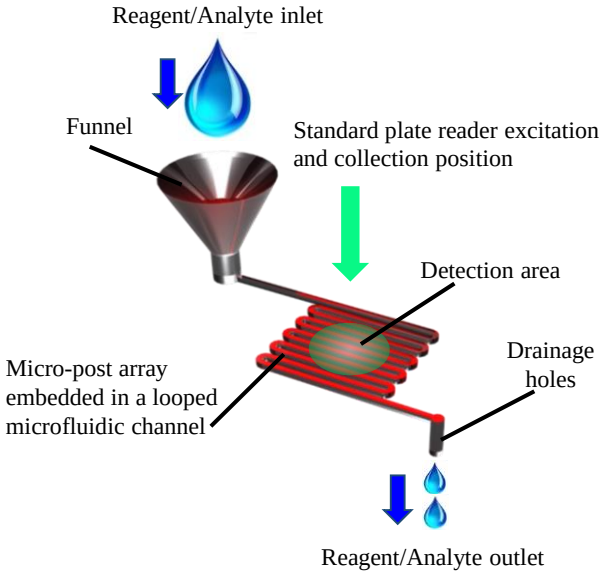


Figure 1. Working principle of a MicroFluere® well plate.

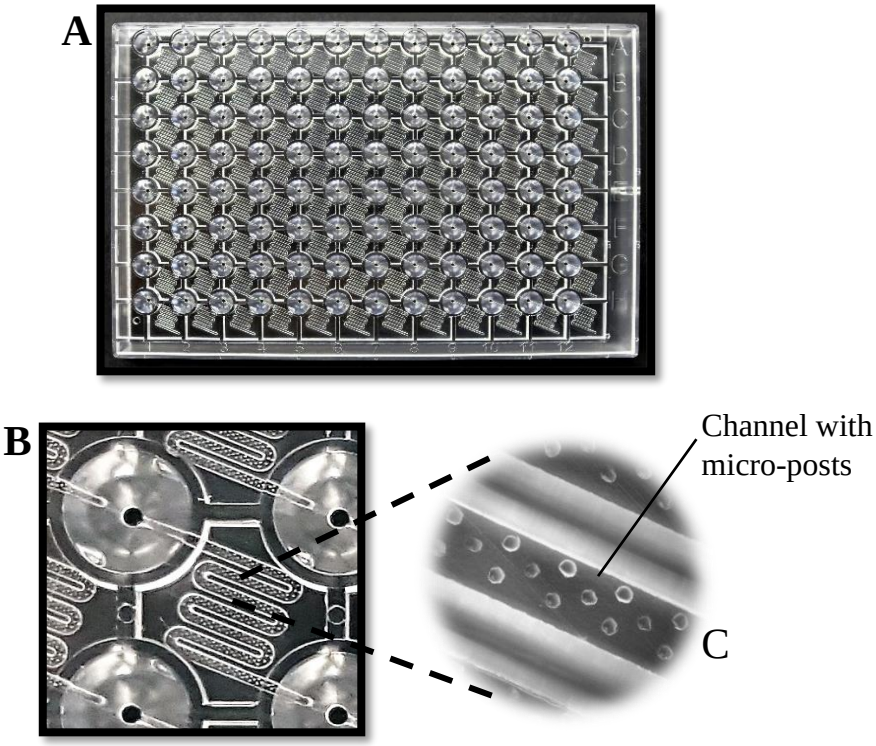


Figure 2. (A) A picture of MicroFluere® well plate. (B) Enlarged view at one of the microfluidic units. (C) Further zoomed-in picture of a microfluidic channel embedded with micro-posts.

In the looped microfluidic channel, an array of optically-transparent micro-posts are arranged in a symmetric pattern. The micro-posts extend perpendicularly from the top of the channel. The micro-posts are equally distributed throughout the entire detection area, and each micro-post has a generally cylindrical shape. The unique arrangement of the channel and the micro-posts within each microfluidic unit assures consistent optical measurements even in the presence of as large as 1 mm positive or negative lateral shift of the plate in the horizontal (X- axis) and/or vertical (Y-axis) direction with respect to the optical detection center, as exhibited in **Figure 3**. The results show that CVs (coefficient of variations) due to both X and Y movements are less than 5% (note: the plate reader itself may already cause 5% variation in optical signal reading).

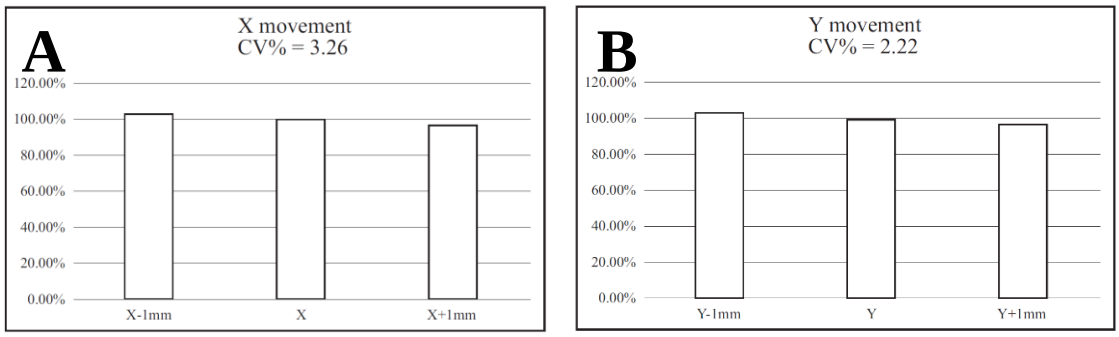


Figure 3. Fluorescence intensity from microfluidic units read by a standard plate reader at each specific X and Y position when each of the microfluidic units is filled with 10 µL of 5 µM Rhodamine 6G. (A) Relative lateral shift of +/- 1 mm in the X-axis. (B) Relative lateral shift of +/- 1 mm in the Y-axis.

Human Interleukin 6 (IL-6) ELISA in buffer and serum

As an example of the MicroFluere® plate performance, we tested various concentrations of Human IL-6 dissolved in buffer solution and serum with fluorescence detection method. MicroFluere® plates were validated by comparing results to traditional 96-well plates. A

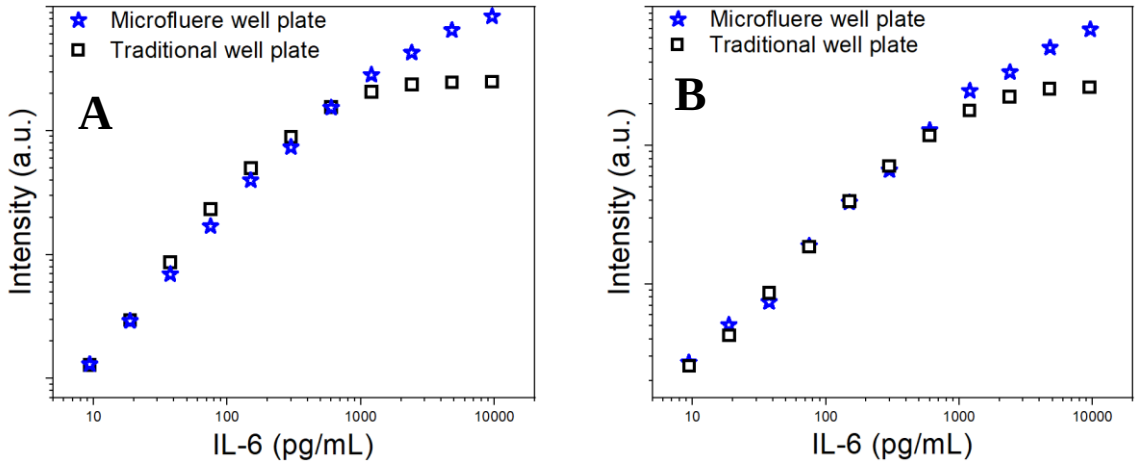


Figure 4. Comparison of IL-6 detection using a traditional 96-well plate (~300 minutes of assay time) and a MicroFluere® plate (<60 minutes of assay time). (A) IL-6 in buffer. (B) IL-6 in serum. The upper detection limit for the traditional and MicroFluere® plates are 1200 pg/mL and 9600 pg/mL, respectively. Note: since the readings for the traditional plate and the MicroFluere® are different, they are adjusted so that the readings for 9.6 pg/mL match.

conventional ELISA protocol of R&D Systems (Kit #DY-206) was used for the traditional 96-well plate and the same protocol (but with less sample and reagent volume and shorter incubation times) was used for the MicroFluere® plate. The results of IL-6 in buffer and serum are shown in **Figure 4A** and **4B**, respectively. Both well plates have the same lower detection limit; however, the dynamic detection rage is further extended with the MicroFluere® plate. The advantages of MicroFluere® plates are summarized in **Table 1**.

Table 1. Comparison of human IL-6 ELISA using traditional and MicroFluere® well plates

	Traditional	**MicroFluere®**
Capture Antibody coating	Over night	1 hr
From adding analytes to recording results	~ 6 hrs	<1 hr
Limit of Detection (IL-6)	9.6 pg/mL	9.6 pg/mL
Dynamic range (IL-6)	9.6-1200 pg/mL	9.6-9600 pg/mL
Reagent/sample consumption	More than 100 µL	Less than 20 µL

Additional biomarkers testing results

MicroFluere® well plates have been validated against traditional well plates with multiple biomarkers, including:

No.	**Tested DuoSet name**	**Catalog code**
1	Human CXCL10/IP-10	DY266
2	Human GDNF	DY212
3	Human IFN-gamma	DY285
4	Human IL-2	DY202
5	Human IL-6	DY206
6	Human IL-8/CXCL8	DY210
7	Human TNF-alpha	DY208
8	Human VEGF	DY293B
9	HIV-1 Gag p24	DY7360-05
10	Rat TIM-1/KIM-1/HAVCR	DY3689

11	Rat VEGF	DY564
12	Human IFN-alpha	DY9345-05
13	Human IL-10	DY217B
14	Human IL-15	DY247
15	Human TGF-beta 1	DY240
16	Human C-Reactive Protein/CRP	DY1707

The list is growing. In all of the above biomarkers (and kits), MicroFluere® plates have the same (or better) lower detection limits and larger dynamic ranges compared to traditional plates, while using only 20% of the sample/reagents and completing the assay 5-10X faster.

Compatibility with Existing ELISA Equipment

MicroFluere® well plates are compatible with existing ELISA microplate readers. In this regard, we have tested the following readers:

EnSpire 2300 Multimode Plate Reader (PerkinElmer)

Infinite F200 Fluorescent Microplate Reader System (Tecan)

Synergy Neo2 Hybrid Multi-Mode Reader (BioTek Instruments)

Synergy H1 Hybrid Multi-Mode Reader (BioTek Instruments)

Synergy HT (BioTek Instruments)

GloMax®-Multi+ Detection System (Promega)

Varioskan Flash (Thermo Fisher Scientific)

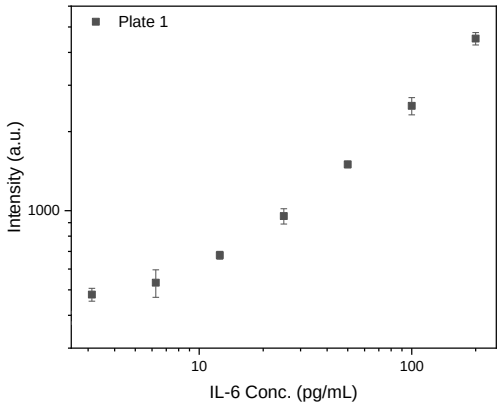
Since MicroFluere® well plates have a flow-through design, the washing and drainage step is slightly different. OptoBio has developed an inexpensive drainage device ($50) that can be used either with the included manual hand-operated pump or through a connection to the vacuum pump. Please be sure to watch the videos included on the website, specifically, https://www.optobio.com/wp-content/uploads/2021/04/Drainage-device-1.mp4

Batch-to-Batch Consistency

OptoBio has finalized the mass manufacturing process to ensure high batch-to-batch consistency with a CV less than 10%.

Well-to-Well Consistency:

Conc.	Plate 1			Avg	STD	CV%
	Rep 1	Rep 2	Rep 3			
0	399	365	412	392	24	6.2%
3.125	511	464	464	480	27	5.7%
6.25	585	550	461	532	64	12.0%
12.5	661	667	704	677	23	3.4%
25	923	1027	911	954	64	6.7%
50	1452	1510	1536	1499	43	2.9%
100	2287	2619	2607	2504	188	7.5%
200	4638	4672	4234	4515	244	5.4%



Conc.	Plate 2			Avg	STD	CV%
	Rep A	Rep B	Rep C			
0	450	379	369	399	44	11.1%
3.125	454	439	401	431	27	6.3%
6.25	592	471	474	512	69	13.5%
12.5	763	587	680	677	88	13.0%
25	1008	993	872	958	75	7.8%
50	1710	1581	1514	1602	100	6.2%
100	2957	2334	2665	2652	312	11.8%
200	4702	4807	5020	4843	162	3.3%

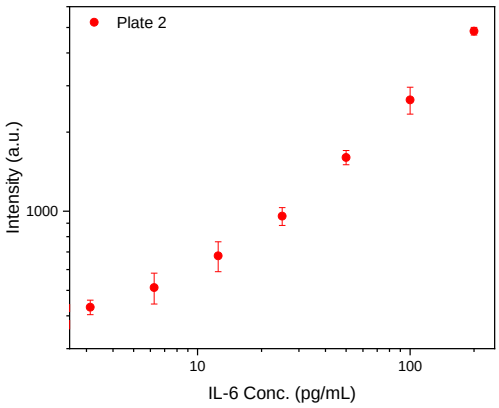
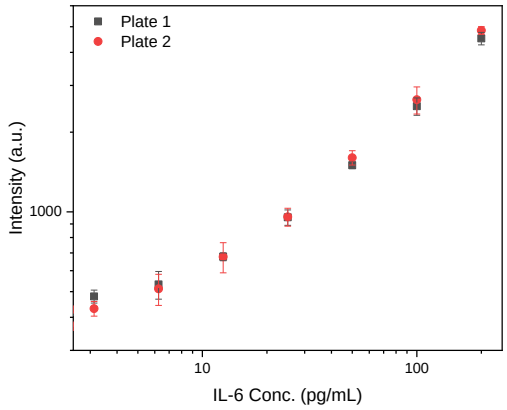
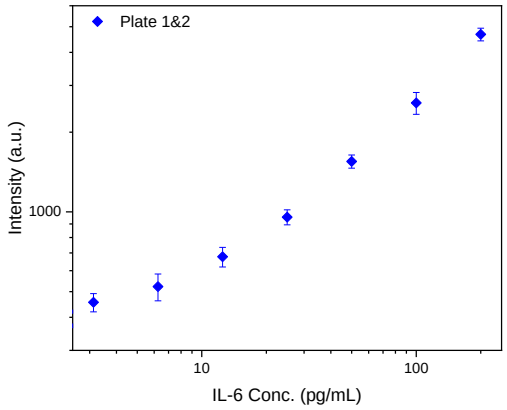


Plate-to-Plate Consistency:

Conc.	Plate 1			Plate 2			Avg	STD	CV%
	Rep 1	Rep 2	Rep 3	Rep A	Rep B	Rep C			
0	399	365	412	450	379	369	396	32	8.1%
3.125	511	464	464	454	439	401	456	36	7.9%
6.25	585	550	461	592	471	474	522	60	11.6%
12.5	661	667	704	763	587	680	677	58	8.5%
25	923	1027	911	1008	993	872	956	62	6.5%
50	1452	1510	1536	1710	1581	1514	1551	89	5.7%
100	2287	2619	2607	2957	2334	2665	2578	244	9.5%
200	4638	4672	4234	4702	4807	5020	4679	258	5.5%





Chemiluminescence

The results of the MicroFluere® plate presented here are based on fluorescence detection method. We have also validated its performance based on chemiluminescence detection method when the MicroFluere® well plate is made of an opaque material such black polystyrene. Currently, all of the MicroFluere® based ELISA are done with manual pipette injection and a manual drainage device.

Summary

OptoBio has successfully utilized microfluidics in 96-well microplate to make ELISA faster, less costly and have higher dynamic range than traditional microplate:

- 5X-10X faster assay
- 5X reduction in reagent and sample required
- 10X increase in dynamic range