



Zoom Plate™ Dot Blot

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Zoom Plate is a novel 96 well assay platform for fast and sensitive membrane-based assays such as dot blot and other sandwich assays. Based on Vitrozm's proprietary vertical flow technology, Zoom Plate significantly simplifies the washing and incubation process in membrane-based assays, allowing fast and sensitive analysis without lengthy incubation or vacuum/centrifuge-assisted filtration. Users only need to pipette samples and reagents sequentially into the Zoom Plate wells, and get results in about 1 hour. Compared to traditional dot blot, Zoom Plate only needs 1/1000 amount of antibody for each sample, a significant saving for large scale screening tests. Compatible with automatic liquid handling, Zoom Plate enables sensitive fluorescent, luminescent and colorimetric assays with excellent signal-to-noise ratio.

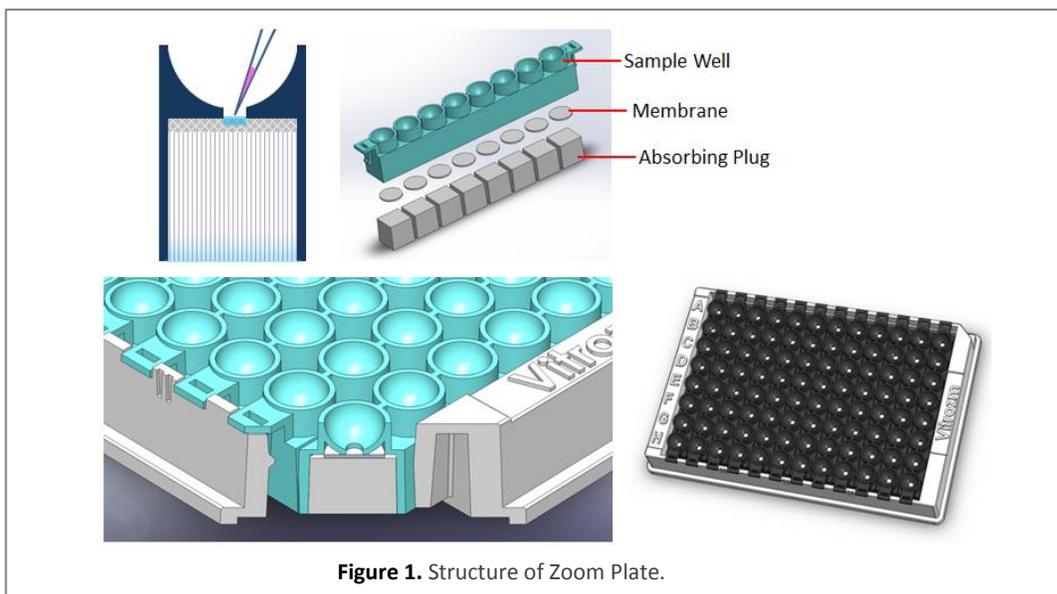


Figure 1. Structure of Zoom Plate.

Dot blot is a widely used membrane-based biomolecule analysis method¹⁻⁵. It can be used for fast detection and identification of proteins and nucleic acids. In a typical dot blot assay, samples containing the target antigens are spotted on a nitrocellulose membrane in an array of small confined area. The blotted membrane is then blocked and incubated sequentially with primary antibody and enzyme-labeled secondary antibody. After applying appropriate enzyme substrates, chemiluminescent or chromogenic signal is detected and analyzed qualitatively or semi-quantitatively. Conventional

protocol requires placing the membrane in a serial of buffer and solutions for membrane blocking, affinity binding, rinsing, and signal development. The entire process, including multiple prolonged incubations and washing steps, takes several hours or even overnight to finish. A commonly used dot blot apparatus Bio-Dot (Bio-Rad) relies on a vacuum source to suck solution through the membrane, but the apparatus set up is complicated, expensive, and need to be assembled, disassembled and cleaned for every use. Leaking and cross-contamination is hard to avoid in these methods because one continuous piece of membrane is used for

multiple assay dots. Because of these limitations, dot blot is mostly used for preliminary screening or confirmation, not widely used for high throughput analysis or clinical diagnostics.

Zoom Plate technology significantly simplifies dot blot assay's washing step and has transformed the time-consuming assay into a fast and easy process. Advantageously, Zoom Plate provides more easier and efficient washing in a matter of minutes, without using shaker, centrifuge, or vacuum. Zoom Plate allows over 99% savings by using much less amount of assay reagent and antibody than traditional methods, while providing 10+ times more sensitive results with zero cross-contamination. Fluorescent, luminescent and colorimetric signals generated from Zoom Plate can be easily detected and analysed using common plate reader or gel imager.

Vertical Flow Technology

Zoom Plate is a vertical flow assay platform for membrane-based assays. It utilizes porous absorbing material to drive assay reagents vertically through a porous membrane by capillary force. The large surface area of the porous membrane allows large number of affinity binding reactions to occur, and the vertical flow enforces close proximity of the reactive molecules in the solution to their targets bound on the membrane, therefore the binding reactions happen much more efficiently than conventional incubation methods. The easy-bind-easy-block membrane materials provide high capacity protein adsorption during spotting, and efficient blocking afterwards. Together with the low fouling absorbing plug, the system ensures ultra-low background for signal detection.

As shown in **Fig. 1**, each Zoom Plate well is composed of a sample well, a porous membrane, and an absorbing plug. In a typical Zoom Plate membrane-based assay, such as dot blot, direct ELISA, indirect ELISA, and other types of sandwich assays, 1-2 μ l of a capture antibody or antigen is first spotted onto the membrane and let air dried for about 20-30 min.

Afterwards, the membrane need to be blocked for 20-30 min. Additional reagents, such as antigen, primary antibody, and secondary antibody are added sequentially into the well and let react with the previously immobilized molecules for no more than 5 min. After each incubation, add rinsing buffer to the well and the unbound molecules will be removed by the absorbing plug underneath the membrane. If the antibody is labeled with a fluorophore or a colored-agent, the plate can be examined immediately after the final rinse. If the antibody is labeled with an enzyme, such as HRP or AP, a small amount of enzyme substrate can be added onto the membrane to allow luminescent or chromogenic signal detection.

Chemiluminescent Dot Blot

An IL6 dot blot assay was performed to demonstrate the capability of Zoom Plate. In this application, we used black Zoom Plate strips with 1.6 μ m pore size glassfiber membrane (cat# GF16B). 1 μ l of human IL6 standard (R&D systems) dilution at 0.1 - 100 μ g/ml was spotted on the membrane and let dried for 20 min. After 20 min blocking with 10% BSA, 2 μ l of 2 μ g/ml primary antibody monoclonal mouse anti-IL6 antibody (R&D Systems) was added onto the membrane and let incubate for 5 min, followed by a rinsing with 50 μ l rinsing buffer 1%BSA-PBST. Afterwards, 2 μ l of 0.5 μ g/ml HRP-conjugated secondary antibody polyclonal goat anti-mouse IgG (KPL) was added for 5 min, followed by final rinsing of four times 50 μ l rinsing buffer PBST. Immediately after 10 μ l of HRP ECL substrate SuperSignal Dura (Thermo) was added, chemiluminescent image was captured in a gel imaging system FluorChem (ProteinSimple).

The entire assay, including reagent preparation and signal detection, was completed comfortably within 1 hour. As shown in **Fig. 2**, the results demonstrate pg sensitivity for dot blot assay, with excellent linearity ($R^2 = 0.997$) and consistency over at least 4 order of detection magnitude from 100 pg to 100 ng.

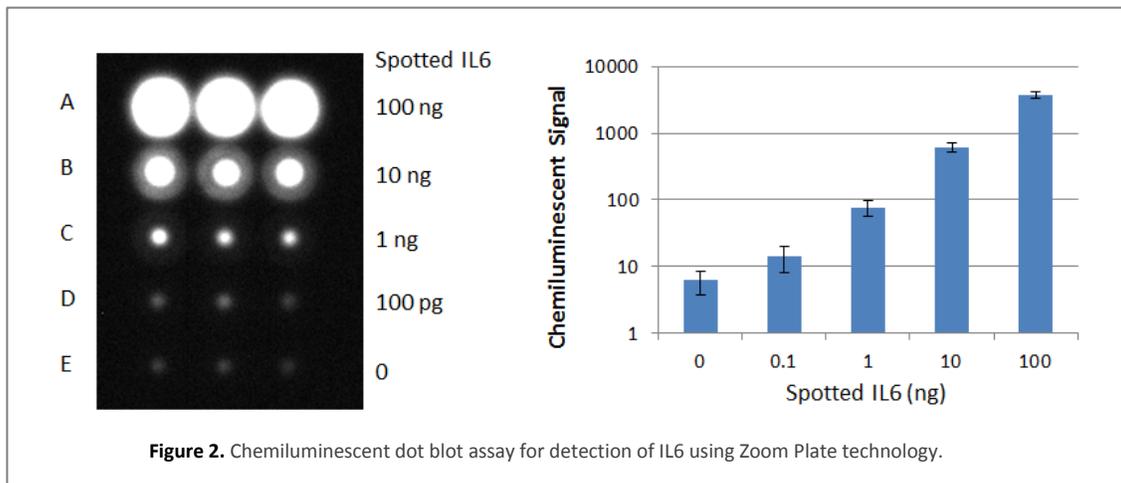


Figure 2. Chemiluminescent dot blot assay for detection of IL6 using Zoom Plate technology.

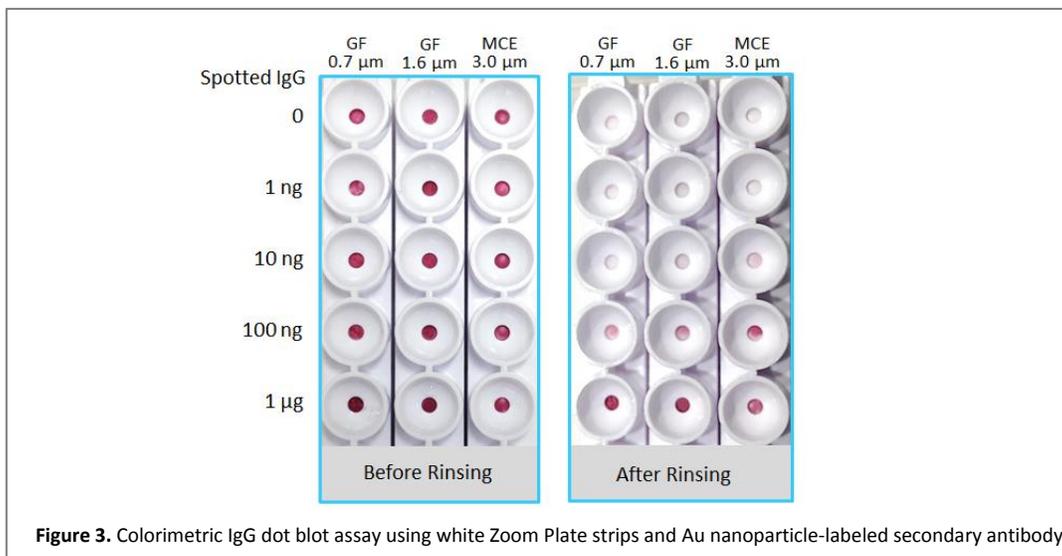


Figure 3. Colorimetric IgG dot blot assay using white Zoom Plate strips and Au nanoparticle-labeled secondary antibody.

Colorimetric Dot Blot Assay

White Zoom Plate is ideal for colorimetric membrane-based assays using detection antibodies labeled with gold nanoparticles or colored latex beads, or HRP-labeled antibody with chromogenic enzyme substrate. We tested three different white color Zoom Plate strips made with 0.7 μm pore size glassfiber, 1.6 μm pore size glassfiber, and 3.0 μm MCE membrane (cat# GF07W, GF16W and MC30W respectively) in a mouse IgG direct dot blot assay using Au nanoparticle-labeled antibody.

1 μl mouse IgG (R&D Systems) from 1-1000 $\mu\text{g}/\text{ml}$ was spotted in the Zoom Plate wells. After 20 min blotting, 50 μl 10% BSA was added for a 20 min blocking. 1 μl goat anti-mouse IgG labeled with 40 nm Au nanoparticle (Abcam) was added into each well and incubated for 5 min. Afterwards, the wells were rinsed with 50 μl PBST four times. Color Images of the strips were taken before and after the rinsing step (**Fig. 3**). Distinctive red color can be clearly visualized against the white background in all three strips. Intensity of the red color is correlated with the concentration of the dotted proteins. In this experiment, MCE membrane demonstrated better detection sensitivity (10 ng) than the glassfiber membranes (100 ng) for colorimetric assay.

Product Summary

Comparing to conventional dot blot assays methods, Zoom Plate can save >99% of antibody by using μl 's instead of ml's of reagents. Sensitive and quantitative results can be obtained with 1 hr. Zoom Plate eliminates cross-contamination between samples, making detections results more reliable. The stand-alone and ready to use 96 well plate is compatible with automatic liquid handler, facilitating high throughput analysis and screening.

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