Near infrared fluorescent detection of a human cytokine array using the Sapphire FL

Introduction

Membrane-based arrays present a way to carry out immunodetection of multiple targets in parallel. The ability to assess dozens of proteins in one experiment increases throughput and saves time and money while conserving sample when compared to analyzing each target individually. Antibody arrays provide the functional opposite of Western blots; where free antibodies are washed over immobilized targets for detection in Westerns, target-containing sample is washed over immobilized antibodies in antibody arrays.

Adding to the differences between the assays, targets in antibody arrays are detected in a native form, while targets in Western blots are usually linearized and denatured before detection.

The antibody array's design is more like a sandwich ELISA than a traditional Western blot. In a sandwich ELISA, immobilized antibodies capture targets within a sample and a second set of labeled antibodies is then used for detection. Though similar in concept to ELISAs, antibody membrane arrays provide a faster and easier way to assess multiple targets.

This application note demonstrates detecting a human cytokine array using near infrared (NIR) fluorescence on the Sapphire FL. Often, antibody arrays are detected using chemiluminescence, which requires only film or a chemiluminescence imaging system. In this note, NIR fluorescence detection was used instead of traditional chemiluminescence. NIR fluorescence detection allows sensitive and quantitative detection compared to chemiluminescence.

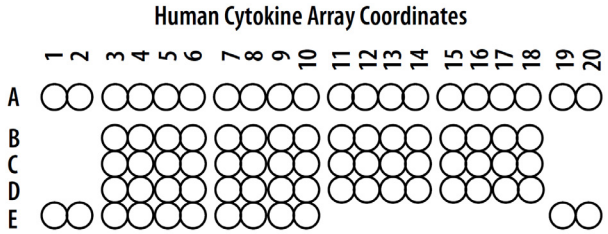


Figure 1. Array coordinates. Diagram showing the layout of the Proteome Profiler™ Human Cytokine Array (R&D Systems, catalog #ARY005B). The array consists of detection antibodies for 36 cytokines, chemokines, and acute phase proteins, spotted in duplicate. In addition, reference spots are present in three corners for alignment and negative control spots are present at the fourth corner (coordinates E19 and E20).

Materials and Methods

Sample Preparation

Peripheral blood mononuclear cells (PBMCs) were collected from whole blood. Four vials of whole venous blood were collected and allowed to equilibrate to ambient temperature for 20 minutes. The vials were centrifuged at 1700 x g for 15 minutes and the PBMC layer transferred to clean 15 mL tubes. The PBMCs were counted and plated on a 24-well plate with $5x10^5$ cells per well. Cells were cultured for 1 day at 37°C in 5% CO_2.

The PBMCs were treated in triplicate with either 10 µg/mL phytohemagglutinin (PHA), 50 ng/mL phorbol myristate acetate (PMA), or 1 µg/mL lipopolysaccharide (LPS). Control cells were left untreated. The media was aspirated from the cells before media containing each treatment was added. After incubation in the treatment for 48 hours, the supernatant from each well was collected and used to probe the cytokine array.

Labeled Streptavidin

Streptavidin (Thermo Scientific) was labeled with IR800-NHS (Advansta Inc.) by incubating at ambient temperature for 30 minutes. Labeled streptavidin (SA-IR800) was purified using a PD-10 desalting column (Cytiva) and concentrated using a centrifugal filter unit (Amicon).

Array Hybridization

Four arrays were probed with the supernatants collected from the treated PBMCs. Proteome Profiler™ Human Cytokine Arrays (R&D Systems) were probed according to the manufacturer's instructions. Arrays were blocked for one hour with rocking in Array Buffer 4.

The treated PBMC supernatants were incubated with Human Cytokine Array Detection Antibody Cocktail in Array Buffer 4 for one hour at ambient temperature.

The blocking buffer was aspirated from the arrays and the supernatant/detection antibody mixtures added to the arrays. Arrays were hybridized overnight at 4°C with rocking. Arrays were washed three times for 10 minutes each in Wash Buffer and then incubated with the SA-IR800 conjugate for 30 minutes at ambient temperature with rocking.

Arrays were transferred to a clean tray containing Azure Fluorescent Blot Washing Buffer (Azure Biosystems) and washed three times for 10 minutes each, and then once in 1X PBS. Arrays were allowed to dry completely and then imaged on the Sapphire FL.

Imaging

Arrays were imaged on the Sapphire FL Biomolecular Imager using the 784 Standard Optical Module which includes the 784 nm laser and 829/62 nm emission filter. The Membrane Focus setting was used (0.00 mm), Quality setting was High, Intensity setting was 2, and Resolution was set to 10 microns.

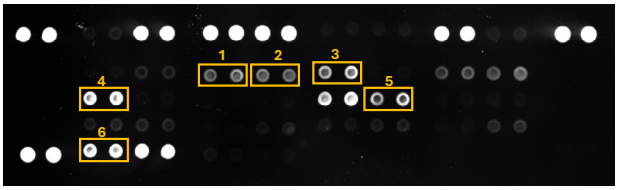
Results and Discussion

Antibody cytokine arrays were probed with supernatant from untreated PBMCs and from PBMCs treated with PHA, PMA, or LPS. The layout of the array is shown in Figure 1. The array consists of detection antibodies for 36 cytokines, chemokines, and acute phase proteins, spotted in duplicate on nitrocellulose. Each spot is about 1 mm in diameter, and the center-to-center distance for spots on the array is 2 to 3 mm.

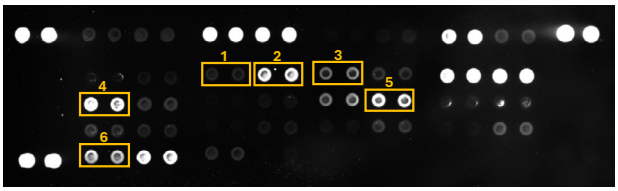
The Proteome Profiler™ Human Cytokine Array kit comes with a biotin-labeled cocktail of detection antibodies which are typically detected using HRP-labeled streptavidin. For this application note, IR800-labeled streptavidin was used instead of HRP-labeled, and the arrays were imaged using the 784 Standard Optical Module of the Sapphire FL. Images of the four arrays are shown in Figure 2A.

A) Untreated

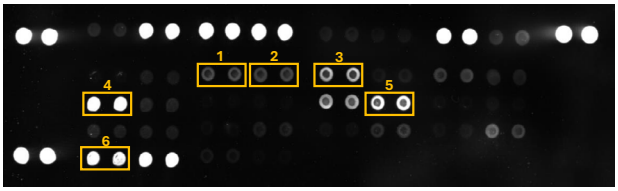
1 µg/mL LPS-Treated



50 ng/mL PMA-Treated



10 µg/mL PHA-Treated



B)

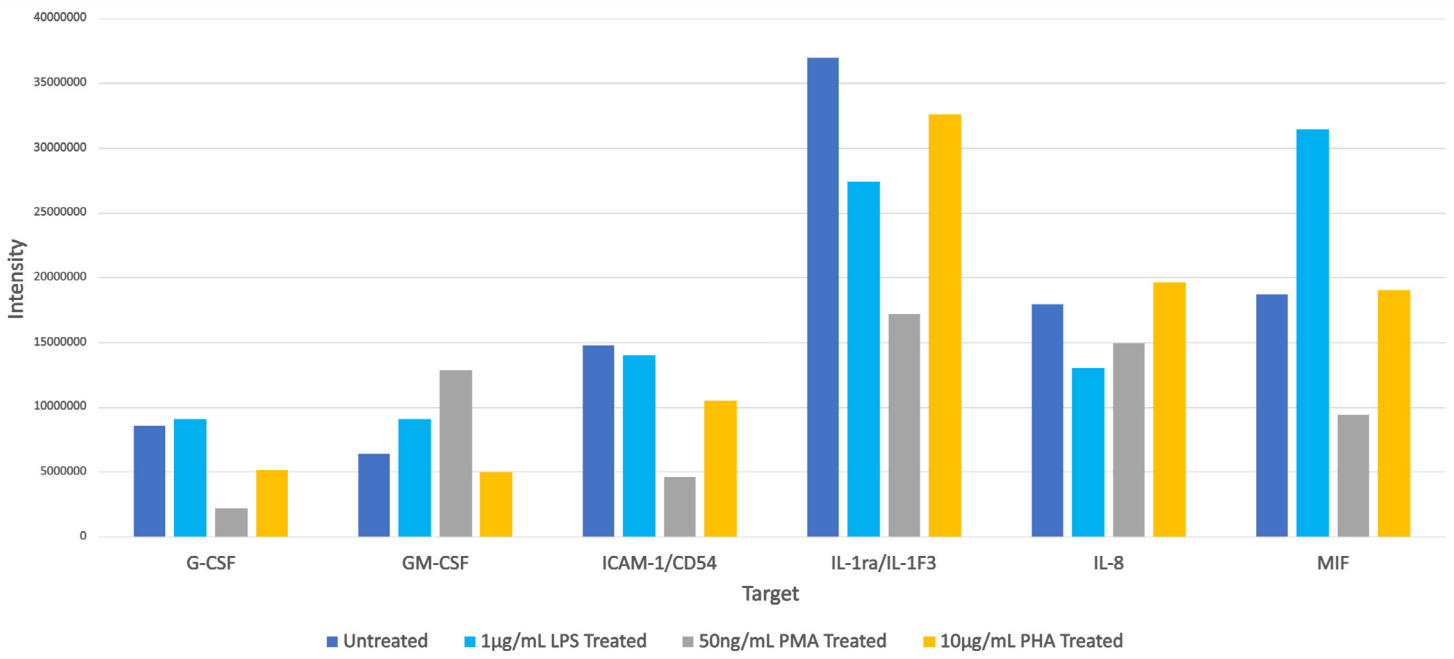


Figure 2. Cytokine array detected using the Sapphire FL. A. Images of the four blots probed with the supernatants of control PBMCs or PBMCs stimulated with the indicated treatments. Six targets were selected for further analysis: 1, G-CSF; 2, GM-CSF; 3, ICAM-1/CD554; 4, IL-1ra/IL-1F3; 5, IL-8; and 6, MIF. The fluorescence intensities of these spots are shown in Figure 2B.

Six targets were selected for further analysis. The selected spots are outlined in Figure 2A and correspond to the following targets: 1, G-CSF; 2, GM-CSF; 3, ICAM-1/CD54; 4, IL-1ra/IL1F3; 5, IL-8; and 6, MIF. The fluorescence intensities of these spots were quantified, and the intensities are shown in Figure 2B. The results indicate that treatment affected the amounts of these cytokines present in the cell supernatants.

The results demonstrate the ability of the Sapphire FL to capture images of membrane arrays using NIR fluorescence, with excellent sensitivity and resolution. The donut or ring shapes of some of the spots are not an artifact but the result of how the capture antibodies dry on the membrane after spotting (1). Changes in several targets are apparent in the images and the differences are confirmed by quantifying the fluorescence intensity. The Sapphire FL simplifies analysis of membrane arrays and similar arrayed samples, such as multi-well plates or dot blots, with software to quickly map and assign labels to each spot.

With user-changeable UV, visible fluorescence, NIR fluorescence, and phosphor modules, the Sapphire FL provides flexible compatibility with a wide range of imaging applications, including NIR fluorescence detection of membrane antibody arrays.

References

1. FAQ. R&D Systems website. https://www.rndsystems.com/resources/faqs/proteome-profiler-antibody-arrays

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