CyAn™ ADP Detection of Soluble Cytokines Using Multiplexed Beads Assays

Introduction

Multiple soluble molecules in body fluids or culture supernatants can be measured by Flow Cytometry using multiplex bead-based assays. This method requires a smaller sample volume (about 25 µL) and has a higher sensitivity and speed compared to other techniques, such as ELISA.[1]

This application note illustrates the use of the CyAn ADP multicolor flow cytometer to acquire 11-plex samples prepared with the Bender MedSystems FlowCytomix Kit used on the culture supernatant of a CD8+ T cell line, HLA-A*0201-specific, obtained with repeated NV9 peptide (part of CMV pp65 antigen, NLVPMVATV) pulsing and IL-2 stimulation (see Figures 2 and 3).

This kit uses two sets of size-coded beads (5,5 microns shown in R1, and 4,4 microns, shown in R2) composed of 5 / 6 different beads subsets, fluorescence-coded by different intensities of the Starfire Red** dye (excitation from UV to red, emission max at 675nm). Each bead is coated with a different antibody, specific for a single analyte.

The analyte concentration is measured staining the cytokine + bead complex with a secondary, biotin-conjugated antibody, followed by streptavidin-PE staining.

Different PE intensities represent different analyte concentrations.

The kit includes a set of standards for each analyte. Thus, the measured intensities can easily be translated into concentration units.

Materials and Methods

A Th1/Th2 11-plex Kit was used to provide the concentration of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α, TNF-β, IFN-γ.

The procedure method in summary is:

Step 1: Incubate beads, sample and biotin conjugates
Step 2: Wash
Step 3: Incubate with PE-streptavidin
Step 4: Wash
Step 5: Acquire sample on CyAn ADP 9 Color with Summit software v4.3.

Results

FSC and SSC were used to identify the two bead populations (Figure 1: R1 and R2). Two plots with FL2 (PE) vs. FL8 (Starfire Red) were created and gated on the two beads sets to separate the different Starfire Red levels, each representing an analyte, and to monitor the PE mean fluorescences

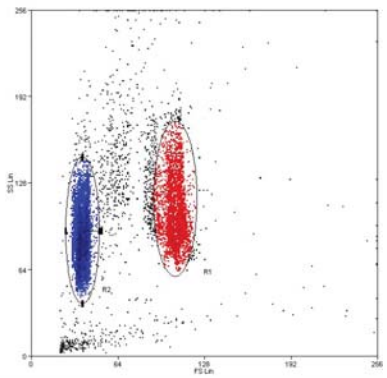

Figure 1

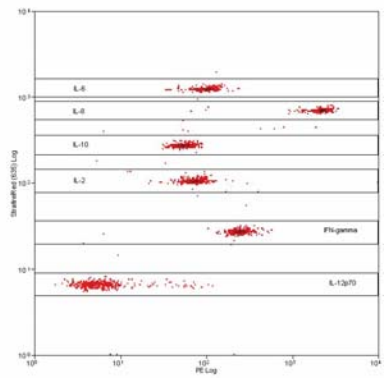

Figure 2

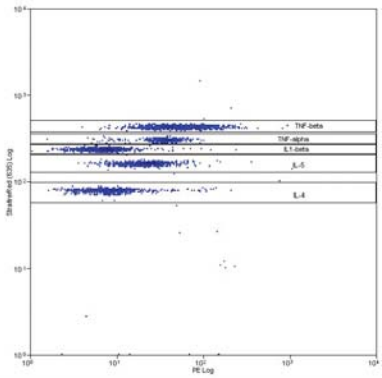

Figure 3

(Figures 2 and 3) representing the analyte concentration.

The FL8 channel gave the necessary resolution and compensation was not required between Starfire Red and PE. For each analyte the required minimum of 300 beads was acquired, 5,000 beads were collected in total.

Discussion

As shown, CyAn ADP can easily resolve the two different sized beads and analytes. A dedicated software (Bender FCMix Pro 2.1, included in the Kit) automatically calculates the cytokine concentration (pg/mL) by defining and applying 11 reference curves generated with corresponding 11 Standards set (e.g. for IL-4, see Figure 4).

Note

Please refer to the manuals and website provided by Bender MedSystems for detailed sample preparation and analysis procedures.

References

1. Paul WE and Seder RA. Lymphocyte responses and cytokines. Cell 76: 241-51 (1994).
2. Rooney JW et al. A common factor regulates both Th1- and Th2-specific cytokine gene expression. EMBO Journal 13:625-33 (1994).
3. Lederer JA et al. Regulation of cytokine gene expression in T helper cell subsets. Journal of Immunology 152: 77-86 (1994).

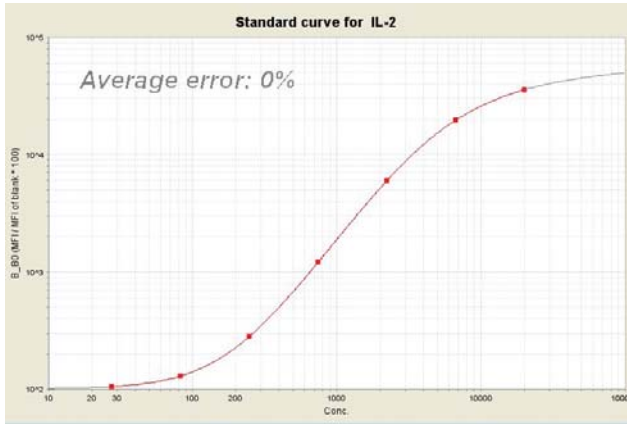

Figure 4

Contributors

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Technical Tips

- Beads agglutination may occur in lipemic samples. Centrifugation of lipemic samples (5 minutes, 16,000 rcf) before analysis may help creating the needed sample fraction located between the pellet and the lipemic layer at the top.

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