



FLOW CYTOMETRY

CyAn™ ADP | Detection of Soluble Cytokines Using Multiplexed Beads Assays

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 ELISA¹.

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 set 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 LX 9 Color with Summit software v4.3.

For more detailed information see manual and website of Bender MedSystems.

Results

FSC and SSC were used to identify the two beads population (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 (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.

Figure 1

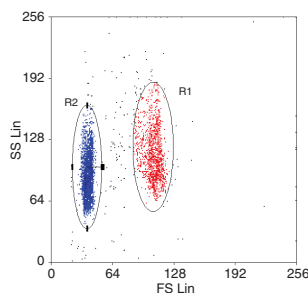


Figure 2

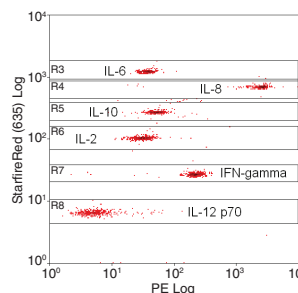
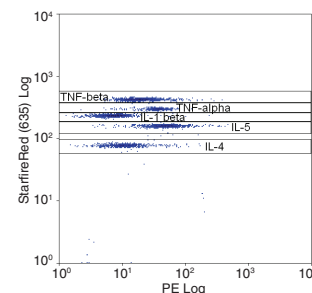


Figure 3



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).

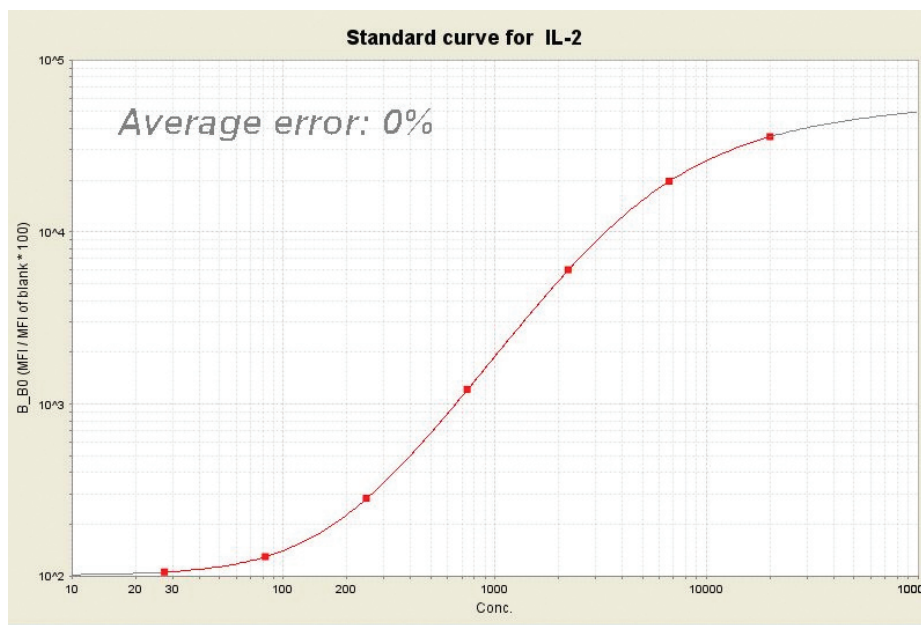
Notes

1. 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.

PRODUCT

CyAn ADP LX 9 Color.....

CODE

CY201

For research use only – not to be used in diagnostic procedures. Other vendor products used in this application: FlowCytomix Th1/Th2 11plex Kit, code number BMS810FF (Bender MedSystems GmbH).

The protocols in this application note are for explanation purposes only and might deviate from the normal recommended protocol/specification guidelines that are included with the Dako product or any other non-Dako product.



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