

## Flow Cytometric Multiplexing Multiple Analyte Detection System

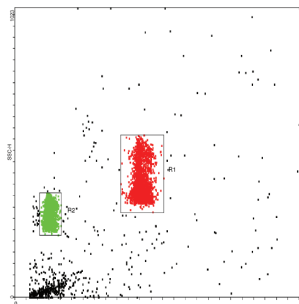
**C**ytokines are a category of signalling proteins and glycoproteins that are used extensively in cellular communication. They are produced by a wide variety of hematopoietic and non-hematopoietic cell types and can have effects on both nearby cells or throughout the organism, sometimes strongly dependent on the presence of other chemicals. They are critical to the functioning of both innate and adaptive immune responses, often secreted by immune cells which have encountered a pathogen as a way to activate and recruit more immune cells and increase the system's response. However, apart from their role in the development and functioning of the immune system, their aberrant modes of secretion in a variety of immunological, inflammatory and infectious diseases makes the monitoring and measurement of these soluble proteins increasingly important.

In the past ELISA methodology was used to detect and measure individual cytokines. This technology has now been updated for use on a flow cytometer where up to 20 cytokines (multiplexing) can be measured simultaneously in a small sample volume.

Flow cytometry is a powerful tool that simultaneously measures and then analyses multiple physical characteristics of single particles as they flow in a fluid stream through a beam of light. The multiplexing technology is based on a sandwich immunoassay, but uses fluorescent polystyrol beads coated with cytokine specific antibodies. A mixture of up to 20 different coated beads is incubated with a patient's sample; the cytokines present in the sample will adhere to the relevant antibody on the bead surface. A biotin-conjugated secondary antibody mixture is added, binding to the captured cytokines on the beads, finally, Streptavidin-Phycoerythrin is placed in the mixture and attaches to the biotin conjugate and emits fluorescent signals, directly proportional to the amount of cytokine present, detected on the flow cytometer. The raw data from the cytometer is then analysed by a programme that measures the amount of each cytokine.

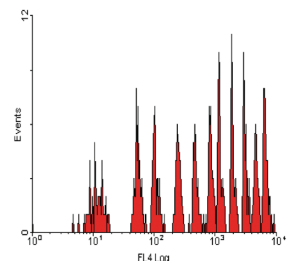
### Principles:

- Two sets of microspheres of different size are used for multiplexing:
  - Size A: 5.5um
  - Size B: 4.4um

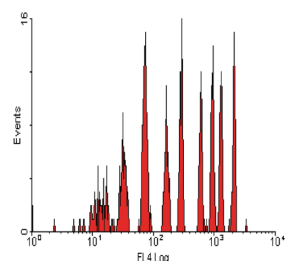


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- Size A consists of 11 bead populations, Size B consists of 9 bead populations internally dyed with different intensities of a fluorescent dye. The dye excites the laser and emits in the far red spectra (690nm)

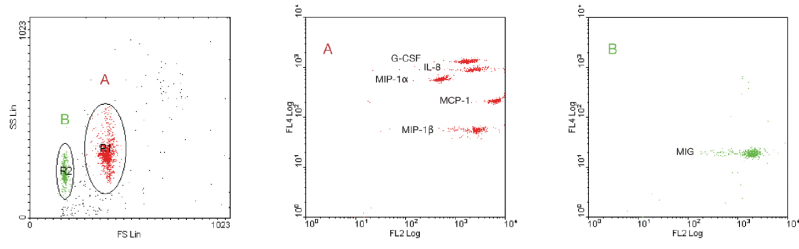


*Size A bead showing 11 populations*



*Size B bead showing 9 populations*

- The 2 different bead sizes make it possible to distinguish 20 bead sets in one fluorescent channel. So that 20 different bead sets distinguished by internal dye intensity and bead size allow simultaneous quantification of 20 analytes in a single 25ul volume sample using the same principle as ELISA.



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