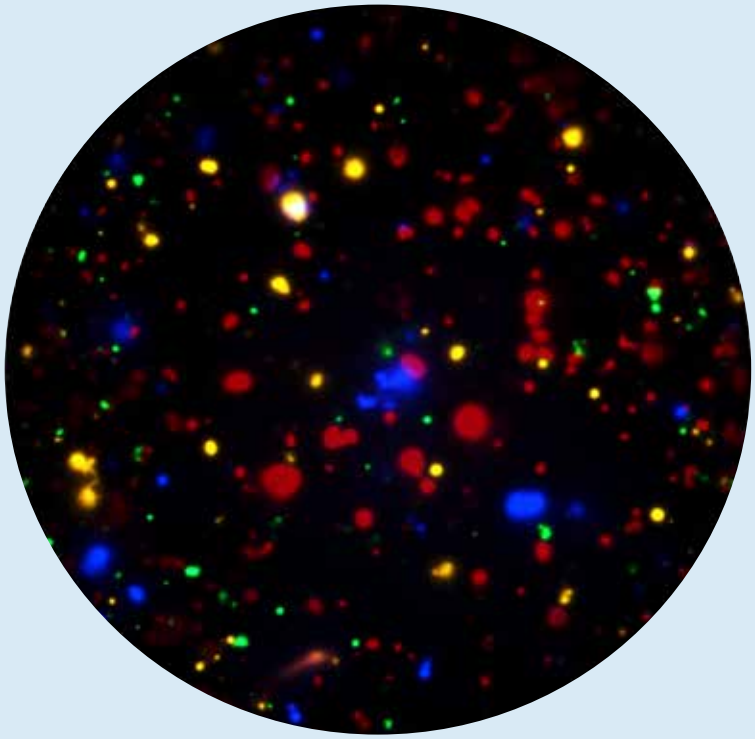
ImmunoSpot®

Four-color B Cell FluoroSpot for Immunoglobulin Isotyping

Traditional measure of antibody titers using ELISA involve the detection of individual immunoglobulin (Ig) classes or IgG subclasses in separate assays. The ELISPOT technique, originally developed by Cecil Czerkinsky in 1983 to detect immunoglobulin-secreting B cells, is a highly-sensitive tool for monitoring immunoglobulins of a single class. However, single-target assays make comprehensive studies of multiple Ig classes/subclasses difficult — requiring additional cell material, antigen, and labor. Since dual-color B cell FluoroSpot assays have become available, facilitating the detection of different combinations of Ig classes or IgG subclasses simultaneously has greatly expanded our capacity to gain relevant insight about the functionality of B cell responses to antigen stimulation.

Even though various multiplex immunoglobulin isotyping technologies are currently available, they employ strategies that suffer from an inherent lack of sensitivity in detecting low-frequency, antigen-specific responses, as opposed to B cell ELISPOT and FluoroSpot assays. Without the ability to measure such antigen-specific responses, the more relevant memory B cell potential, and thus the conferred protective immunity, remains unknown.



IgG1: Red IgG2: Yellow IgG3: Blue IgG4: Green

A representative four-color well is shown along with the fluorescent tags for the individual IgG subclasses.

Four-color B Cell FluoroSpot for Multiplex Analysis

Measure all four classes/ subclasses simultaneously & in various combinations

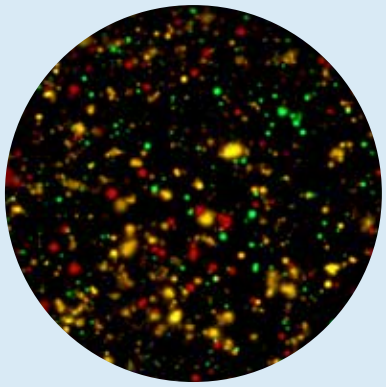
Detect combinations of up to four different Ig classes, or all four IgG subclasses *simultaneously* with the same sensitivity, accuracy and reproducibility as individual detection.

The B cell FluoroSpot assay reveals the frequency of antigen-specific B cells within a given cell population. Immunoglobulin classes/subclasses of the Antibody Secreting Cells (ASC) can be detected using Ig class/subclass specific ELISPOT/FluoroSpot assays, giving valuable evidence about the function of the secreted antibody:

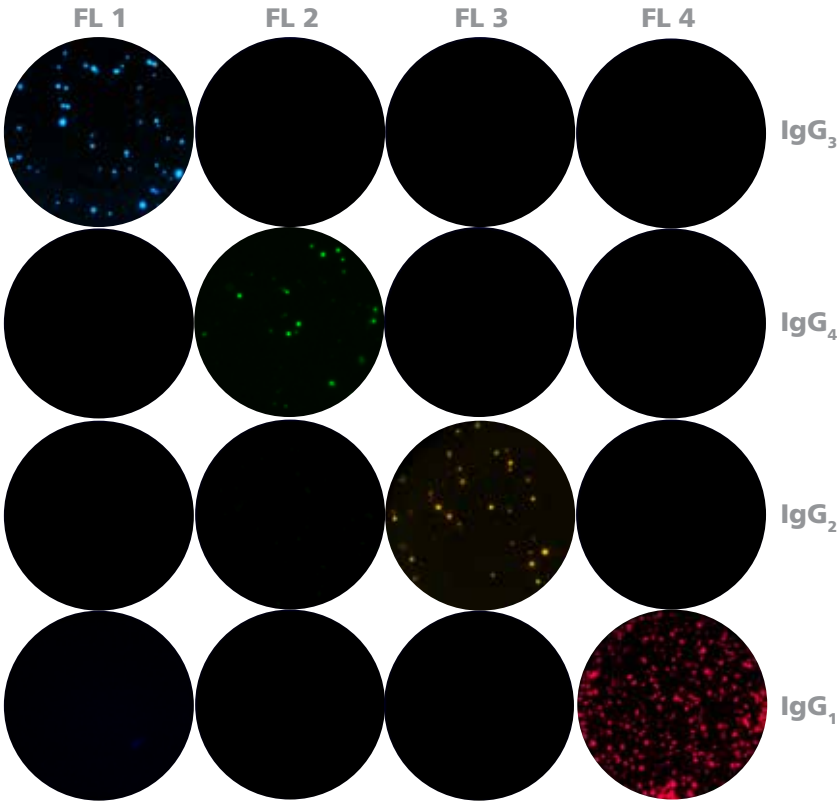
IgE	Degranulation of basophils, mast cells, type 1 allergy
IgG4	Allergen-specific "blocking"
IgA	Mucosal defense
IgG1	Most prevalent Ig, complement fixation, opsonization
IgG2	Low-affinity, activation of complement, opsonization
IgG3	High-affinity binding, complement activation; spontaneously secreted by B1 cells
IgM	Initial pathogen response, agglutination; spontaneously secreted by B1 cells

IMMUNOSPOT® Four-color B Cell FluoroSpot for Immunoglobulin Isotyping

Another unique metric of ELISPOT/ FluoroSpot assays is the spot morphology. For example, the extent to which the secreted antibody has diffused away from an ASC indicates the relative affinity of the secreted antibody for its target antigen. Also, the size and intensity of the detected spot is directly related to the magnitude of the B cell response.



Dissecting the Ig class response: Three-color detection of HCMV-specific antibody classes produced by polyclonally stimulated B cells. All 3 classes – IgG, IgM, and IgA – are produced in response to stimulation. Yellow: IgG; Green: IgM; Red: IgA



In a 4-Color B cell FluoroSpot assay, each subclass-specific detection antibody is labeled with a fluorochrome that has an emission wavelength that clearly distinguishes it from the other fluorochromes it is used in combination with. Using an ImmunoSpot® Analyzer allows clear, unambiguous detection of all four wavelengths, and thus all four IgG subclasses can be visualized simultaneously.

	IgG1	IgG2	IgG3	IgG4
SC Enzymatic	241 +/- 13	60 +/- 8	67 +/- 8	29 +/-7
SC Fluorescent	248 +/- 16	59 +/- 3	43 +/-5	25 +/- 3
4C Fluorescent	250 +/-9	37 +/-7	55 +/- 10	21 +/- 5

Single-color (SC) enzymatic and SC fluorescent B cell ELISPOT assays performed to detect individual IgG subclasses provide the same ASC frequencies as the four-color (4C) fluorescent assay.

CTL can tailor B cell FluoroSpot Assays to meet your research needs. Select different combinations of Ig classes and IgG subclasses, depending on your specific interests; for example, IgG1, IgG3, IgM, IgA. Dual-color FluoroSpot assays for all combinations of classes or subclasses are also now available!

C.T.L.

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