

NEW Interferon- γ ELISPOT Assays

By Qi Guan, David Sehy, M.S. and David Ernst, Ph.D.

The enzyme-linked immunospot (ELISPOT) assay is a powerful tool for detecting and analyzing individual cells that secrete a particular protein *in vitro*¹. Although originally developed for analyzing specific antibody-secreting cells^{2,3}, the assay has been adapted for measuring the frequencies of cells that produce and secrete a variety of other effector molecules such as cytokines^{4,5,6}. The ELISPOT assay has the advantage of being capable of detecting cytokine-producing cells from both activated naive and memory T cell populations, as well as a variety of other cell types. Cytokine release can be detected at the single cell level, allowing direct determination of cytokine-producing cell frequencies⁵. Furthermore, this assay has been found to be more sensitive than ELISA and intracellular staining^{1,7,8}. The sensitivity of the assay enables measurement of even very low frequencies of cytokine-producing cells (*e.g.*, 1/300,000)¹. Recent developments in assay plate design and in ELISPOT plate-reader instrumentation have significantly improved the utility of the ELISPOT method for objective and rapid analysis of cytokine-producing cells¹.

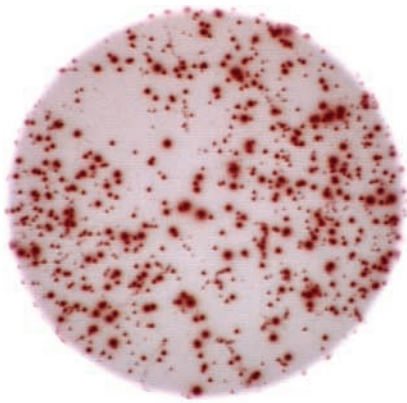
The ELISPOT derives its specificity and sensitivity by employing high affinity capture and detection antibodies and enzyme-amplification. Since 1989, Pharmingen has produced and supplied high quality antibody pairs for cytokine analysis. Numerous publications cite BD Pharmingen™ cytokine ELISA pairs for ELISPOT assay, including *Current Protocols in Immunology* (John Wiley & Sons, Inc., NY). Recent collaborations with CTL Analyzers LLC (www.immunospot.com; Cleveland, OH), the developers of state-of-the-art ImmunoSpot™ plate reader instrumentation and software, have led to the identification of novel, optimal antibody clone pairings for ELISPOT assays. In the course of screening many antibody clones for the ELISPOT application, we ascertained that some antibody pairs which perform very well for ELISA are not necessarily effective for ELISPOT assay. ELISPOT-compatible antibody clones were selected based on sensitivity and ability to produce discrete and densely-colored spots. In some cases, best ELISPOTs were found

to result from cocktails made from multiple capture antibody clones combined. To avoid the effects of sodium azide and endotoxin on cultured cells, the BD Pharmingen™ ELISPOT capture antibodies are special formulations which contain no sodium azide and minimal endotoxin (NA/LE™).

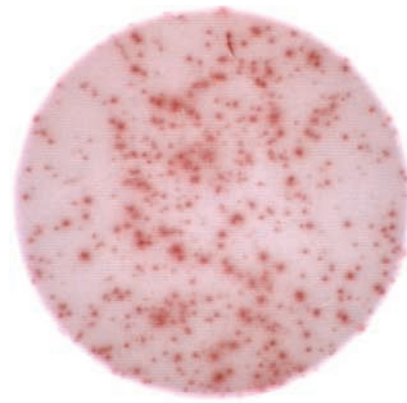
The IFN- γ ELISPOT assay is one important tool for characterization of T cell effector function. To identify the best IFN- γ ELISPOT reagents, we have screened a panel of new anti-mouse and anti-human IFN- γ antibody clones in ELISPOT assays with a variety of target cell preparations, including naïve T cells, memory T cells, and cell lines. We are proud to announce that BD Biosciences Pharmingen now has available specially screened and formulated high sensitivity antibody pairs for human IFN- γ and mouse IFN- γ ELISPOT assay applications. These ELISPOT-compatible antibody pairs have also proven to be very effective for conventional sandwich ELISA.

The IFN- γ ELISPOT reagents are to be available in three different formats, which provide varying degrees of flexibility and time saving convenience: Pairs, Sets, and Kits.

- **ELISPOT Reagent Pairs:** Sufficient antibody for 5 plates of assays. These reagents are tested with, and recommended for use with, high sensitivity/low background ImmunoSpot™ ELISPOT plates available from CTL Analyzers LLC (Cleveland, OH). www.immunospot.com.
- **ELISPOT Reagent Sets:** 10 ImmunoSpot™ ELISPOT plates (not pre-coated with antibody), along with sufficient antibody for 10 plates of assays.
- **ELISPOT Kits:** Designed for maximal time savings and convenience, supplying 2 plates, precoated with antibody, along with all buffers, substrates, enzymes, and detection reagents needed for those 2 plates of assays.



Panel A



Panel B

Figure 1. Superior Human IFN- γ ELISPOTS Are Obtained Using a novel anti-human IFN- γ Antibody for Capture. Primed human PBMC were restimulated (overnight) with PMA (5 ng/ml; Sigma, Cat. No. P-8139) and ionomycin (500 ng/ml; Sigma, Cat. No. I-0634) in the microwell of an ImmunoSpot™ ELISPOT plate that was pre-coated with the NA/LE anti-human IFN- γ . Biotinylated anti-human IFN- γ antibody was used to detect the captured IFN- γ . Spots were visualized using avidin-HRP enzyme and AEC substrate. Image analysis and spot enumeration were carried out using the ImmunoSpot™ Series I Analyzer (CTL Analyzers LLC, Cleveland, OH). Panels A and B were derived from experiments conducted using the same activated cells in the same ImmunoSpot™ plate.

Panel A: Human IFN- γ ELISPOT Set (BD Biosciences Pharmingen, Cat. No. 2554KI).

Panel B: Alternative human IFN- γ ELISPOT reagent pairing.

BD Pharmingen™ ELISPOT Products

ELISPOT Reagent Pairs

- Unlabelled anti-cytokine capture antibody (NA/LE™ format); sufficient reagent for coating 5 plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 5 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations

ELISPOT Reagent Sets

- 10 ImmunoSpot™ ELISPOT plates
- Unlabelled anti-cytokine capture antibody (NA/LE™ format); sufficient reagent for coating 10 plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 10 plates

- Avidin horseradish peroxidase; sufficient for 10 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations

ELISPOT Kits

- 2 pre-coated ImmunoSpot™ ELISPOT plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 2 plates
- Avidin horseradish peroxidase; sufficient for 2 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations
- Assay diluent
- Wash Buffer
- AEC substrate

ELISPOT Reagent Product List

ELISPOT Reagent Sets

New Cat. No.	Cat. No.	Description	Format	Size
551083	2510KI	Mouse IFN- γ	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551849	2554KI	Human IFN- γ	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551017	2446KI	Mouse IL-4	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551084	2511KI	Human IL-4	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551075	2450KI	Mouse IL-5	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551085	2512KI	Human IL-5	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551491	2538KI	Mouse TNF- α	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551446	2534KI	Human TNF- α	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551076	2509KI	Mouse IL-2	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551282	2515KI	Human IL-2	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551445	2533KI	Mouse IL-10	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551018	2449KI	Human IL-10	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates

ELISPOT Reagent Pairs

New Cat. No.	Description	Format	Size
551881	Mouse IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551873	Human IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551945	Monkey IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551878	Mouse IL-4	Pairs	Antibody reagent for 5 plates of assays
551885	Human IL-4	Pairs	Antibody reagent for 5 plates of assays
551949	Monkey IL-4	Pairs	Antibody reagent for 5 plates of assays
551880	Mouse IL-5	Pairs	Antibody reagent for 5 plates of assays
551886	Human IL-5	Pairs	Antibody reagent for 5 plates of assays
551946	Monkey IL-5	Pairs	Antibody reagent for 5 plates of assays
551875	Mouse TNF- α	Pairs	Antibody reagent for 5 plates of assays
551882	Human TNF- α	Pairs	Antibody reagent for 5 plates of assays
551948	Monkey TNF- α	Pairs	Antibody reagent for 5 plates of assays
551876	Mouse IL-2	Pairs	Antibody reagent for 5 plates of assays
551884	Human IL-2	Pairs	Antibody reagent for 5 plates of assays
551947	Monkey IL-2	Pairs	Antibody reagent for 5 plates of assays
551874	Mouse IL-10	Pairs	Antibody reagent for 5 plates of assays
551883	Human IL-10	Pairs	Antibody reagent for 5 plates of assays

Complete ELISPOT Kits

New Cat. No.	Description	Format	Size
Please inquire	Human IFN- γ	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-4	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-5	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human TNF- α	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-2	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers

Ancillary Reagents

New Cat. No.	Description	Size
551951	AEC Substrate	100 ml, sufficient for 10 plates of assays
551950	Avidin-HRP	1 ml, sufficient for 10 plates of assays