

BD™ ELISPOT Research Products

Including Human and Non-Human Primate
IFN- γ Kits with Pre-coated Plates

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



BD ELISPOT Reagents

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 following advantages:

- Cytokine release can be detected at the single cell levels, allowing for direct determination of cytokine-producing cell frequencies.⁵
- This assay has been found to be more sensitive than ELISA and intracellular staining.^{1,7} The sensitivity of the assay enables measurement of even very low frequencies of cytokine-producing cells (eg, 1/300,000).¹
- Recent developments in assay plate design and in high-throughput ELISPOT plate-reader instrumentation have significantly improved the utility of the ELISPOT method¹. Objective and rapid analyses of cytokine-producing cell numbers (spots), and relative amounts of cytokine produced per cell (spot size) are now possible.

BD Biosciences Pharmingen offers an expanded line of research reagents for performing enzyme-linked immunospot (ELISPOT) assays. Our BD ELISPOT assays enable high-sensitivity and high throughput analyses for direct *ex vivo* monitoring of T cell function, including Ag-specific T cells. We offer high-quality reagents with the flexibility of choosing among several formats such as: Ready-to-use Kits with pre-coated BD ELISPOT plates, coat-it-yourself Sets, and matched antibody pairs.

Quality Antibody reagents result in crisp, clear results.

Our BD ELISPOT reagents have been carefully screened to ensure the generation of crisp, clearly-defined immunospots.

(BD NA/LE™: no azide/low endotoxin)

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High-affinity BD Pharmingen™ NA/LE™ capture antibodies safeguard against nonspecific cellular activation. Detection antibodies have been carefully selected, along with pre-optimized preparations of avidin-horseradish peroxidase (HRP), substrate, and chromogen to produce optimal BD ELISPOT reagents.

Automated coating process ensures consistent results.

For the Kit format, BD Biosciences Discovery Labware, the leader in biological coating and an ISO 9001 facility, uses state-of-the-art automation to coat our BD ELISPOT plates to guarantee high quality assay results with minimal well-to-well and plate-to-plate variation (See Table 2). The plates are coated with BD NA/LE™ capture antibodies, are UV processed, and are tested and found negative for the presence of bacteria and fungi.

Reagent performance validated using a leading-edge analyzer.

We validate our reagents by using the ImmunoSpot® Series 1 Analyzer (Cellular Technology, Ltd., of Cleveland, OH). This system, available now, features the premier ELISPOT plate reader and analysis software.

Broad Range of Reagents

Our BD ELISPOT Kits with pre-coated plates, Sets, and Pairs are available for human, non-human primate, and mouse research.

BD ELISPOT Reagent Pairs:

- Sufficient capture antibody and detection antibody for 5 plates of assays

BD ELISPOT Reagent Sets:

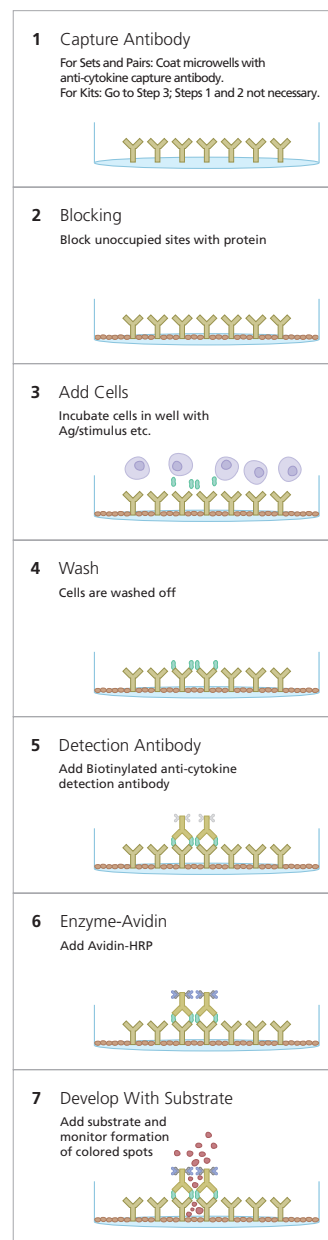
- Sufficient capture antibody, detection antibody, and avidin-HRP for 10 plates of assays
- Includes 10 uncoated ImmunoSpot® ELISPOT plates

BD ELISPOT Kits with Pre-coated Plates:

Designed for optimal spot quality, maximum time savings, and convenience, the kit includes all necessary reagents such as:

- 2 Antibody-Coated Plates
- Detection Antibody
- Avidin-HRP
- AEC Substrate/Chromogen
- Wash Concentrate
- PBS Concentrate
- Assay Diluent

BD ELISPOT Assay Procedure



Unless otherwise specified, all products are for Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD™ ELISPOT Human IFN-γ Kits with pre-coated plates

Quality data comes from quality tools.

This premium-quality BD ELISPOT Kit (Cat. No. 552138) can be used to enumerate and characterize the nature of individual IFN-γ-producing cells within activated human and non-human primate blood and lymphoid cell populations. The BD ELISPOT Human IFN-γ Kit employs specific, high-affinity capture and detection antibodies, and pre-titered, specially formulated enzyme and substrate solutions. This optimized reagent system enables accurate and reproducible measurements of crisp, clearly-defined immunospots.

The BD ELISPOT Human IFN-γ Kit has demonstrated cross-reactivity with non-human primates, including Rhesus and Cynomolgus macaques, Baboons, Chimpanzees, and Pigtail Monkeys.

Also available are BD ELISPOT Human IFN-γ Antibody Pairs (Cat. No. 551873) and Sets (Cat. No. 551849).

BD ELISPOT Human Granzyme B

The BD ELISPOT Granzyme B assay is an important high-throughput assay to monitor cytotoxic T cell function.⁸ As a non-radioactive alternative to the Cr⁵¹ Release test, this assay is especially suitable for large-scale testing of peptide reactivity in freshly isolated human PBMC (or cultured cell populations). Please inquire for availability.

Table 2
Consistent Performance of BD ELISPOT Human IFN-γ Kits (with pre-coated plates)

| Plate | No. of Spots/Well (Mean of 36 wells) | SD | %CV |
|-------|---|-----|-----|
| 1 | 432 | 19 | 4.4 |
| 2 | 466 | 18 | 3.8 |
| 3 | 432 | 22 | 5.0 |
| 4 | 338 | 26 | 7.8 |
| 5 | 365 | 19 | 5.2 |
| 6 | 363 | 20 | 5.6 |
| 7 | 303 | 13 | 4.3 |
| 8 | 311 | 13 | 4.2 |
| 9 | 301 | 12 | 4.1 |
| | Average | 4.9 | |

Note: Results of three independent experiments performed at same cell concentration (8×10^4 cells/ml) and culture conditions.

Table 1

| Key Features and Benefits of the BD ELISPOT Human IFN-γ Kit | |
|---|--|
| Features | Benefits |
| Automated BD BioCoat™ coating process | Consistent results (Table 2) |
| Pre-coated plates | Minimal well-to-well and plate-to-plate variation |
| High quality antibody pairs (NA/LE™ capture Ab and specially formulated detection Ab) | Crisp, clearly-defined immunospots with low background (see cover photo and below) |
| Cross-reactive with NHP* | Broad application including NHP studies |
| Contains all the necessary reagents | Convenient |
| Validated in leading edge ImmunoSpot® Analyzer | Total solution for your ELISPOT research |

*The BD ELISPOT Human IFN-γ Kit detects (i.e., crossreacts with) activated IFN-γ-producing cells prepared from Non-human Primates including Rhesus and Cynomolgus Macaques, Baboons, Chimpanzees, and Pigtail Monkeys.

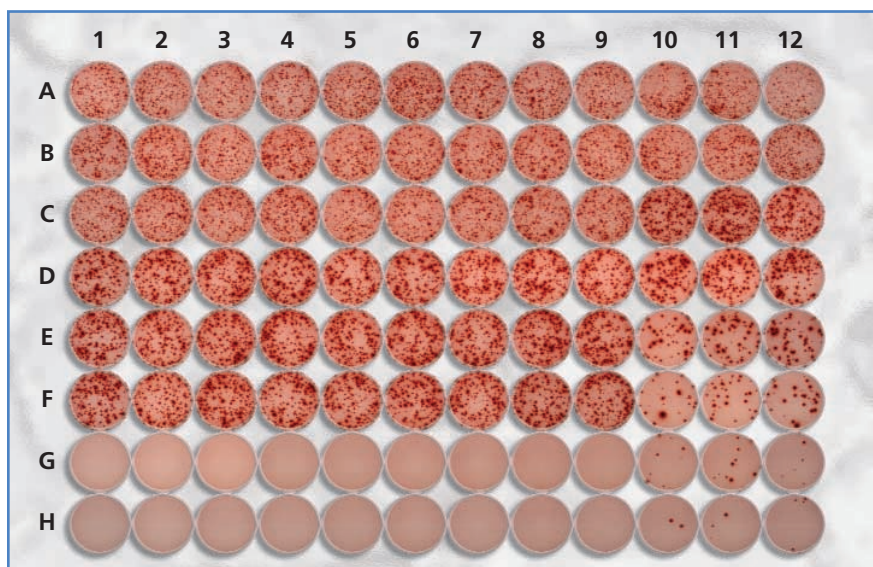


Figure 1. Detection of IFN-γ-secreting cells by BD ELISPOT Human IFN-γ Kit (Cat. No. 552138). Human PBMCs were stimulated with PMA and ionomycin for 18 hours. (blank controls: G 1-9: cells without stimuli; H 1-9: stimuli without cells)

Also Available

| BD ELISPOT Reagents | | |
|---------------------|----------------------|-------|
| Human | Non-human primates** | Mouse |
| IFN-γ | IFN-γ | IFN-γ |
| IL-2 | IL-2 | IL-2 |
| IL-4 | IL-4 | IL-4 |
| IL-5 | IL-5 | IL-5 |
| IL-10 | TNF-α | IL-10 |
| TNF-α | | TNF-α |
| Granzyme B*** | | |

** Tested on Rhesus and Cynomolgus Macaques, Baboon, Chimpanzees, and Pigtail Monkey

***Coming Soon, Please Inquire

BD ELISPOT Reagent Product List

| Cat. No. | Description | Format | Size |
|--|---------------------|--------|--|
| BD ELISPOT Human Reagents | | | |
| 552138 | Human IFN- γ | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551849 | Human IFN- γ | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551873 | Human IFN- γ | Pair | Antibody reagents for 5 plates |
| 552142 | Human IL-2 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551282 | Human IL-2 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551884 | Human IL-2 | Pair | Antibody reagents for 5 plates |
| <i>please inquire</i> | Human IL-4 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551084 | Human IL-4 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551885 | Human IL-4 | Pair | Antibody reagents for 5 plates |
| 552139 | Human IL-5 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551085 | Human IL-5 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551886 | Human IL-5 | Pair | Antibody reagents for 5 plates |
| <i>please inquire</i> | Human IL-10 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551018 | Human IL-10 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551883 | Human IL-10 | Pair | Antibody reagents for 5 plates |
| <i>please inquire</i> | Human TNF- α | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551446 | Human TNF- α | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551882 | Human TNF- α | Pair | Antibody reagents for 5 plates |
| BD ELISPOT Non-human Primate (NHP) Reagents (Human reagents verified to cross-react with Rhesus and Cynomolgus Macaques, Baboons, Chimpanzees, and Pigtail Monkeys) | | | |
| 552138 | Human IFN- γ | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551849 | Human IFN- γ | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551873 | Human IFN- γ | Pair | Antibody reagents for 5 plates |
| 552142 | Human IL-2 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551282 | Human IL-2 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551884 | Human IL-2 | Pair | Antibody reagents for 5 plates |
| <i>please inquire</i> | Human IL-4 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551084 | Human IL-4 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551885 | Human IL-4 | Pair | Antibody reagents for 5 plates |
| 552139 | Human IL-5 | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551085 | Human IL-5 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551886 | Human IL-5 | Pair | Antibody reagents for 5 plates |
| <i>please inquire</i> | Human TNF- α | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551446 | Human TNF- α | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551882 | Human TNF- α | Pair | Antibody reagents for 5 plates |
| BD ELISPOT Mouse Reagents | | | |
| <i>please inquire</i> | Mouse IFN- γ | Kit | 2 pre-coated ImmunoSpot® plates and all necessary reagents and buffers |
| 551083 | Mouse IFN- γ | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551881 | Mouse IFN- γ | Pair | Antibody reagents for 5 plates |
| 551076 | Mouse IL-2 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551876 | Mouse IL-2 | Pair | Antibody reagents for 5 plates |
| 551017 | Mouse IL-4 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551878 | Mouse IL-4 | Pair | Antibody reagents for 5 plates |
| 551075 | Mouse IL-5 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551880 | Mouse IL-5 | Pair | Antibody reagents for 5 plates |
| 551445 | Mouse IL-10 | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551874 | Mouse IL-10 | Pair | Antibody reagents for 5 plates |
| 551491 | Mouse TNF- α | Set | Antibody reagents, Av-HRP, and 10 uncoated Immunospot® Plates |
| 551875 | Mouse TNF- α | Pair | Antibody reagents for 5 plates |
| BD ELISPOT Ancillary Reagents | | | |
| 551951 | AEC Substrate | | 100 ml, sufficient for 10 plates of assays |
| 551950 | Avidin-HRP | | 1 ml, sufficient for 10 plates of assays |

ELISPOT Instrumentation and Software

BD Biosciences Pharmingen is now co-marketing the state-of-the-art ImmunoSpot® Series I Analyzer from Cellular Technology, Limited. This Series I Analyzer provides high speed, accurate acquisition and analysis of even the most complex BD ELISPOT data. We deliver a comprehensive solution for your ELISPOT research.

Unique features of the Immunospot® Series I Analyzer

- Fast, fully automated acquisition and storage of high-resolution images
- Fully automatic well centering
- Software handles the complexities of everyday use
- Custom imaging
- Automated analysis of data
- Versatility



Figure 2. The ImmunoSpot® Series 1 Analyzer (CTL Analyzers LLC, Cleveland, OH)



Sophisticated ImmunoSpot® software

- Background balance
- Overdeveloped area handling
- Spot size gating
- Spot separation tolerance

For a free trial of the ImmunoSpot® software and ordering information

Please visit www.immunospot.com, or contact your BD Biosciences Reagent Sales Representative.

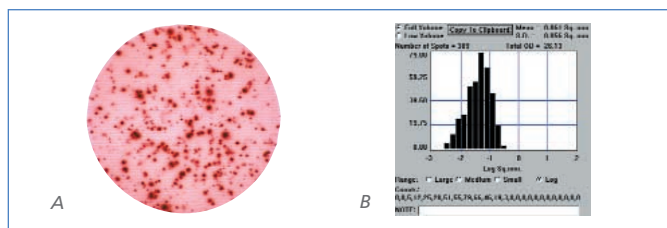


Figure 3. BD ELISPOT analysis of human IL-10-secreting cells. Primed human PBMCs were restimulated in culture with PMA and ionomycin in the microwell of an ImmunoSpot® ELISPOT plate, which was precoated with unlabeled NA/LE™ anti-human IL-10 antibody (BD ELISPOT Human IL-10 Reagent Set, Cat. No. 551018). Biotinylated anti-human IL-10 antibody was used to detect the captured IL-10. Spots were visualized using avidin-HRP enzyme and AEC substrate, followed by image analysis and spot enumeration using an ImmunoSpot® Series 1 Analyzer. The spot size distribution of the PMA and ionomycin-induced response, as measured by image analysis, is shown in Panel B.

BD ELISPOT Custom Products and Services

Please contact your local BD Biosciences Reagent Sales Representative

References:

1. Helms, T., B. Boehm, R. Asaad, R. Trezza, P. Lehmann, and M. Tary-Lehmann. 2000. Direct visualization of cytokine-producing recall antigen-specific CD4 memory T cells in healthy individuals and HIV patients. *J. Immunol.* 164: 3723-3732.
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ImmunoSpot® ELISPOT plates and ImmunoSpot® Series I Analyzer are trademarks of Cellular Technology Limited, Cleveland, Ohio. The BD Biosciences Pharmingen Cytokine ELISPOT Reagent Sets and protocols were developed in collaboration with Cellular Technology Limited, Cleveland, Ohio.

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