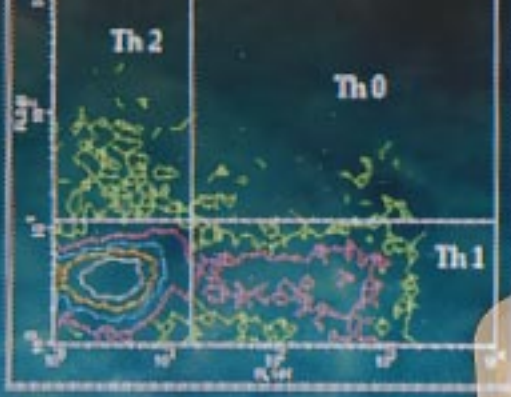


BECTON DICKINSON  
IMMUNOCYTOLOGY SYSTEMS



# FastImmune™ Cytokine System



Intracellular

Cytokine

Detection

in Hours,

Not Days



**BECTON  
DICKINSON**

## FastImmune Cytokine System

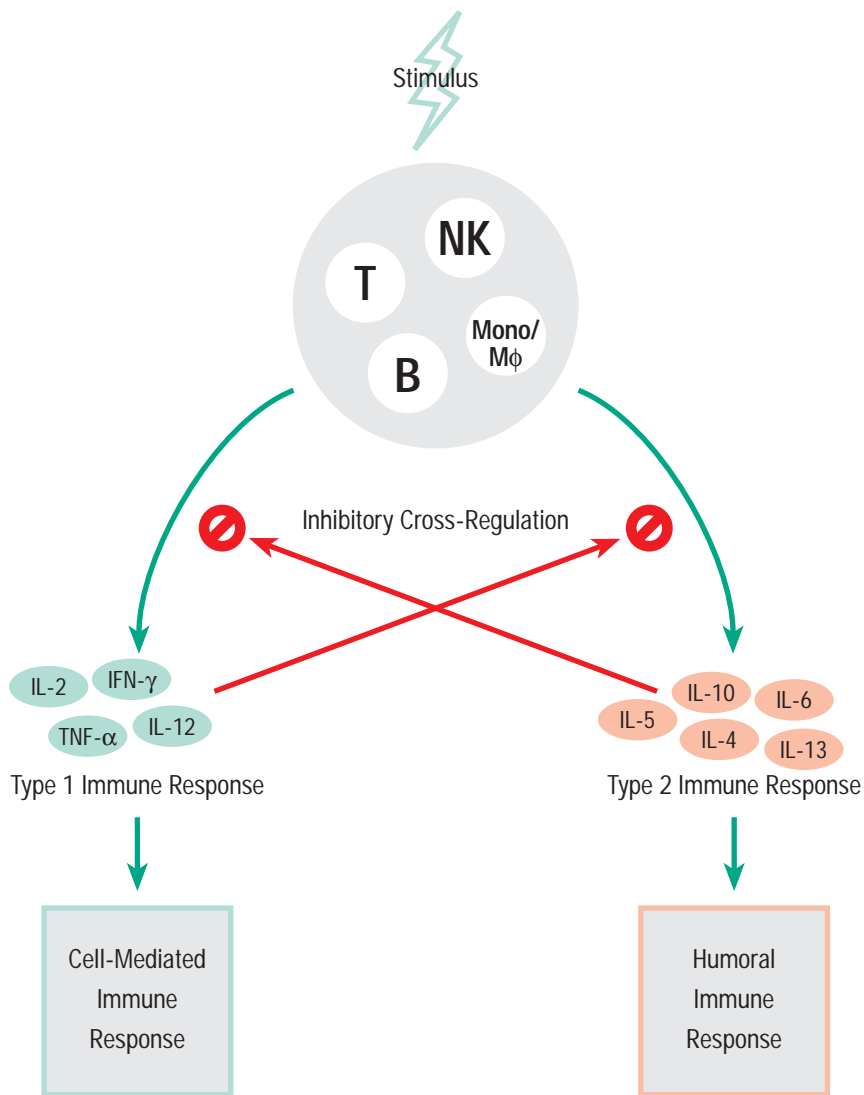
Becton Dickinson Immunocytometry Systems (BDIS), the leader in flow cytometric cellular analysis and sorting, continues the innovative tradition of the FastImmune family of products with the introduction of the FastImmune Cytokine System. This novel reagent system, like the FastImmune Activation System, combines the power of multiparameter flow cytometry with an elegant method featuring whole blood activation. Unlike bulk ELISA methods, the FastImmune Cytokine System yields comprehensive, subset-specific results in just a few hours rather than days.

Cytokines are soluble proteins that play a significant role in the immunoregulation of lymphocyte functions. For example, cytokines regulate growth, differentiation, and function of a wide variety of cells, and mediate normal and pathological immune responses. Cytokine responses—changes in cytokine synthesis in response to physiological and non-physiological immunomodulators—have become an important tool in understanding basic immunological function and disease pathogenesis.

T cells may be functionally defined by their cytokine profiles. Cells participating in a Type 1 ( $T_H1$ -like) response typically produce IL-2, Interferon- $\gamma$ , and TNF- $\alpha$ . Type 1 cytokines participate in cellular immune responses against intracellular pathogens, such as the HIV virus. Cells participating in a Type 2 ( $T_H2$ -like) response produce IL-4, IL-5, IL-6, IL-10, and IL-13. Type 2 cytokines regulate humoral immune responses against extracellular infections, such as multicellular parasitic organisms. T cells have also been identified which produce mixed profiles of the hallmark Type 1 and Type 2 cytokines.

Lymphocyte function has been investigated and implicated in a wide variety of immune function phenomena:

- Immunoregulation
- Lymphocyte activation and function
- Modulation of physiological functions (*e.g.*, lipid and glucose metabolism, thyroid function, and aging)
- Infectious disease and sepsis
- Cancer
- Organ-specific diseases



*The Roles of Cytokines in Human Type 1 and Type 2 Immune Responses.*

The emerging picture of cytokine regulation in human immune response is more complex than the original  $T_H1/T_H2$  model described by Mossman *et al.*<sup>1</sup> Newer models include a larger population of participating cell types that express cytokines, including T, B, and NK lymphocytes and monocytes/macrophages.<sup>2,3</sup>

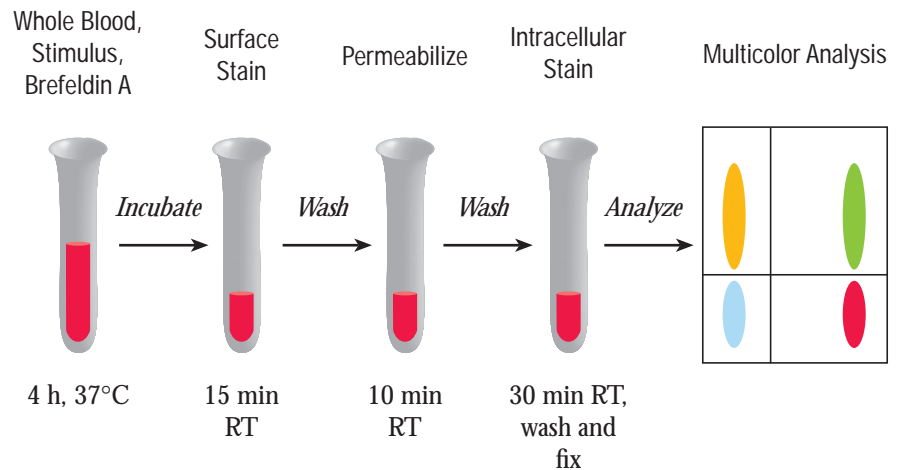
<b>Type 1</b>	<b>Type 2</b>
<b>Cell-Mediated Immune Response</b>	<b>Humoral Immune Response</b>

<i>Physiological Results</i>	<ul style="list-style-type: none"> <li>• Delayed-type hypersensitivity</li> <li>• Macrophage activation</li> <li>• Type 2 response downregulation</li> </ul>	<ul style="list-style-type: none"> <li>• B-cell proliferation</li> <li>• Polyclonal Ig secretion</li> <li>• Type 1 response downregulation</li> </ul>
------------------------------	--	---

<i>Typical Stimuli</i>	<ul style="list-style-type: none"> <li>• Viruses</li> <li>• Some bacteria</li> </ul>	<ul style="list-style-type: none"> <li>• Multicellular parasites</li> <li>• Allergens</li> </ul>
------------------------	--	--

## Results in Hours, Not Days

The FastImmune Cytokine System provides a superior solution to cytokine production analysis by leveraging the power of multiparameter flow cytometry in a simple, one-day procedure. The assay is typically completed in 6 to 8 hours with only 1 to 2 hours of actual hands-on work.



*FastImmune Cytokine System method for intracellular staining in whole blood.*

Many biological and analytical assays have been developed for quantifying and assessing cytokine synthesis. Most methods, such as ELISA, involve measuring the amount of secreted cytokine present in serum or culture supernatant. Bulk methods do not allow investigators to determine the phenotypes of the cytokine-producing cells. These methods suffer from several limitations, as shown in the table below.

Benefit	FastImmune Cytokine System	Limitations of Bulk Methods
<i>Fast</i>	<ul style="list-style-type: none"> <li>&lt;8 hours start to finish</li> <li>Only 1 to 2 hours hands-on time</li> </ul>	<ul style="list-style-type: none"> <li>3 to 10 days</li> </ul>
<i>Easy</i>	<ul style="list-style-type: none"> <li>Whole blood assay</li> <li>No PBMC fractionation</li> <li>No tissue culture</li> </ul>	<ul style="list-style-type: none"> <li>PBMC fractionation and tissue culture typically required</li> </ul>
<i>Safe</i>	<ul style="list-style-type: none"> <li>Reduced sample manipulation and biohazard risk</li> </ul>	<ul style="list-style-type: none"> <li>Extensive sample manipulation during PBMC fractionation and tissue culture</li> </ul>
<i>Comprehensive</i>	<ul style="list-style-type: none"> <li>Multiparameter results accelerate discovery</li> <li>Functional activity determined simultaneously with phenotype of each cell</li> </ul>	<ul style="list-style-type: none"> <li>Yield bulk results for entire population</li> <li>Individual or subpopulation responses cannot be discerned</li> </ul>
<i>Sensitive</i>	<ul style="list-style-type: none"> <li>Highly sensitive fluorescent labels/detection system</li> </ul>	<ul style="list-style-type: none"> <li>Less sensitive alkaline phosphatase or other enzyme-linked detection systems</li> </ul>
<i>Biologically Relevant Assay Conditions</i>	<ul style="list-style-type: none"> <li>Whole blood conditions retain cellular and biochemical environment</li> <li>More accurately represents in vivo conditions</li> </ul>	<ul style="list-style-type: none"> <li>PBMC fractionation and resuspension in synthetic tissue culture environment required</li> <li>Native cell populations and biochemical environment lost</li> </ul>

# Complete, Flexible System

## Whole Blood Activation

The FastImmune Cytokine System begins with activation in whole blood.\* Whole blood activation preserves the *in vivo* cellular and biochemical environment and eliminates artifacts that may be introduced through tedious density-gradient fractionation of blood used in other methods.

## Superior Antibodies and Conjugates

Successful intracellular staining demands antibodies optimized for intracellular targets. Detection of intracellular cytokines requires cell permeabilization and fixation, processes which can change the immunoreactivity of the cytokines. Intracellular assays that utilize secretory inhibitors, such as Brefeldin A, detect developing protein in the Golgi apparatus. Conformationally, the nascent protein may be different from the secreted form. Therefore, antibodies that work well in detecting secreted cytokine may perform poorly in intracellular assays.

Stringent selection of antibodies with high affinity for nascent, fixed cytokines is paramount to formulating superior intracellular detection systems. BDIS screens many monoclonal antibodies for each cytokine under actual intracellular assay conditions to ensure that the FastImmune Cytokine System will provide superior sensitivity and very low background staining.

Multicolor reagents are similarly formulated to provide optimum concentrations of all reagents. Additional anti-cytokine reagents are under development. Contact your BDIS representative for the latest information on the FastImmune Cytokine System reagents.

## Proprietary Permeabilizing Reagent

BDIS has developed FACS™ Permeabilizing Solution to ensure consistent sensitivity and low background staining. This proprietary reagent is specifically designed to overcome the limitations of saponin-based permeabilizing reagents common to many intracellular protocols. Saponin, a compound derived from plants, is a common source of variability in intracellular immunophenotypic staining because of its heterogeneous nature and broad range of source material. FACS Permeabilizing Solution also eliminates overnight freezing of cells, which some protocols recommend to increase permeability.

## Intuitive Control Reagents

The FastImmune Cytokine System saves time and resources by utilizing conventional† isotype control reagents, eliminating the need for expensive and tedious cross competition with unlabeled recombinant cytokines.

\* Activation with provocative stimuli can be routinely performed in whole blood. When activating with PMA and ionomycin, best results are obtained by diluting the whole blood 1:1 in serum-free tissue culture medium (RPMI-1640 with 2 mM glutamine).

† The FastImmune Cytokine System controls are standard anti-KLH antibody controls specially formulated for intracellular detection systems.

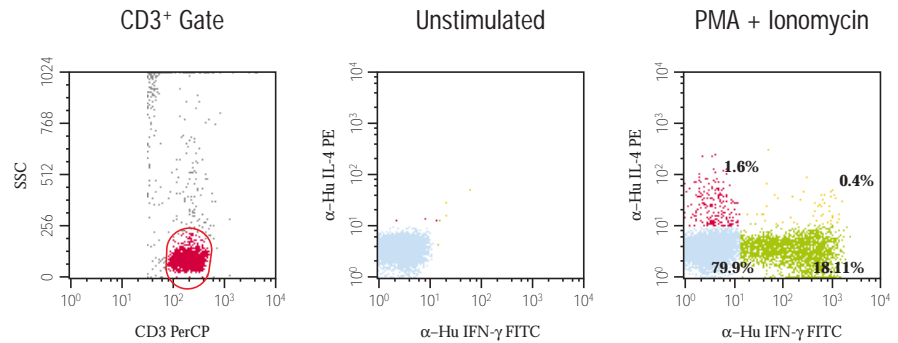


# Research Applications

One of the significant advantages of measuring cytokine production using multiparameter flow cytometry is that individual subset responses to specific stimuli are monitored even in the presence of other cell populations. The following examples show three applications in which subset-specific analysis can be used in important areas of research.

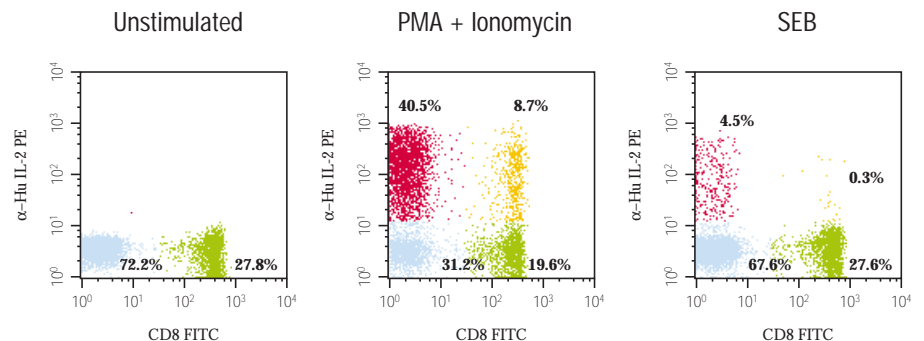
## *Analysis of Type 1 and Type 2 Immune Responses*

The FastImmune Cytokine System is designed to provide a fast and easy means of determining the functional status of T cells. The results below were produced by following the protocol outlined earlier, including surface staining with CD3 PerCP to detect T cells and the two-color FastImmune Anti-IFN- $\gamma$  FITC/Anti-IL-4 PE reagent and isotype controls. Two-color analysis of the CD3-gated events yielded the dot plots shown. The subset expressing only IL-4 (upper-left quadrant) is exhibiting a Type 2 response, while the population expressing only IFN- $\gamma$  (lower-right quadrant) is displaying a Type 1 response. The population co-expressing IL-4 and IFN- $\gamma$  (upper-right quadrant) participates in both Type 1 and Type 2 responses.



## *Functional Subsets of CD8<sup>+</sup> T Cells*

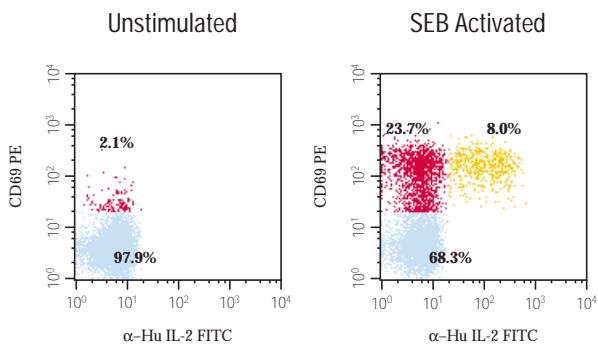
The data below demonstrate how the FastImmune Cytokine System may be used to delineate functionally distinct subsets of CD8<sup>+</sup> T cells. The two-color dot plots compare the results of very general activation with PMA and ionomycin (PMA+I) versus more specific activation with superantigen staphylococcal enterotoxin B (SEB). Activation with PMA+I clearly induces IL-2 expression in the CD8<sup>+</sup> and CD8<sup>-</sup> (nominally CD4<sup>+</sup>) populations. In contrast, SEB stimulates IL-2 production in a less frequent population which is more strongly biased toward CD8<sup>-</sup> cells. These subset-specific results cannot be observed in a bulk assay.



### Cytokine Expression in Lymphocyte Activation

The FastImmune Cytokine System is part a family of novel reagents for exploring immune function. The FastImmune family includes the FastImmune Activation System, a unique assay for assessing activation of T, B, and NK lymphocytes by the cell-surface expression of CD69, a very early activation marker. The FastImmune Activation System yields specific, multiparameter flow cytometric results in less than 5 hours with very little hands-on time per sample.

The relationship between intracellular CD69 expression and cytokine production is shown below. The two-color analysis of SEB-activated CD4 PerCP-gated T cells clearly demonstrates that only the CD69<sup>+</sup> cells are producing IL-2.



Additional FastImmune products are planned to expand the range of tools for studying the function of the human immune system. Contact your BDIS representative for the latest information on the FastImmune product family.

### References

1. Mossman TR, Cherwinski HM, Bond MW, Giedlin MA, and Coffman RL. Two types of murine helper T-cell clone. I. Definition according to profiles of lymphocyte activities and secreted proteins. *J Immunol.* 1986;136:2348–2357.
2. Clerici M and Shearer G. The T<sub>H</sub>1-T<sub>H</sub>2 hypothesis of HIV infection: new insights. *Immunol Today.* 1994;15:575–581.
3. Mossman TR and Sad S. The expanding universe of T-cell subsets: T<sub>H</sub>1, T<sub>H</sub>2 and more. *Immunol Today.* 1996;17:138–146.





## Worldwide Headquarters

<b>United States</b> Becton Dickinson Immunocytometry Systems Ordering Information Tel (800) 223-8226 Fax (408) 954-BDIS (2347) <b>www.bdfacs.com</b>	<b>Asia Pacific</b> Becton Dickinson and Company Tel (65) 860-1478 Fax (65) 860-1590	<b>Canada</b> Becton Dickinson Canada, Inc. Tel (905) 822-4820 Fax (905) 855-1243	<b>Europe</b> Becton Dickinson European HQ Tel (32) 53-720211 Fax (32) 53-720450	<b>Japan</b> Nippon Becton Dickinson Company, Ltd. Tel (81) 3-5413-8251 Fax (81) 3-5413-8155	<b>Mexico</b> Becton Dickinson Mexico Tel (525) 237-1200 Fax (525) 237-1288
---	---	--	--	--	--

## International Offices

<b>Africa</b> Becton Dickinson Worldwide Inc Kenya Tel (254) 2 449 608 Fax (254) 2 449 619	<b>Denmark</b> Becton Dickinson AS Tel (45) 43 434566 Fax (45) 43 434166	<b>Hong Kong</b> Becton Dickinson Asia Ltd Tel (852) 2575-8668 Fax (852) 2803-5320	<b>Israel</b> Bactlab Diagnostics Ltd. Tel (972) 6-6309600 Fax (972) 6-6230777	<b>Norway</b> Laborel A/S Tel (47) 23 05 19 30 Fax (47) 22 63 07 51	<b>Sweden</b> Becton Dickinson AB Tel (46) 8 775 5100 Fax (46) 8 645 08 08
<b>Australia</b> Becton Dickinson Pty Ltd Tel (612) 9978-6800 Fax (612) 9978-6850	<b>Eastern Europe</b> Becton Dickinson International Germany Tel (49) 6221 3050 Fax (49) 6221 305 388	<b>Hungary</b> Becton Dickinson Tel (36) 1 216 48 93 Fax (36) 1 216 48 93	<b>Italy</b> Becton Dickinson Italia SpA Tel (39) 02 482401 Fax (39) 02 48200323	<b>Philippines</b> Becton Dickinson Philippines, Inc Tel (632) 818 9727 Fax (632) 810 5687	<b>Switzerland</b> Becton Dickinson AG Tel (41) 61 385 44 22 Fax (41) 61 385 44 00
<b>Austria</b> Becton Dickinson Austria Tel (43) 1 706 36 60-0 Fax (43) 1 706 36 60-11	<b>Finland</b> Oriola OY Prolab Tel (358) 942 92 520 Fax (358) 942 92 080	<b>Iceland</b> Icelandic American Trad Co Tel (354) 168 27 00	<b>Korea</b> Becton Dickinson Korea Inc Tel (822) 5694030 Fax (822) 5694048/9	<b>Poland</b> Becton Dickinson Polska Tel (48) 22 6517921 Fax (48) 22 6517924	<b>Taiwan</b> Becton Dickinson Worldwide Inc Tel (8862) 722-5660 Fax (8862) 725-1772
<b>Benelux</b> N.V. Becton Dickinson S.A. Belgium: Tel (32) 53 720211 Fax (32) 53 720200 The Netherlands: Tel (31) 76 5037720 Fax (31) 76 5014133	<b>France</b> Becton Dickinson SA Division Immunocytométrie Tel (33) 476 683730 Fax (33) 476 683544	<b>India</b> Becton Dickinson India Pvt Ltd Tel (91-11) 6913092 Fax (91-11) 6831783	<b>Latin America</b> Becton Dickinson Immunocytometry Systems USA Tel (408) 954-2157 Fax (408) 526-1804	<b>Portugal</b> ENZIfarma, Diagnostica e Farmaceutica Tel (351) 1 4420100 Fax (351) 1 4420110	<b>Thailand</b> Becton Dickinson Thailand Ltd Tel (662) 643 1371-80 Fax (662) 643 1381
<b>China</b> Becton Dickinson Asia Ltd. Tel 8610-6593 3072-77 Fax 8610 6593 3070	<b>Germany</b> Becton Dickinson GmbH Tel (49) 6221 3050 Fax (49) 6221 303798	<b>Indonesia</b> Becton Dickinson Asia Ltd Tel (6221) 577 1920 Fax (6221) 577 1925	<b>Malaysia</b> Becton Dickinson Sdn Bhd Tel (0203) 7571323 Fax (0203) 7571153	<b>South Africa</b> BD Immunocytometry Systems Tel (27) 11 807 1531 Fax (27) 11 807 1953	<b>Turkey</b> BD Turkey Tel (90) 212 222 87 77 Fax (90) 212222 87 76
	<b>Greece</b> Becton Dickinson Hellas SA Tel (30) 1 940 77 41 Fax (30) 1 940 77 40	<b>Ireland</b> Becton Dickinson Diagnostic Systems Tel (353) 1 285 48 00 Fax (353) 1 285 43 32	<b>Middle East</b> Becton Dickinson Tel (971) 4 379525 Fax (971) 4 379551	<b>Spain</b> Becton Dickinson España SA Tel (34) 91 8488100 Fax (34) 91 8488104	<b>United Kingdom</b> Becton Dickinson UK Ltd Tel (44) 1865 748844 Fax (44) 1865 781635

Printed on recycled paper  
using soy-based ink



**BECTON  
DICKINSON**



For research use only. Not for use in diagnostic or therapeutic procedures.  
FastImmune and FACS are trademarks of Becton Dickinson and Company.  
©1996 Becton Dickinson and Company.  
11/98 23-3253-01