FACService
Customer Focused Solutions

TECHNOTES

Vol 8 No. 2 May 2002

BD FastImmune Cytokine Flow Cytometry

BD FastImmune™ Cytokine flow cytometry (CFC) is a powerful research tool for quantitating and characterizing antigen-specific T-cell responses.

At BD Biosciences, we have developed a step-by-step and highly reproducible methodology for performing antigen-specific CFC assays. Our protocols are based on the use of individual antibody reagents, multicolor reagent products, and cytokine detection kits that include all processing reagents for sample preparation, thus providing optimal assay standardization.

In this issue of BD FACService™ TECHNOTES, the identification and relevance of cytokine-producing $CD8^+$ T cells is explored, as well as important factors in the analysis of cytokine-producing T cells. The BD FastImmune handbook, *Performance Characteristics of Antigen-Specific Cytokine Flow Cytometry Assays*, will soon be available through BD Biosciences and provides much more detailed information on this exciting topic.

BD Biosciences
http://www.bdbiosciences.com

BD

Identifying Antigen-Specific CD8+ T-Cell Responses

by Smita Ghanekar, KerstinWillmann, and Holden T. Maecker

Originally published by BD Biosciences in *Hotlines, Vol 6. No. 3, Winter 2001*

CD8+ T cells are the primary effector cells of acquired cellular immunity. They possess cytolytic activity against virally infected cells (or in some cases tumor cells) and can elaborate anti-viral cytokine.[1] In HIV disease, a powerful demonstration of the importance of CD8+ T cells was made in a macaque model of SIV.[2] Depletion of CD8+ T cells from macaques completely abolished their ability to be protected from disease. Evidence for the importance of CD8+ T cells in HIV also has come from studies of HIV-exposed, but uninfected, individuals who share strong CTL activity against particular epitopes of HIV.[3-5] These studies have raised interest in detecting and monitoring antigen-specific CD8+ T cells in HIV and cancer.

CD8+ T cells recognize peptide antigens bound to class I MHC molecules on host antigen-presenting cells. In order to detect CD8+ T-cell cytokine responses to complex antigens, it is necessary to present the relevant peptide epitopes of these antigens on host class I MHC molecules. Fortunately, this is easily accomplished by exogenous addition of peptides to whole blood or PBMCs.[6] In fact, a large number of peptides can be added as a mixture to a single tube or well. Studies by Kern and colleagues[7] have demonstrated the utility of using a mixture of overlapping 15 amino acid peptides to detect CD8+ T-cell responses to an entire protein. We, and others, have adapted this approach to a variety of protein antigens from CMV and HIV.[8,9] An advantage of using 15 amino acid peptides is that they are able to stimulate CD4+ T cells, while still stimulating CD8+ T cells with relative efficiency.[9] Using multiparameter cytokine flow cytometry (CFC), CD4+ and CD8+ functional responses can be easily resolved in a single assay.

Other methods for detecting antigen-specific CD8+ T cells include phenotypic analysis using recombinant, multivalent MHC-peptide complexes. The two most common constructs of this type are MHC-peptide tetramers[10] and MHC-Ig dimers[11] (DimerX, BD Biosciences Pharmingen). These structures can be loaded with a specific recombinant peptide of choice and added to whole blood or PBMCs. Antigen-specific T cells can be detected by flow cytometry after MHC-peptide complexes bind to a specific T-cell subset. Both dimer and tetramer technologies enable interrogation of T cells of just one MHC-peptide specificity at a time. The response to a complex antigen such as HIV involves T-cell clonotypes specific for a number of different MHC-peptide combinations that are different in each individual.[8,12] It is important to note that dimer and tetramer technologies used in isolation do not provide a direct readout of a cell's functional capacity. These drawbacks are overcome by the use of functional assays, such as CFC or ELISPOT, that detect cytokine production by antigen-specific T cells and are suggested to correlate with cytotoxic activity.[13]

WEB LINKS

Flow Cytometry on the Web
http://pingu.salk.edu/flow/sitelink.html

By combining functional assays with tetramer or dimer analysis, functionally anergic cells of a particular specificity can be identified.[14-16] Anergic cells could contribute to the lack of immune responsiveness in chronic diseases like cancer. The dimer or tetramer approach is easily combined with functional CFC, since both techniques can be applied to the same sample tube and analyzed by flow cytometry. In contrast, ELISPOT technology uses an optical plate reader for sample analysis and is restricted to one- or two-parameter detection. Therefore, subsetting of functional and non-functional T-cell populations is easiest to address with CFC.

BD FastImmune Product Highlight

BD Biosciences Immunocytometry Systems has developed a step-by-step, highly reproducible methodology for performing CFC assays. Our BD FastImmune™ protocols are based on the use of individual antibody reagents, multicolor reagent products, or cytokine detection kits that include all reagents for sample preparation, thus providing assay standardization.

The new BD *FastImmune CD8 Intracellular Cytokine Detection Kit* directly identifies antigen-activated $CD8^+$ T-cell IFN-γ responses. The kit is developed for use with human whole blood or PBMCs. It includes a specific antibody combination, matching isotype control, and necessary sample processing reagents for optimal and highly reproducible results. The BD FastImmune methodology is compatible with the requirements of vaccine monitoring in clinical trials.[17] For example, whole blood samples can be activated and automatically cooled at the end of the 6-hour activation period by means of a programmable water bath or similar device. Also, samples activated and fixed with BD FACS™ Lysing Solution* can be frozen at –80°C for shipment to a different site where processing and analysis are done in a batch process. Maecker et al found that cytokine responses to peptide antigens can be efficiently obtained with fresh and cryopreserved samples.[9] Streamlined procedures for analysis of $CD8^+$ T-cell responses in clinical trials have the potential to accelerate the identification of surrogate immunological endpoints in diseases such as HIV and cancer.[18] An overview of the BD FastImmune CFC assay using whole blood is shown in Figure 1.

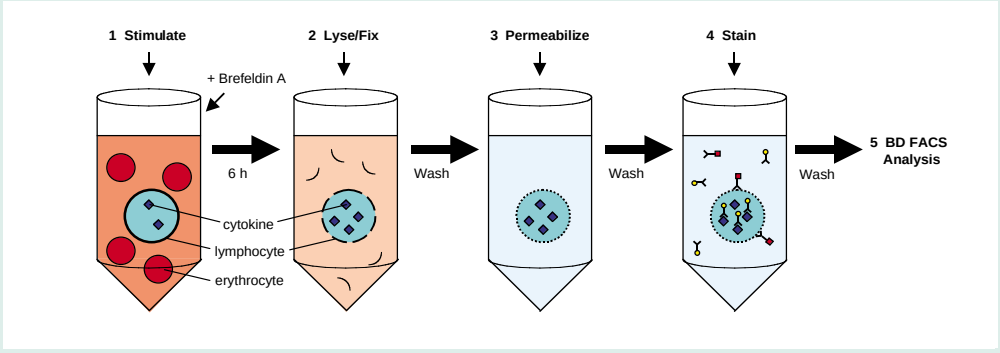

Figure 1 Overview of BD FastImmune CFC methodology for whole blood

WEB LINKS

Molecular Cytometry Unit, UTMB
http://stem.utmb.edu/

Whole blood is subjected to antigenic stimulus in the presence of a secretion inhibitor, Brefeldin A (BFA). This leads to intracellular accumulation of newly synthesized protein. After a stimulation period of 6 hours, the leucocytes are fixed and erythrocytes lysed, simultaneously. The cells are then washed and permeabilized. Finally, intracellular, as well as surface antigens are stained in the same protocol step with Anti-Hu–IFN-γ FITC,CD69 PE, CD8 PerCP-Cy5.5, and CD3 APC, followed by flow cytometric analysis. The BD FastImmune CD8 Kit includes CD3 APC to avoid misidentification of NK-cell responses (CD8dim) upon antigenic stimulus. $CD8^+$ T cells are identified as $CD3^+CD8^+$ events, and their functional responses are measured as cells positive for both CD69 PE and Anti–IFN-γ FITC. The use of CD69, an early activation marker, provides more confidence in identifying cytokine-positive cells as having been recently activated, either in vivo, or as a result of the in vitro stimulation. CD69 staining also permits better visual clustering of cytokine-positive cells in a CD69 vs Anti–IFN-γ dot plot. This can aid in the identification of rare populations of antigen-specific cells. A typical example of $CD8^+$ T-cell responses using a peptide-mix antigen is shown in Figure 2, using mixtures of overlapping 15 amino acid peptides spanning the CMV pp65 or HIV p55 glycoproteins. All plots are gated on $CD3^+CD8^+$ cells. For methods, see reference 9.

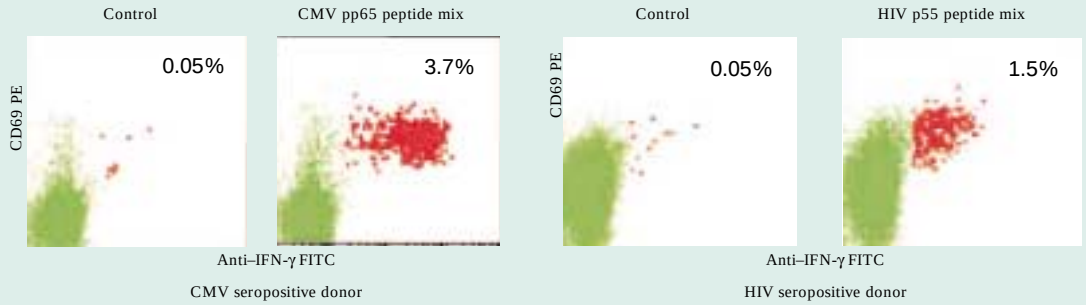

Figure 2 Detection of CMV– and HIV–specific $CD8^+$ T-cell responses

In intracellular staining procedures, the requirements for optimal staining intensity are very stringent. BD Biosciences has selected monoclonal antibody clones and optimized conjugation chemistry to achieve bright signals and minimal background. Table 1 provides a general reference of typical ranges of cytokine responses observed using various stimuli in healthy donors (estimated by BD Biosciences Research and Development group). While individual results might vary, antigen-specific CFC assays demand the use of reagents and techniques that provide very low background in order to detect responses of this magnitude.

Table 1 Typical $CD8^+$ T cell response ranges

Stimulus	Typical %$CD3^+CD8^+CD69^+IFN\text{-}\gamma^+$cells
PMA + ionomycin	70–90
Staphylococcal enterotoxin B (SEB)	1–15
Specific antigen (eg, CMV pp65 peptide mix)	0.2–5

References

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WEB LINKS

Flow Cytometry Core Facility
http://facs.scripps.edu/

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WEB LINKS

Fluorochomes
http://home.t-online.de/home/Bohlen.Hintzhismisc.htm#Fluorochomes

NEW

BD FastImmune Specific Multicolor Cytokine Reagents

BD FastImmune three- and four-color cytokine reagents can be used alone or in conjunction with the *Intracellular Cytokine Detection Kit* for additional cytokine measurements. These reagents have already been optimized to work together, so you don't have to perform tedious titrations or additional pipeting steps during your assay. CD4 and CD8 are conjugated to PerCP-Cy5.5 for better separation of $CD4^{dim}$ and $CD8^{dim}$ T cells from the negative cell population.

Catalog Nos.		Product Description*
346048	NEW	BD FastImmune Anti-Hu–IFN-γ FITC/CD69 PE/CD8 PerCP-Cy5.5/CD3 APC
346047	NEW	BD FastImmune IgG_{2a} FITC/IgG_1 PE /CD8 PerCP-Cy5.5/CD3 APC
340962		BD FastImmune Anti-Hu–IFN-γ FITC/CD69 PE/CD4 PerCP-Cy5.5
340963		BD FastImmune Anti-Hu–IL-2 FITC/CD69 PE/CD4 PerCP-Cy5.5
340964		BD FastImmune Anti-Hu–TNF-α FITC/CD69 PE/CD4 PerCP-Cy5.5
340965		BD FastImmune IgG_{2a} FITC/IgG_1 PE/CD4 PerCP-Cy5.5

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To place an order, or to obtain technical support, call BD Biosciences at 1.877.232.8995 (toll free) and follow the prompt for flow cytometry instruments and reagents from BD Biosciences Immunocytometry Systems.

*Use of these products to measure activation antigens expressed on mononuclear cell subsets for purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US 5,445,939; 5,656,446; and 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australia Patent No. 615,880; and Japan Patent No. 2,769,156.

Identifying Antigen-Specific $CD8^+$ T-Cell Responses

WEB LINKS

FACS Laboratory at London Research Institute
http://www.icnet.uk/axp/facs/davies/flow.html

BD FastImmune CFC Acquisition and Analysis—Practical Tips

Gating

There is an inaccurate perception of flow cytometry as a subjective discipline, in which subtle manipulation of instrument settings and gates can materially alter the results obtained. It is true that basic conventions of instrument setup and gating must be followed or the results will not be interpreted. One way to achieve basic instrument settings for BD FastImmune CFC assays is to use BD CaliBRITE™ beads and BD FACSComp™ software, using lyse/no wash (LNW) settings.

Once an optimal setup is achieved (PMT values and compensation settings), it is necessary to set gates or regions of cells to acquire or analyze, or both. The placement of gates in CFC assays can quantitatively (but not qualitatively) alter the result. An example is shown in Figure 3. Elimination of $CD4^{dim}$ lymphocytes can lower the reported percentage of cytokine-positive CD4 cells because activated T cells tend to down-modulate CD4 (or CD8). This alters the result from 8.90% to 6.98% in this example. Although this indicates that consistency in gating is important, it should not be concluded that gating could qualitatively affect the answer obtained (ie, gating variations cannot turn a negative result into a positive result, or vice versa).

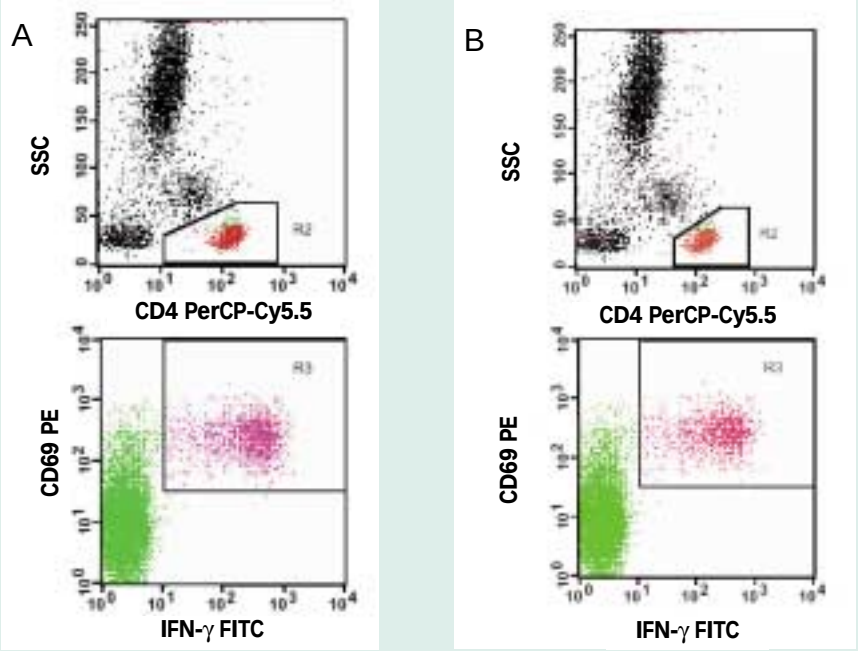

Figure 3 A. Optimally gated (%gated = 8.90%) B. $CD4^{dim}$ cells excluded (%gated = 6.98%)

WEB LINKS

Cytometry Journal Information
http://www.interscience.wiley.com/jpages/0196-4763/

Number of Events to Collect

Since antigen-specific CFC assays require the identification of potentially very small populations of cytokine-producing cells, they can accurately be called rare-event assays. As such, it is important to collect enough cells to be able to determine, with high statistical significance, that a given result is accurate. Statistical analyses can be performed to aid in this process. We have established a sample-size algorithm for this purpose, commonly used to determine the statistical power of a study.[1] *Power* is related to the ability to detect a significant difference between two test samples (in our case, between the background and the relevant antigen response in a CFC assay). Using this algorithm, the recommended number of events to collect (eg, CD4- or CD8-gated lymphocytes) can be determined for various levels of statistical power and P values (where P is the probability that the indicated result is not true). Table 1 shows a number of relevant events to collect in a CFC assay, for two different power and P values, given various levels of background and expected positive percentages.

The relevant variables that affect this calculation are: (1) the level of background cytokine production in an unstimulated or control-stimulated sample; and (2) the level of cytokine production observed in the test sample. This will require some estimation on the part of the user as to what level of background and response is expected. Thus, from Table 1, one can see that to detect a 0.1% response over a background of 0.01%, with 90% power and P<0.05, 12,000 relevant cells need to be collected. On the other hand, when the background increases to 0.04%, 33,000 cells need to be collected to detect the same level of response with this power and P value. It is possible to collect more events in order to improve one's ability to detect low-level responses. However, these calculations will help to determine the reasonable number of events to collect. This is to avoid gathering an unnecessarily large number of events in every sample. An electronic copy of the sample-size calculator is available from BD Biosciences Immunocytometry Systems Applications Support.

Table 1 Relevant events collected in a CFC assay

% Background	Lowest % Positive	90% Power P <0.05	99% Power P <0.005
0.01	0.02	260,000	720,000
0.01	0.05	32,000	90,000
0.01	0.1	12,000	32,000
0.02	0.05	67,000	190,000
0.02	0.1	16,000	45,000
0.03	0.05	170,000	480,000
0.03	0.1	23,000	63,000
0.04	0.1	33,000	93,000
0.05	0.1	52,000	140,000
0.06	0.1	86,000	240,000
0.07	0.1	160,000	450,000
0.08	0.2	17,000	46,000
0.1	0.2	26,000	72,000

BD FastImmune CFC Acquisition and Analysis Practical Tips

WEB LINKS

About Flow Cytometry
http://130.189.200.66/AboutFlow/

Interpretation of Results as Positive or Negative

Once data has been collected, it is often necessary to categorize results as either positive or negative (or indeterminate, if too few events have been collected to make an accurate determination). For this, we have used statistics[2] to calculate a confidence interval (CI) around a CFC result. A result is defined as the difference in response to test antigen versus control, that is, a difference between two proportions. This CI depends on the number of relevant events collected in each sample, the amount of background, and positive staining. See Table 2 for calculated CI for a CFC assay, based on the number of relevant events in the sample.

BD FastImmune CFC Acquisition and Analysis Practical Tips

Table 2 CI intervals for a CFC assay

25,000 relevant events

% background	%positive	lower limit of 95% CI using normal theory	difference between proportions	upper limit of 95% CI using normal theory
0.01	0.11	0.057	0.1	0.143
0.02	0.11	0.045	0.09	0.135
0.01	0.2	0.133	0.19	0.247
0.09	0.2	0.043	0.11	0.177

35,000 relevant events

% background	%positive	lower limit of 95% CI using normal theory	difference between proportions	upper limit of 95% CI using normal theory
0.01	0.11	0.064	0.100	0.136
0.02	0.11	0.052	0.090	0.128
0.01	0.2	0.142	0.190	0.238
0.09	0.2	0.054	0.110	0.166

We have noted that CFC results from CMV-seronegative donors yield 95% confidence intervals that are entirely below 0.05%, while results from seropositive donors yield 95% confidence intervals that are entirely above 0.05%. With this as a guide, one can formulate the following rule: If the calculated 95% confidence interval of a CFC result lies entirely above 0.05%, the result can be called positive. If it lies entirely below 0.05%, the result can be called negative. And if the 95% confidence interval overlaps with 0.05%, the result is labeled indeterminate. In Table 2, note that the values in red represent results whose confidence intervals overlap with 0.05%, and would therefore be considered indeterminate. Also note that with 25,000 relevant events, and a threshold value of 0.2% positive, the result does not become indeterminate until the background reaches 0.09%.

An electronic copy of the confidence interval calculator is available from BD Biosciences Immunocytometry Systems Applications Support.

WEB LINKS

Boston Users Group for Cytometry
http://users.primushost.com/~bugbytes/

Frequently Asked Questions

Q **What is the most sensitive assay for detecting antigen-specific T cells?**

ELISPOT, CFC, tetramer, and DimerX assays are all sensitive ways of measuring small populations of antigen-specific cells. While the detection limit of ELISPOT assays is lower than for CFC or tetramer, CFC and tetramer[3] technology generally detects higher frequencies of antigen-specific cells than ELISPOT technology.[4,5] In addition, the multiparameter nature of flow cytometry allows discrimination of responses, ie, CD4 versus CD8 T-cell subsets, which is more difficult to resolve with ELISPOT.

Q **Which is better for intracellular cytokine visualization, Brefeldin A or monensin?**

Both agents can be used to block transport of newly synthesized proteins, allowing intracellular accumulation and staining.[6] For the cytokines targeted by the BD FastImmune product line, we have found that Brefeldin A at 10 µg/mL is highly efficient for visualizing intracellular cytokine staining.

Q **What's the difference between BD Biosciences Pharmingen Cytofix/Cytoperm™ and BD FACS Lysing Solution and BD FACS Permeabilizing Solution?**

The Cytofix/Cytoperm system is based on permeabilization of cells with saponin while BD FACS Permeabilization Solution is based on a proprietary detergent formulation. When saponin is used, the detergent must be present throughout the staining and washing procedure, as saponin-based permeabilization is at least partially reversible. The BD FastImmune product line has been developed with BD FACS Lysing Solution and BD FACS Permeabilizing Solution to detect activation responses in human blood. We also prefer using the BD FastImmune product line in antigen-specific non-human primate (NHP) systems. For mouse CFC systems, we have found that the BD Cytofix/Cytoperm products perform best. Do not use fixation, permeabilization, and staining protocols from different commercial systems.

Q **What's the difference between BD FACS Permeabilizing Solution and BD FACS Permeabilizing Solution 2?**

The detergent in BD FACS Permeabilizing Solution 2 was developed for antigen-specific CFC assays, and results in brighter cytokine staining. In addition, we tested and compared both solutions in stimulation experiments using whole human blood with mitogens like PMA, ionomycin, and LPS for cytokine detection in T cells, monocytes, and peripheral blood dendritic cells. We found that BD FACS Permeabilization Solution 2 performed

WEB LINKS

Multirules and Westgard Rules
http://www.westgard.com/mltirule.htm#westgard

equally or better than its counterpart.

Q **Can I use BD Biosciences Pharmingen antibodies instead of BD Biosciences FastImmune antibodies for antigen-specific CFC assays?**

Do not use antibodies from other sources with the BD FastImmune System. Antibodies from BD Biosciences Immunocytometry Systems have been optimized for the BD FastImmune Cytokine System. They are produced with high purity and optimal fluorochrome-to-protein ratios and perform consistently well in intracellular staining experiments with minimal background. This is particularly important for rare-event assays like antigen-specific CFC.

Q **Do I need to use Fc-blocking reagents in CFC assays?**

Because we have selected very high affinity antibodies for the BD FastImmune system, they are used at such low concentrations that Fc binding is not a problem. The same can be said for the non-human primate CFC assay. In mouse CFC assays, there is a greater tendency for Fc binding because very high affinity antibodies are not available; so use of Fc block is recommended in mouse assays.

Q **Should I use unlabeled cytokines as a blocking agent to check the specificity of cytokine staining?**

This is not necessary in the BD FastImmune System. All antibodies have been carefully tested for cytokine specificity, so routine use of unlabeled cytokines to block specific binding is not required.

Q **How long can samples be stored after staining, before acquisition on a flow cytometer?**

Acquire specimens within 24 hours. Staining separation can begin to degrade with longer storage times. We recommend that stained samples be stored in 1% paraformaldehyde in PBS at 4°C in the dark if they are not acquired immediately after staining.

Q **What is the best way to standardize the flow cytometer setup for BD FastImmune CFC assays?**

If you run BD FACSComp software using the LNW setup, you will obtain settings that are

WEB LINKS

Interdisciplinary Center for Biotechnology Research, UFL
http://www.biotech.ufl.edu/FlowCytometry/

appropriate for running intracellular staining assays such as CFC. However, it is necessary to adjust or remove the FL3 threshold. If preferable, further fine adjustment of the PMT settings can be done using an isotype control–stained sample, and further adjustment of the compensation settings can be done using single-color stained samples.

Q What if my isotype control staining intensity does not match the negative population of my cytokine-stained cells?

Isotype control cocktails will usually not match the exact staining intensity of the relevant negative population in cytokine-stained samples. They are only meant as approximations to check instrument setup. It is for this reason that isotype controls should not be used to set the boundaries of positive and negative staining.

Q What about automation of BD FastImmune CFC assays?

CFC assays are compatible with sample loading devices such as the BD FACS™ Loader and the BD Multiwell™ AutoSampler. We are currently developing software for automated data analysis that will be compatible with BD FastImmune CFC assays and will greatly increase throughput.

References

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WEB LINKS

Salk Institute Flow Ctyometry Laboratory
http://www.ccmi.salk.edu/

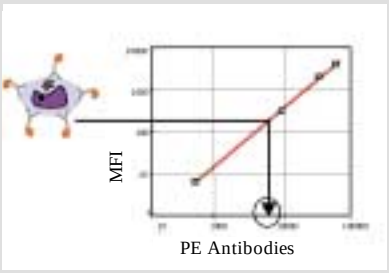
New Workshop at BD Biosciences

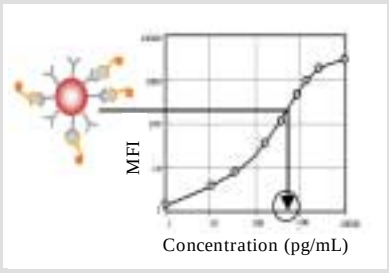
Quantitation Tools for Flow Cytometry

What is the BD QuantiBRITE System?

The BD QuantiBRITE™ System offers the first completely standardized approach to fluorescence quantitation. BD QuantiBRITE PE Beads are lyophilized pellets of beads conjugated with four levels of PE. These beads calibrate the FL2 fluorescence axis of a flow cytometer in terms of numbers of PE molecules. All of our BD QuantiBRITE reagents are guaranteed ≥ 95% one PE molecule per antibody, making your flow cytometric quantitation studies more dependable, efficient, and flexible.

New Courses and Workshops at BD





What is BD CBA?

The BD Cytometric Bead Array system (CBA) employs a series of particles with discrete fluorescence intensities to simultaneously detect multiple soluble analytes. Each bead in a CBA provides capture-surface for a specific protein and is analogous to an individually coated well in an ELISA plate.

Course Content

- Quantitate soluble cytokine levels using the BD CBA kit.
- Calibrate the flow cytometer using BD QuantiBRITE PE Beads.
- Report antibody-binding capacity (ABC) as a cell-surface quantitation unit.
- Troubleshoot sample prep, data acquisition, and analysis problems.

Prerequisites

- basic understanding of immunology and flow cytometry
- proficiency in BD FACSCalibur/BD FACScan™/BD FACSort™ operation
- experience with data acquisition and analysis using BD CellQuest software

Schedule	May 17, San Jose, CA August 9, Mansfield, MA	**Tuition**	$495

To register call 1.877.232.8995, prompt #2-2-5-2.

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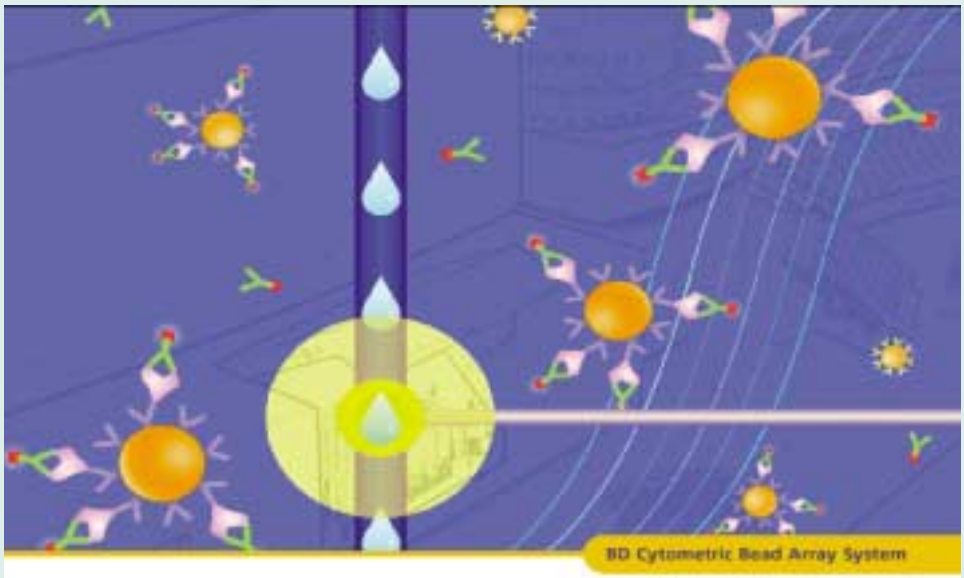
WEB LINKS

Current Contents on the Web
http://connect.isihost.com/



A Multiplex Bead System You Can Count On

The innovative BD Cytometric Bead Array (CBA) technology allows for quantitative detection of multiple analytes. The BD CBA provides reproducible data; gets more results from a single small volume sample; runs one standard curve for all your analytes; avoids artifacts associated with enzyme dependent signal generation. Use a complete system with ready-to-use kits and analysis software.

The following products are covered by the listed patents held or licensed by BD Biosciences.

PE, APC	US Patent Nos. 4,520,110; 4,859,582; 5055,556; European Patent No. 76,695; Canadian Patent No. 1,179,942
PerCP	US Patent No. 4,876,190
Cy5.5	US Patent Nos. 5,268,486; 5,486,616; 5,569,587; 5,569,766; and 5,627,027
FACS Lysing Solution	US Patent Nos. 4,654,312; 4,902,613; and 5,098,849

BD Patents and Trademarks

WEB LINKS

Conference on Guidelines for Laboratories Performing HIV-related CD4 T-Cell Determinations
http://www.phppo.cdc.gov/mlp/cd4.asp

Upcoming Courses and Workshops 2002

Workshops	Intracellular Cytokine Detection	Techniques in Cell-Cycle Analysis	BD CellQuest Pro Software	Sorting Review/Options BD FACSVantage SE Flow Cytometer
Course Content	• In vitro activation of whole blood • Cell permeabilization and labeling techniques • Flow cytometric analysis of cytokine-producing lymphocyte subsets • Troubleshooting data and sample preparation	• Paraffin-embedded tissue for single-color DNA analysis • BrdU-labeled cells for two-color DNA analysis • Cells for two-, three- or four-color DNA analysis	• Data acquisition menu features • Data analysis using one- and two-parameter plots • Logical gating strategies • Histogram overlays • Design of Experiment documents • Annotation features • Exporting data	• Sorting theory including drop-delay optimization, sort modes, and sterile-sort setup • Sorting options including BD TurboSort Option™ option, large-particle sorting using BD MacroSORT™ Plus sorter, sorting into wells, or onto slides using BD CloneCyt™ Plus software Because this course is customized for participants, the sorting option covered can vary.
Prerequisites	A basic understanding of immunology and completion of an operator course, or equivalent experience that includes data acquisition and analysis using BD CellQuest software	Completion of an operator course, or equivalent experience that includes proficiency with BD CellQuest and ModFit™ *LT* software	Basic Macintosh® skills and a basic understanding of flow cytometry, and the analysis of flow cytometry data	Completion of a BD FACS Vantage™ SE operator course, or equivalent experience
Who Should Attend	Users proficient in BD FACSCalibur/ BD FACScan/ BD FACSort operation and who want to gain experience in preparing and analyzing specimens used in studying immune function	Users proficient in BD FACSCalibur/ BD FACScan/ BD FACSort operation and those who want to expand their repertoire of DNA analysis techniques	Users who have upgraded to BD FACStation™ system, users who deal mainly with data analysis rather than cytometer operation, or users who are familiar with BD CellQuest software but require more in-depth training	Users who have a working knowledge of the BD FACS Vantage SE instrument and who want to obtain more experience with cell sorting
Duration	1 day	2 days Contact hours [a]	1 day	2 days
Schedule	May 16, San Jose, CA August 8, Mansfield, MA	May 14–15, San Jose, CA August 6–7, Mansfield, MA	May 13, San Jose, CA August 5, Mansfield, MA	May 13–14, San Jose, CA August 5–6, Mansfield, MA
Tuition	$495	$990	$495	$990

[a] BD Biosciences is approved as a provider of continuing education programs in the clinical laboratory sciences by the ASCLS P.A.C.E.

Call the customer education coordinator at 1.877.232.8995, prompt# 2-2-5-2 for details. For information on other courses and BD FACSAcademy products check out our Customer Education website.

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WEB LINKS

Customer Education
http://www.biosciences.com/immunocytometry_systems support/education/

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