Kits and Templates for the BD Accuri™ C6

As Easy as Cell Analysis is Going to Get

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The analytical power and versatility of today's laser-based flow cytometry systems have unlocked the mysteries of cell biology and empowered entirely new fields of research.

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Reagent Kits

BD reagent kits are preconfigured to conduct many common flow cytometry assays for applications such as immunophenotyping, apoptosis, cell cycle, stem cells, microbiology, intracellular cytokines, and more. In addition to preselected fluorescent antibodies, the kits include buffer systems, other reagents, and protocols needed for acquisition and analysis.

Software Templates

Free BD Accuri™ C6 software templates are matched to each kit. A template is a predefined workspace that includes markers, regions, gates, labels, parameter names, run criteria, and compensation settings for an assay using the kit. These settings provide a shortcut to quick and easy setup and analysis.

As a result, flow cytometry has become a staple of modern laboratories around the world. Now, BD makes it even easier to apply the power of flow cytometry to your research with ready-to-go reagent kits, protocols, and free software templates for the BD Accuri™ C6 personal flow cytometer.

BD Accuri C6

The analytical power and versatility of today's laser-based flow cytometry systems have unlocked the mysteries of cell biology and empowered entirely new fields of research. As a result, flow cytometry has become a staple of modern laboratories around the world. Innovations in ease of use reflected in the BD Accuri C6 flow cytometer make these powerful capabilities accessible and affordable.

The compact footprint and transportable weight of the BD Accuri C6 also make it a valuable personal use tool for experienced researchers who want a cytometer to be easily available when and where they need it.

Ease of use is a hallmark of the system because it is designed for the non-flow expert. Using the BD Accuri C6, customers can begin collecting and analyzing data with the help of a quick start guide. The intuitive interface of the software guides the user through workflows.

The system takes in data digitally over a 7-decade dynamic range, allowing researchers to easily re-analyze data after it is collected. This helps ensure that, should data need to be re-examined to accommodate new research, the data is always available.

The BD Accuri C6 flow cytometer is small enough to easily fit on a benchtop and can be placed in a laminar flow hood. It measures 11 x 14.75 x 16.5 inches (H x W x D) (27.9 x 37.5 x 41.9 cm) and weighs just 30 pounds (13.6 kg).

Using Kits and Templates

Template Home Page

Reagent kits and their associated software templates are listed at bdbiosciences.com/go/templates. There you can browse and order the kits, download the templates, examine sample data, replay webinars, and review product information sheets.

Using Templates

Once you have downloaded a template, select **File > Open workspace or Template** and open it. Gate positions, zoom level, thresholds, and compensation, run, and fluidics settings are all fully adjustable and may require optimization for different sample types. Once the settings are optimized for your experiment, click **Run** to begin data acquisition.

Opening a template

Select **File > Open workspace or template**. All settings are fully adjustable.

Cell Biology: Apoptosis

BD apoptosis kits, protocols, and software templates for the BD Accuri C6 simplify the detection of apoptosis at different stages. The kits support studies involving Annexin V, JC-1, and active caspase-3, and include buffers and other agents needed for acquisition and analysis.

The **BD Pharmingen™ Annexin V FITC and PE Apoptosis Detection Kits** (Cat. Nos. 556570 and 559763) detect externalization of phosphatidylserine (PS) molecules on the plasma membrane, one of the first signs of the apoptotic process.

The **BD Pharmingen™ BD™ MitoScreen (JC-1) Kit** (Cat. No. 551302) detects apoptosis from changes in mitochondrial membrane potential ($\Delta\Psi m$) due to the accumulation of JC-1 aggregates.

The **BD Pharmingen™ Caspase-3 PE Assay Kit** (Cat. No. 550914) detects apoptosis from the presence of cleaved (activated) caspase-3, a key apoptotic protease that cleaves and activates other caspases as well as other cellular targets.

Apoptosis (programmed cell death) is an important biological process for both development and normal tissue homeostasis. Dysregulation of apoptotic pathways can lead to disease.

Flow cytometry is an especially powerful method for detecting apoptosis because researchers can gain quantitative data on both apoptotic and dead cells within whole populations and cell subsets. BD offers multiple methods for detecting cells at various stages of apoptosis, as well as a broad portfolio of reagents to identify cell subsets.

For a broader review of apoptosis detection on the BD Accuri C6, see the BD Biosciences technical bulletin, *Multiple Methods for Detecting Apoptosis on the BD Accuri™ C6 Flow Cytometer* (March 2012), available online at bdbiosciences.com.

BD Pharmingen Annexin V FITC Apoptosis Detection Kit II	Quantity	Number of Tests	Cat. No.
Purified Recombinant Annexin V	100 µg	100 tests	556570
FITC Annexin V	0.5 mL		
Propidium Iodide Staining Solution	2.0 mL		
10X Annexin V Binding Buffer	50 mL		

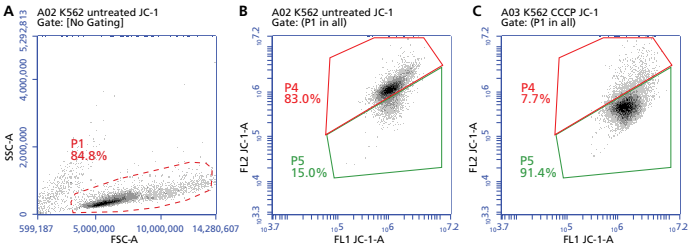
BD Pharmingen Annexin V FITC Apoptosis Detection Kit I	Quantity	Number of Tests	Cat. No.
PE Annexin V	0.5 mL	100 tests	559763
7-AAD	2.0 mL		
10X Annexin V Binding Buffer	2.0 mL		

BD MitoScreen (JC-1) Kit	Quantity	Number of Tests	Cat. No.
JC-1	50 mL	100 tests	551302
10X Assay Buffer	60 mL		

BD Pharmingen Caspase-3 PE Apoptosis Kit	Quantity	Number of Tests	Cat. No.
PE Rabbit Anti-Active Caspase-3	20 µL/test	100 tests	550914
BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution	65 mL		
BD Perm/Wash™ Buffer (10X Solution)	65 mL		

All kits and their associated software templates are available at bdbiosciences.com/go/templates.

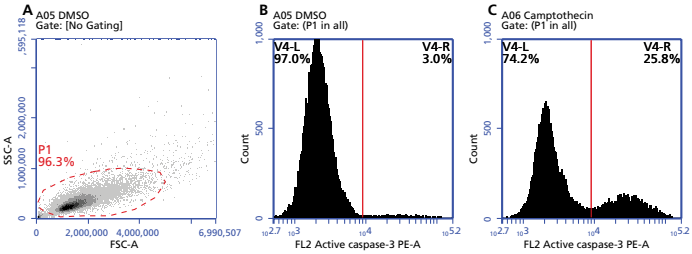
Detecting Mitochondrial Membrane Changes Using JC-1



BD MitoScreen (JC-1) Kit (Cat. No. 551302) analysis on the BD Accuri C6.

K562 cells (human chronic myelogenous leukemia; ATCC CCL-243) were treated with 100 µM of CCCP (in DMSO) for 5 minutes at 37°C to induce mitochondrial membranes to decouple. The cells were stained with JC-1 and washed according to the kit protocol, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. **Results:** Compared to untreated controls **(B)**, CCCP treatment **(C)** resulted in a shift in mitochondrial membrane potential (red to green). Because JC-1 staining patterns can vary for different cell types and experimental conditions, P4 and P5 gate boundaries should be adjusted according to the kit guidelines.

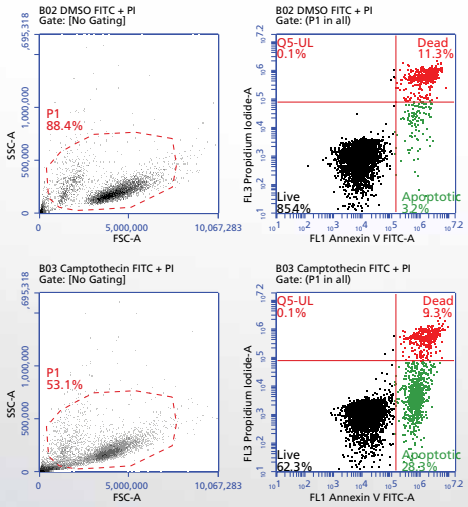
Detecting Apoptosis Using Caspase Activation



BD Pharmingen Caspase-3 Assay Kit (Cat. No. 550914) analysis on the BD Accuri C6.

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for 4 hours to induce apoptosis. Cells were permeabilized, fixed, and stained according to the kit protocol, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. **Results:** Camptothecin treatment **(C)** resulted in an increase in active caspase-3 expression compared to the DMSO control **(B)**, which was almost completely negative.

Detecting Early-Stage Apoptosis Using Annexin V



BD Pharmingen Annexin V FITC Apoptosis Detection Kit (Cat. No. 556570) analysis on the BD Accuri C6.

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for 4 hours to induce apoptosis. Cells were stained with FITC Annexin V and PI according to the kit staining protocol, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. **Results:** Camptothecin treatment **(lower plots)** resulted in an increase in early apoptotic cells (PI^-Annexin V^+, shown in green) compared to the DMSO control **(upper plots)**. Dead or late-stage apoptotic cells (PI^+Annexin V^+, red) and live cells (PI^- Annexin V^-, black) were easily distinguished.

Cell Biology: Cell Cycle and DNA

BD cell cycle/DNA kits, protocols, and software templates for the BD Accuri C6 simplify the assessment of cell cycle and DNA status.

The **BD Cycletest™ Plus DNA Reagent Kit** (Cat. No. 340242) uses PI and other active agents to obtain precise ploidy and cell cycle measurements using isolated cell nuclei. Researchers can use it to estimate the DNA index (DI) and cell cycle distribution of DNA stemlines and identify those with abnormal ploidy.

The **BD Pharmingen™ FITC and APC BrdU Flow Kits** (Cat. Nos. 559619 and 552598) use 7-AAD and BrdU to provide high-resolution cell cycle measurements. Researchers can use them to identify and analyze actively cycling cell subpopulations, and to examine cell cycle kinetics.

Flow cytometry has become an essential methodology for assessing cell cycle, cell proliferation, and DNA content. Multicolor flow cytometric assays allow researchers to investigate these facets of cell status—along with other cellular events, such as apoptosis, DNA damage, protein phosphorylation, or cytokine secretion—within heterogeneous cell populations.

Dyes such as PI and 7-AAD, which bind to DNA, can trace changing DNA levels and generate characteristic cellular DNA content profiles. To determine aneuploidy of abnormal cells, normal or peripheral blood mononuclear cells (PBMCs) can be stained simultaneously as a reference.

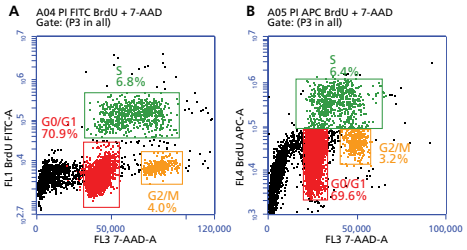
When cells are incubated in the presence of BrdU, an analog of the DNA precursor thymidine, the molecule is incorporated into newly synthesized DNA. BrdU assays can assess cell proliferation and apoptosis as well as cell cycle distribution.

The kits include key DNA reagents such as propidium iodide (PI), 7-amino actinomycin D (7-AAD), and bromodeoxyuridine (BrdU), along with buffers and antibodies needed for acquisition and analysis. The panels are compatible with other markers that allow researchers to characterize the subpopulations found.

BD Cycletest Plus DNA Reagent Kit	Quantity	Number of Tests	Cat. No.
Solution A: Trypsin in spermine tetrahydrochloride detergent buffer	10 mL	40 tests	340242
Solution B: RNase A and trypsin inhibitor in spermine buffer	8 mL		
Solution C: Propidium iodide (PI) in spermine buffer	8 mL		
Buffer Solution: Dimethyl sulfoxide (DMSO) in sucrose-sodium citrate	3 x 50 mL		

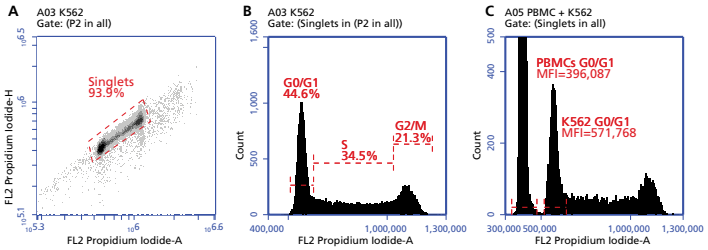
BD Pharmingen FITC or APC BrdU Flow Kit	Quantity	Number of Tests	Cat. No.
FITC or APC BrdU (1o mg/mL)	5 x 0.5 mL	50 tests	559619 (FITC) 552598 (APC)
DNase	5 x 300 µL		
Fluorochrome-conjugated anti-BrdU antibody	1 x 65 µL		
BD Cytofix/Cytoperm buffer	1 x 25 mL		
BD Perm/Wash buffer (10X)	2 x 25 mL		
BD Cytofix/Cytoperm™ Plus permeabilization buffer	1 x 10 mL		
7-AAD	1 x 1 mL		

Identifying Cell Cycle Phases Using BrdU and 7-AAD



BD Pharmingen FITC and APC BrdU Flow Kits (Cat. Nos. 559619 and 552598) analysis on the BD Accuri C6.
Human peripheral blood mononuclear cells (PBMCs) were stimulated, expanded, restimulated, and labeled with 20 µM of BrdU during the final hour. After harvesting and staining the cells with 7-AAD and either FITC or APC anti-BrdU according to the kit protocol, samples were acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. Cell cycle phases are clearly distinguished in plots showing **(A)** 7-AAD vs BrdU FITC and **(B)** 7-AAD vs BrdU APC. Cells in black (to left of G_0/G_1 gate) contain less DNA, which may indicate cell death.

Assessing Cell Cycle and Ploidy Using PI



BD Cycletest Plus DNA Reagent Kit (Cat. No. 340242) analysis on the BD Accuri C6.
K562 leukemia cells (incorporating the Philadelphia translocation) were cultured and stained according to the kit protocol, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. **A.** K562 cells were gated to exclude aggregates on a PI FL2-A vs PI FL2-H plot. **B.** A PI histogram of the gated K562 singlets distinguishes cells at the G_0/G_1, S, and G_2+M cycle phases. Gating of cell cycle phases is approximate and can be refined using software analysis. **C.** Staining and analyzing normal PBMCs along with the K562 cells can quantify their aneuploidy by gating on their G_0/G_1 peaks. The ratio of the MFIs of the two peaks, called the DNA Index (DI), serves as a measure of aneuploidy—in this case 1.4.

Immunology: Naïve/Memory T Cells

BD naïve/memory T-cell kits, protocols, and software templates for the BD Accuri C6 simplify the identification of human naïve and memory T cells and their subsets. The kits include antibodies for key T-cell markers such as CD45RA, CD45RO, CD62L, and CD197, which can not only distinguish memory from naïve T cells but subset them into activated vs non-activated or central vs effector cells.

The **BD Multitest™ Human CD45RA/CD45RO/CD3/CD4 Kit** (Cat. No. 340571) is a convenient antibody cocktail to differentiate between naïve and memory T cells using the classic markers CD45RO and CD45RA. The lyse/no-wash protocol is fast and easy to use.

The **BD Multitest™ Human CD45RA/CD62L/CD3/CD4 Kit** (Cat. No. 340977) also distinguishes naïve from memory CD4 T cells in a convenient antibody cocktail, and adds a CD62L (L-selectin) antibody to assess activation of memory cells.

The **BD Pharmingen™ Human Naïve/Memory T Cell Panel** (Cat. No. 561438) also distinguishes naïve from memory CD4 T cells in a convenient antibody cocktail, and adds a CD197 (CCR7) antibody to identify central and effector memory cells. It may be combined with CD27, CD28, and other markers to assess antigen experience, depending on the level of activation.

The immune system's ability to respond with greater intensity upon re-exposure to antigens forms the basis of immunological memory. The understanding of immunological memory is important for the study of vaccine development, infectious disease, and immune reconstitution.

Relative populations of different T-cell subsets, activation markers, and growth factors can provide a useful measurement of immune response to antigens. Memory T cells, derived from naïve T cells upon activation, are characterized phenotypically in humans by high levels of CD45RO expression. Central memory cells retain the lymph node (LN)-homing receptors CD197 (CCR7) and CD62L (L-selectin) as they develop from the naïve T cells. Effector memory cells, on the other hand, downregulate CD62L and express a diverse set of homing receptors, which enables the cells to pass through non-lymphoid tissues.[1]

1. Appay V, van Lier RA, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A*. 2008;73:975-983.

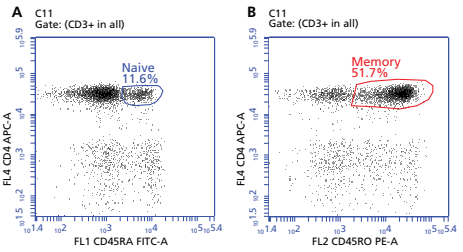
BD Multitest Human CD45RA/CD45RO/Cd3/CD4 Kit	Clone	Quantity	Number of Tests	Cat. No.
Human CD3 PerCP	SK7	20 µL/test	50 tests	340571
Human CD4 APC	SK3			
Human CD45RA FITC	L48			
Human CD45RO PE	UCHL-1			

BD Multitest Human CD45RA/CD62L/CD3/CD4	Clone	Quantity	Number of Tests	Cat. No.
Human CD3 PerCP	SK7	20 µL/test	50 tests	340977
Human CD4 APC	SK3			
Human CD45RA FITC	L48			
Human CD62L PE	SK11			

BD Pharmingen Human Naïve/Memory T Cell Panel	Clone	Quantity	Number of Tests	Cat. No.
Human CD3 APC-H7 (optional drop-in; not used on BD Accuri C6)	SK7	5 µL/test	50 tests	561438
Human CD4 PerCP-Cy™5.5	SK3	5 µL/test		
Human CD45RA FITC	HI100	5 µL/test		
Human CD197 (CCR7) Alexa Fluor® 647	150503	5 µL/test		

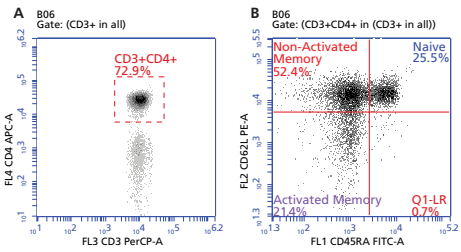
All kits and their associated software templates are available on the BD Biosciences website.

Differentiating Naïve vs Memory T Cells



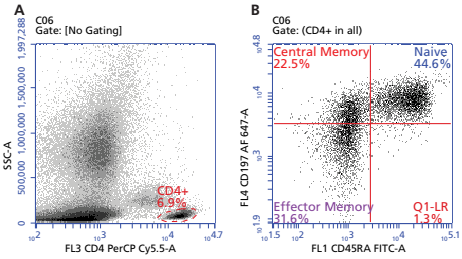
BD Multitest Human CD45RA/CD45RO/CD3/CD4 Kit (Cat. No. 340571) analysis on the BD Accuri C6.
Human peripheral blood was stained according to the kit procedure, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software with a gate set on $CD3^+$ lymphocytes. **Results:** The $CD3^+$ T cells were characterized as either **(A)** naïve ($CD4^+CD45RA^+$, blue) or **(B)** memory cells ($CD4^+CD45RO^+$, red).

Distinguishing Activated vs Non-Activated Memory Cells



BD Multitest Human CD45RA/CD62L/CD3/CD4 Kit (Cat. No. 340977) analysis on the BD Accuri C6.
Human peripheral blood was stained according to the kit procedure, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software with a gate set on $CD3^+$ lymphocytes. **Results: A.** An additional gate was drawn around the $CD3^+CD4^+$ T-cell population. **B.** The $CD3^+CD4^+$ T cells were characterized as either naïve ($CD45RA^+CD62L^+$, blue), non-activated memory ($CD45RA^-CD62L^+$, red), or activated memory cells ($CD45RA^-CD62L^-$, purple).

Distinguishing Central vs Effector Memory Cells



BD Pharmingen Human Naïve/Memory T Cell Panel (Cat. No. 561438) analysis on the BD Accuri C6.
Human peripheral blood was stained according to the kit procedure, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. (The optional drop-in reagent CD3 APC-H7 was not used.) **Results: A.** A gate was drawn around the $CD4^+$ T-cell population. **B.** The $CD4^+$ T cells were characterized as either naïve ($CD45RA^+CD197^+$, blue), central memory ($CD45RA^-CD197^+$, red), or effector memory cells ($CD45RA^-CD197^-$, purple).

Immunology: Intracellular Cytokines

BD T-cell cytokine kits, protocols, and software templates for the BD Accuri C6 simplify the detection of cytokines and cell surface markers. The kits support studies involving the production IFN-γ, IL-4, and IL-17A by activated Th1, Th2, Th17, and other cells. They include antibodies to surface markers that help characterize the cytokine-producing cells, along with buffers and transport inhibitors needed for acquisition and analysis.

The BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit (Cat. No. 560751) is designed to assess the differentiation of naïve T cells into Th1, Th2, and Th17 cells, which secrete IFN-γ, IL-4, and IL-17A, respectively.

The BD FastImmune™ Human IFN-γ/CD69/CD8/CD3 Kit (Cat. No. 346048) measures the response of activated $CD8^+$ cytotoxic T cells to specific antigen stimulation in vitro, or to non-specific stimulation such as PMA+Ionomycin.

The BD FastImmune™ Human IFN-γ/IL-4 Kit (Cat. No. 340456) enables rapid characterization of activated cells expressing cytokines.

Intracellular flow cytometry offers distinct advantages over classical methods for the detection of cytokines secreted by T cells and other cells. It allows for the analysis of cytokines and other inflammatory mediators produced by multiple, phenotypically identified subpopulations within a heterogeneous sample. It can determine whether the cytokine production by an activated cell population is the result of a few cells producing large amounts of cytokine or a large cell population producing small quantities. Finally, it can easily measure multiple cytokines simultaneously for an individual cell.

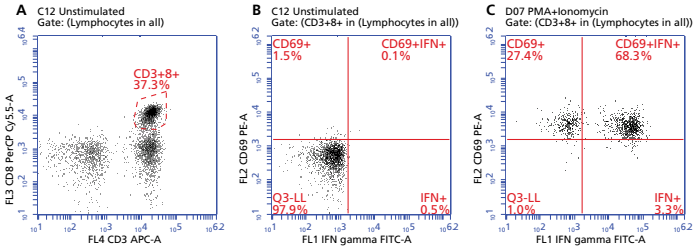
Since cytokines typically are secreted proteins, they must first be trapped inside the cell using a protein transport inhibitor. The best choice of transport inhibitor varies by cytokine and by species. Once trapped, most cytokines are relatively accessible using the gentle BD Cytofix/Cytoperm fixation and permeabilization solution.

BD Pharmingen Human Th1/Th2/Th17 Phenotyping Kit	Clone	Quantity	Number of Tests	Cat. No.
Human CD4 PerCP-Cy5.5	SK3	1 mL	50 tests	560751
Human IL-17A PE	N49-653			
Human IFN-γ FITC	B27			
Human IL-4 APC	MP4-25D2			
BD Cytofix™ Fixation Buffer		100 mL		
BD Perm/Wash Buffer		25 mL		
BD GolgiStop™ Protein Transport Inhibitor		0.7 mL		

BD FastImmune Human IFN-γ/CD69/CD8/CD3 Kit	Clone	Quantity	Number of Tests	Cat. No.
Human IFN-γ FITC	25723.11	1 mL	50 tests	346048
Human CD69 PE	L78			
Human CD8 PerCP-Cy5.5	SK1			
Human CD3 APC	SK7			

BD FastImmune Human IFN-γ/IL-4 Kit	Clone	Quantity	Number of Tests	Cat. No.
Human IFN-γ FITC	25723.11	1 mL	50 tests	340456
Human IL-4 PE	3010.211			

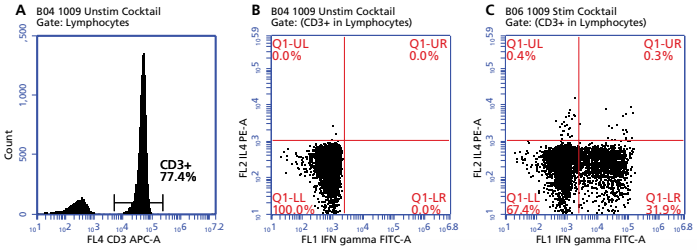
IFN-γ Production by Cytotoxic T Cells



BD FastImmune IFN-γ/CD69/CD8/CD3 Kit (Cat. No. 346048) analysis on the BD Accuri C6.

Human whole blood was drawn into heparinized tubes and stimulated with PMA (10 ng/mL) and Ionomycin (1 µg/mL) for 5 hours at 37°C in the presence of 10 ng/mL of Brefeldin A protein transport inhibitor (BD GolgiPlug™, Cat. No. 555029). Samples were harvested, fixed, lysed, permeabilized, and stained according to the kit procedure. They were acquired on a BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software, gating on lymphocytes using light scatter and then on $CD3^+CD8^+$ cytotoxic T cells **(A)**. **Results:** Compared to unstimulated controls **(B)**, stimulated cells **(C)** were more likely to produce high levels of the activation marker CD69. Most of these $CD69^+$ cells expressed IFN-γ as well. Data is representative of five donors.

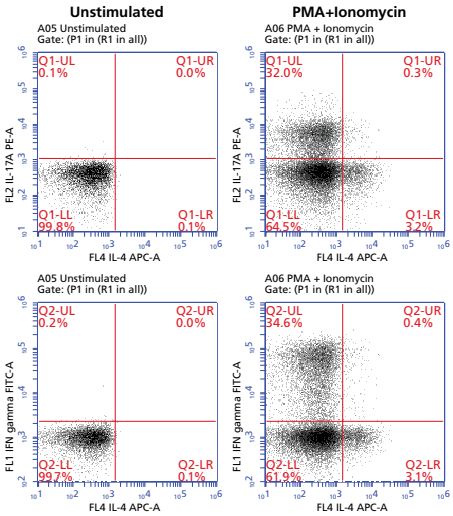
IFN-γ and IL-4 Production by Activated T Cells



BD FastImmune IFN-γ/IL-4 Kit (Cat. No. 340456) analysis on the BD Accuri C6.

Human lysed whole blood was activated with PMA/Ionomycin (at 50 ng/mL and 1 µg/mL, respectively) in the presence of Brefeldin A protein transport inhibitor (BD GolgiPlug, Cat. No. 555029) for 4 hours at 37°C. Cells were washed, fixed, and permeabilized using the BD Cytofix/Cytoperm fixation/permeabilization solution kit procedure (Cat. No. 554714). After two washes with BD Perm/Wash buffer, the cells were stained according to the kit procedure and with CD3 APC (Cat. No. 555335) to identify T cells. They were acquired on a BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software, gating on lymphocytes using light scatter and then on $CD3^+$ T cells **(A). Results:** Compared to unstimulated controls **(B)**, stimulated cells **(C)** were more likely to produce high levels of IFN-γ (lower-right quadrant). Production of high levels of IL-4 was rare (upper-left and upper-right quadrants), but was also more common in stimulated than in unstimulated cells.

Cytokine Production by Stimulated Naïve T Cells



BD Pharmingen Human Th1/Th2/Th17 Phenotyping Kit (Cat. No. 560751) analysis on the BD Accuri C6.

Purified human PBMCs were stimulated with PMA/Ionomycin (at 50 ng/mL and 1 µg/mL, respectively) in the presence of BD GolgiStop protein transport inhibitor (provided in the kit or Cat. No. 554724) for 5 hours at 37°C. Cells were stained according to the kit procedure, acquired on a BD Accuri C6 flow cytometer using the kit template, and analyzed using BD Accuri C6 software. **Results:** Density plots (gated on $CD4^+$ lymphocytes) show that stimulated cells **(right)** were more likely than unstimulated controls **(left)** to produce high levels of IFN-γ, IL-4, and IL-17A as they differentiate into Th1, Th2, and Th17 helper T cells, respectively.

Immunology: Regulatory T Cells

BD human Treg kits, protocols, and software templates for the BD Accuri C6 simplify the detection and characterization of regulatory T cells (Tregs) using intracellular and surface markers. The kits include antibodies for key Treg markers such as FoxP3, CD4, CD25, and CD127, along with buffer systems and other reagents needed for acquisition and analysis. The panels are compatible with other markers to provide a base for Treg studies.

The **BD Pharmingen™ FoxP3 Staining Kit** (Cat. No. 560132) provides two different analysis strategies for identifying Treg populations, using FoxP3 expression in combination with CD4 and CD25.

The **BD Pharmingen™ Human Regulatory T-Cell Cocktail** (Cat. No. 560249) is a one-step, premixed cocktail for convenient, optimized analysis of Treg populations using surface markers, without the need to permeabilize cells.

The **BD Pharmingen™ Human Th17/Treg Phenotyping Kit** (Cat. No. 560762) can identify both Th17 and Tregs from a single sample, and includes optimized reagents necessary for successful intracellular staining.

Regulatory T cells, which suppress the function of other T cells, play an important role in maintaining immune homeostasis. The transcription factor FoxP3 is the classic marker for Tregs.

Because FoxP3 staining requires fixation and permeabilization of cells, the cells cannot be used for further experiments. However, the surface marker CD127 is negatively correlated with FoxP3 and, when combined with CD4 and CD25 (as in the BD Pharmingen Human Regulatory T-Cell Cocktail), enables the identification of Tregs without permeabilization.

The discovery of FoxP3 has led to the characterization of different types of Tregs. For example, natural Tregs (nTregs) emerge from the thymus "naturally" expressing high levels of FoxP3. In contrast, adaptive or inducible Tregs (iTregs) express FoxP3 only after antigenic stimulation in the presence of cognate antigen and specialized immunoregulatory cytokines. iTregs are reported to be more plastic, with the ability to convert to other T-cell subtypes such as Th1 and Th17, which could undermine their eventual therapeutic value. All three Treg kits can be used with antibodies to other markers such as CD45RA to study Treg plasticity.

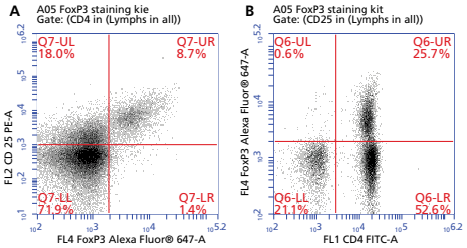
BD Pharmingen FoxP3 Staining Kit	Clone	Quantity	Number of Tests	Cat. No.
Human FoxP3 Alexa Fluor® 647	259D/C7	1 vial	100 tests	560132
Human CD4 FITC	RPA-T4	1 vial		
Human CD25 PE	M-A251	1 vial		
Human FoxP3 Buffer A (10X)		25 mL		
Human FoxP3 Buffer A (50X)		100 mL		

BD Pharmingen Human Th17/Treg Phenotyping Kit	Quantity	Number of Tests	Cat. No.
Human CD4 PerCP-Cy5.5	1 mL	50 tests	560762
Human IL-17 PE			
Human FoxP3 Alexa Fluor® 647			
Human FoxP3 Buffer A (10X)	25 mL		
Human FoxP3 Buffer A (50X)	100 mL		
BD GolgiStop Protein Transport Inhibitor (containing monensin)	0.7 mL		

BD Pharmingen Regulatory T-Cell Cocktail	Clone	Quantity	Number of Tests	Cat. No.
Human CD4 FITC	SK3	20 µL/test	50 tests	560249
Human CD25 PE-Cy™7	2A3			
Human CD127 Alexa Fluor® 647	hIL-7R-M21			

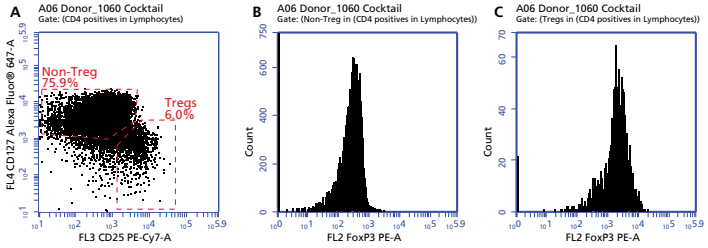
All kits and their associated software templates are available on the BD Biosciences website.

Identifying Tregs Using FoxP3



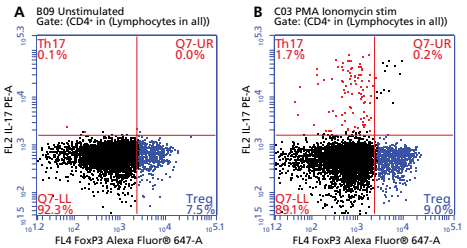
BD Pharmingen FoxP3 Staining Kit (Cat. No. 560132) analysis on the BD Accuri C6. Human PBMCs were stained with CD4 FITC and CD25 PE, fixed, permeabilized, and stained for intracellular content with FoxP3 Alexa Fluor® 647 according to the kit procedure. Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. **Results: A.** Gating on $CD4^+$ lymphocytes, Tregs are $CD25^+FoxP3^+$. **B.** Gating on $CD25^+$ lymphocytes, Tregs are $CD4^+FoxP3^+$. Both plots depict equivalent percentages of Tregs.

Distinguishing Tregs Using CD127



BD Pharmingen Human Regulatory T-Cell Cocktail (Cat. No. 560249) analysis on the BD Accuri C6.
Human PBMCs were stained according to the kit procedure. The cells were then fixed, lysed, and permeabilized using the BD Pharmingen™ Human Transcription Factor Buffer Set (Cat. No. 562574) and stained with PE-conjugated anti-human BD Pharmingen™ FoxP3 monoclonal antibody (Cat. No. 560082). Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. $CD4^+$ lymphocytes were identified and gated by light scatter profile and fluorescence (data not shown). **Results: A.** A CD25 vs CD127 plot was used to identify $CD25^{bright}CD127^{dim}$ Tregs and non-Treg $CD4^+$ cells. **B, C.** Tregs identified in **Panel A** were validated using FoxP3 staining. $CD4^+$ cells identified as Tregs **(C)** expressed higher levels of FoxP3 than did non-Tregs **(B)**.

Distinguishing Th17 Cells and Tregs in a Single Sample



BD Pharmingen Human Th17/Treg Phenotyping Kit (Cat. No. 560762) analysis on the BD Accuri C6.
Human PBMCs were either unstimulated or stimulated with PMA and Ionomycin for 4 hours in the presence of BD GolgiStop protein transport inhibitor (included in the kit or Cat. No. 554724). The cells were fixed, permeabilized, and stained with the antibody cocktail according to the kit procedure. Samples were collected on the BD Accuri C6 flow cytometer using the kit template and analyzed using BD Accuri C6 software. $CD4^+$ lymphocytes were identified and gated by light scatter profile and fluorescence (data not shown). **Results:** Compared to unstimulated cells **(A)**, more PBMCs stimulated with PMA and Ionomycin **(B)** expressed IL-17, and some expressed FoxP3 as well.

Stem Cells: Pluripotent Stem Cells

BD pluripotent stem cell kits, protocols, and software templates for the BD Accuri C6 simplify the characterization of pluripotent stem cells and their derivatives. The kits support studies involving key pluripotency and differentiation markers such as SSEA-1, SSEA-3, SSEA-4, TRA-1-81, Nanog, Oct3/4, and Sox2. They also include buffer systems and controls needed for acquisition and analysis, and are compatible with other markers to provide a base for studies of stem cell pluripotency and differentiation.

The BD Stemflow™ Human Pluripotent Stem Cell Sorting and Analysis Kit (Cat. No. 560461), which includes antibodies to TRA-1-81, SSEA-1, and SSEA-3, provides a comprehensive system for characterizing pluripotent stem cells using flow cytometry.

The B**D Stemflow™ Human Pluripotent Stem Cell Transcription Factor Analysis Kit** (Cat. No. 560589) can simultaneously measure expression of the stem cell master regulators—Nanog, Oct3/4, and Sox2—in heterogeneous stem cell populations.

The **BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit** (Cat. No. 560477) enables reliable, in-depth characterization of cellular pluripotency and differentiation state in heterogeneous human or mouse stem cell cultures using antibodies to both cell surface (SSEA-1, SSEA-4) and intracellular markers (Oct 3/4).

Flow cytometry offers an easy, rapid, and quantitative method of analyzing established markers of stem cell pluripotency and differentiation. Researchers can examine the cells' transcriptional and surface profile to correlate with pluripotency, study their differentiation states, or scrutinize heterogeneous cell populations to identify culture dynamics.

Pluripotent stem cells differentiate into the three primary germ layers and into differentiated tissue, each characterized by certain cell surface proteins and/or intracellular transcription factors. Antibodies to these markers can be combined in many ways to monitor the cells' changing expression patterns. Analysis based on cell surface markers can preserve cell viability for use in additional experiments.

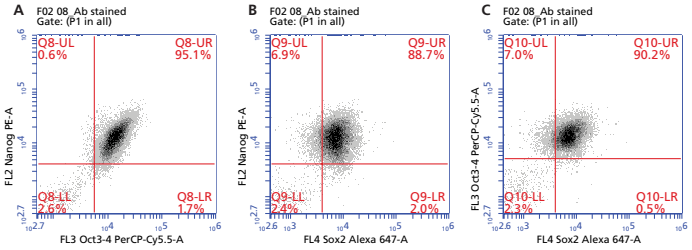
BD Stemflow Human Pluripotent Stem Cell Sorting and Analysis Kit	Clone	Quantity	Number of Tests	Cat. No.
TRA-1-81 Alexa Fluor® 647	TRA-1-81	1.5 mL	50 tests	560461
SSEA-1 FITC	MC480	1.5 mL		
SSEA-3 PE	MC631	1.5 mL		
Controls and compensation particles as detailed in kit manual				

BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit	Clone	Quantity	Number of Tests	Cat. No.
Nanog PE	N31-355	1.5 mL	50 tests	560589
Oct3/4 PerCP-Cy5.5	40/Oct-3	1.5 mL		
Sox2 Alexa Fluor® 647	245610	1.5 mL		
Controls and compensation particles as detailed in kit manual				

BD Stemflow Human and Mouse Pluripotent Stem Cell Analysis Kit	Clone	Quantity	Number of Tests	Cat. No.
Oct3/4 PerCP-Cy5.5	40/Oct-3	1.5 mL	50 tests	560477
SSEA-4 Alexa Fluor® 647	MC813	1.5 mL		
SSEA-1 PE	MC480	1.5 mL		
Controls and compensation particles as detailed in kit manual				

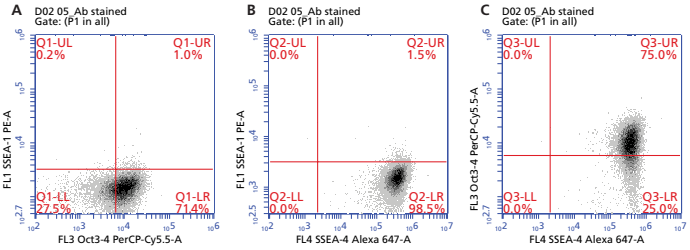
All kits and their associated software templates are available on the BD Biosciences website.

Analyzing Stem Cells for Key Transcription Factors



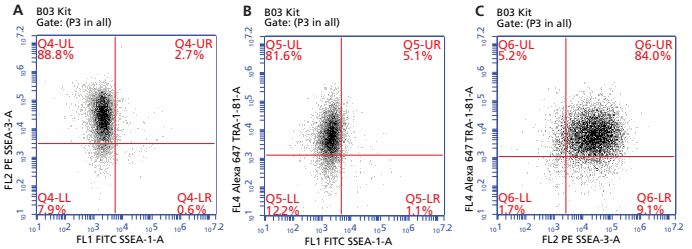
BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit (Cat. No. 560589) analysis on the BD Accuri C6.
H9 human embryonic stem cells (hESCs) were disassociated using BD™ Accutase Cell Detachment Solution (Cat. No. 561527), stained according to kit instructions, and acquired on a BD Accuri C6 flow cytometer using the kit template. Cells were gated on light scatter properties of H9 hESCs and analyzed for expression of core pluripotency transcription factors using BD Accuri C6 software. **Results:** Most analyzed cells expressed the core pluripotency transcription factors Nanog, Oct3/4, and Sox2.

Characterizing Stem Cell Pluripotency and DIfferentiation State



BD Stemflow Human and Mouse Pluripotent Stem Cell Analysis Kit (Cat. No. 560477) analysis on the BD Accuri C6.
H9 hESCs were disassociated using BD Accutase Cell Detachment Solution (Cat. No. 561527), stained according to kit instructions, and acquired on a BD Accuri C6 flow cytometer using the kit template. Cells were gated on light scatter properties of H9 hESCs and analyzed for expression of key pluripotency surface markers and transcription factors using BD Accuri C6 software. **Results:** Most analyzed cells expressed the positive pluripotency surface marker SSEA-4 and the pluripotency transcription factor Oct3/4, while few expressed the negative pluripotency marker (positive differentiation marker) SSEA-1.

Analyzing Stem Cells for Key Pluripotency Markers

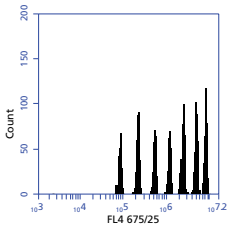


BD Stemflow Human Pluripotent Stem Cell Sorting and Analysis Kit (Cat. No. 560461) analysis on the BD Accuri C6.
H9 hESCs were disassociated using BD Accutase Cell Detachment Solution (Cat. No. 561527), stained according to kit instructions, and acquired on a BD Accuri C6 flow cytometer using the kit template. Cells were gated on light scatter properties of H9 hESCs and analyzed for expression of key pluripotency surface markers using BD Accuri C6 software. **Results:** Most analyzed cells expressed positive pluripotency surface markers SSEA-3 and TRA-1-81, while few expressed the negative pluripotency marker (positive differentiation marker) SSEA-1.

Bead-Based Assays: BD Cytometric Bead Array Kits

Bead-based flow cytometric immunoassays are powerful methods of quantifying proteins because they allow researchers to multiplex many analytes with very little sample.

BD CBA Kit Bead Resolution

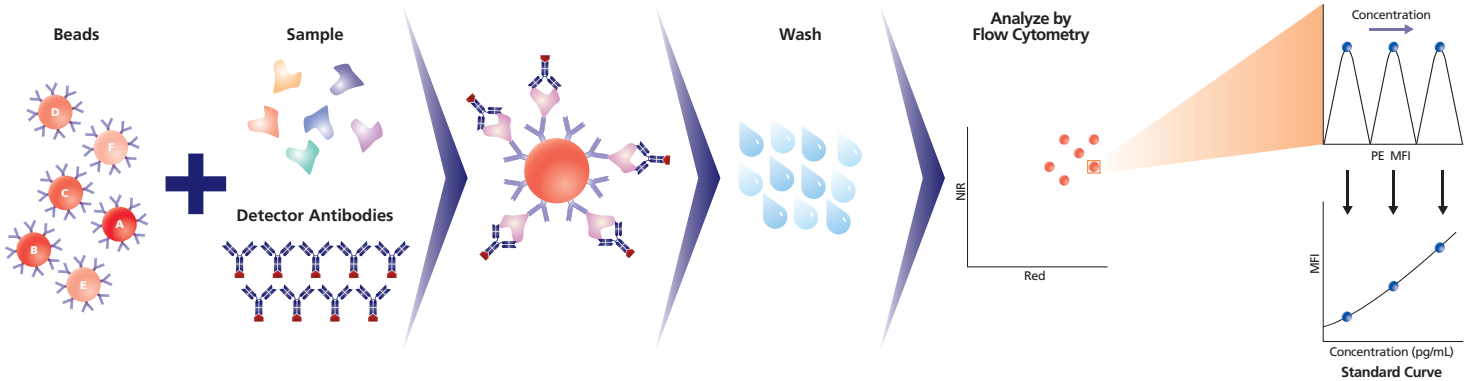


BD CBA kit bead resolution on the BD Accuri C6.
The beads used in BD CBA kits are resolved clearly in the FL4 detector of the BD Accuri C6 using the standard 675/25 filter.

There are two ways to use BD CBA: preconfigured **kits** (described here) and fully configurable **flex sets** (described further at bdbiosciences.com/go/cba/).

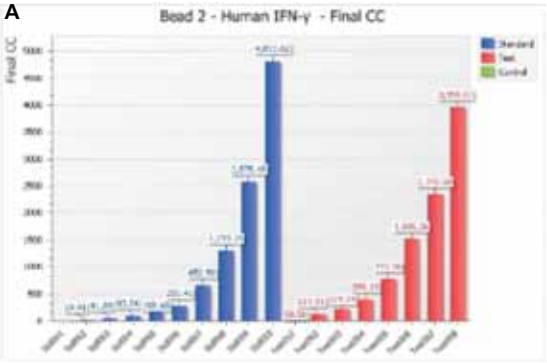
BD CBA kits provide preconfigured panels of 3–7 analytes for ultimate ease of use. For example, the BD™ CBA Human Th1/Th2/Th7 Cytokine Kit can quantitate IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A simultaneously. Other kits include panels of human cytokines, chemokines, and anaphylatoxins; mouse cytokines and immunoglobulins; and non-human primate cytokines.

On the BD Accuri C6, the beads are excited by the red laser and detected in FL4, while the PE reporter is excited by the blue laser and detected in FL2. A BD Accuri C6 software template simplifies setup and acquisition. Results can be displayed in FCAP Array™ software by bead (graphing one analyte across all samples) or by sample (graphing one sample across all analytes).



A BD™ Cytometric Bead Array (CBA) assay contains a cocktail of beads bound with specific capture antibodies that differ slightly in fluorescence intensity. The beads are mixed with samples along with PE-labeled detection antibodies and run on a BD Accuri C6. BD CBA can analyze 300 beads per protein—the equivalent of 300 ELISA wells—for up to 30 cytokines simultaneously. In essence, BD CBA is like running multiple ELISAs at once using flow cytometry.

BD CBA Kit Bead Resolution



BD CBA kit analysis by analyte or sample. Human PBMCs were cultured for several days with plate-bound anti-CD3, soluble anti-CD27, IL-2, and IL-4. Cells were stimulated with PMA+Ionomycin for several hours before collecting culture supernatants. Samples were prepared and stained with the BD CBA Human Th1/Th2/Th17 Cytokine Kit (Cat. No. 560484), acquired on a BD Accuri C6 using the BD CBA Kit Accuri template, and analyzed using FCAP Array software v3.0.1. **A.** Bar chart of human IFN-γ levels for all standards and test samples. **B.** Bar chart of all seven cytokine levels for one sample.

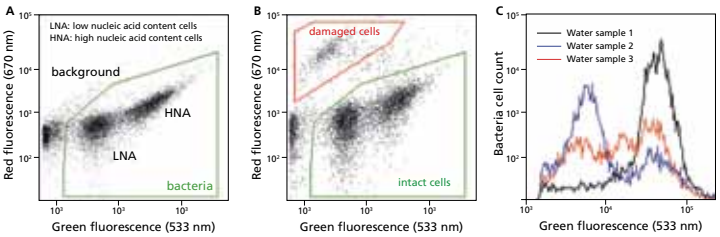
Brand	Kit	Cat. No.
BD™ Cytometric Bead Array	Human Anaphylatoxin Kit	561418
BD™ Cytometric Bead Array	Human Chemokine Kit	552990
BD™ Cytometric Bead Array	Human Inflammatory Cytokine Kit	551811
BD™ Cytometric Bead Array	Human Inflammatory Cytokine Lyophilized Standard	552932
BD™ Cytometric Bead Array	Human Th1/Th2 Cytokine Kit II	551809
BD™ Cytometric Bead Array	Human Th1/Th2 Cytokine Kit	550749
BD™ Cytometric Bead Array	Human Th1/Th2 Cytokine Lyophilized Standard	551810
BD™ Cytometric Bead Array	Human Th1/Th2 Cytokine Standards	561666
BD™ Cytometric Bead Array	Human Th1/Th2/Th17 Kit	560484
BD™ Cytometric Bead Array	Mouse Immunoglobulin Isotyping Kit	550026
BD™ Cytometric Bead Array	Mouse Inflammation Kit	552364
BD™ Cytometric Bead Array	Mouse Inflammation Lyophilized Standard	620280
BD™ Cytometric Bead Array	Mouse Th1/Th2 Cytokine Kit	551287
BD™ Cytometric Bead Array	Mouse Th1/Th2 Cytokine Lyophilized Standard	552967
BD™ Cytometric Bead Array	Mouse Th1/Th2/Th17 Cytokine Kit	560485
BD™ Cytometric Bead Array	Mouse Th1/Th2/Th17 Cytokine Standards	561665
BD™ Cytometric Bead Array	Non-human Primate Th1/Th2 Cytokine Kit	557800
Required Software		
FCAP Array Software v3.0 (Microsoft® Windows® 7, Windows Vista®, XP)		652099

All kits, a product information sheet, and the BD™ CBA Kit Accuri Template are available on the BD Biosciences website.

Microbiology: Water Quality

The BD Accuri™ C6 Eawag Water Quality Template[2] simplifies the enumeration of intact and damaged bacteria in drinking water samples on the BD Accuri C6. The Eawag protocol involves co-staining the samples with SYBR® Green I and (optionally) propidium iodide (PI). The template exploits the ability of the BD Accuri C6 to calculate absolute cell counts and concentrations automatically.

Enumerating Intact and Damaged Bacteria in Drinking Water



Water quality analysis using the Eawag water quality template on the BD Accuri C6.
Drinking water samples were stained according to the Eawag protocol, acquired on a BD Accuri C6 using the Eawag water quality template, and analyzed using BD Accuri C6 software. **A.** When a sample is stained with SYBR® Green I, all bacteria appear within the template's single, fixed gate, while noise and debris are excluded. **B.** When the sample is co-stained with SYBR® Green I and propidium iodide (PI), damaged bacteria are shifted out of the gate, leaving only viable bacteria within. **C.** Each water sample generates a unique flow cytometric fingerprint.

Data Courtesy of Frederik Hammes, Eawag Department of Environmental Microbiology, Dübendorf, Switzerland.

Using nucleic acid dyes, flow cytometry can quantitate microbes and discriminate them from debris much more rapidly than plate methods. SYBR® Green I, which preferentially stains double-stranded DNA, is well suited for staining bacteria in water samples for flow cytometric analysis. PI can be added to differentiate live and damaged bacterial cells. PI is impermeable to healthy cells with intact membranes, but permeates cells with compromised membranes such as dead cells.

For a detailed discussion of the use of the Eawag template and protocol for water quality research, see the BD Biosciences white paper, *Assessing Water Quality with the BD Accuri™ C6 Flow Cytometer* (January 2013), available at bdbiosciences.com.

2. The water quality template and staining protocol were developed in collaboration with Eawag, the Swiss Federal Institute of Aquatic Science and Technology.

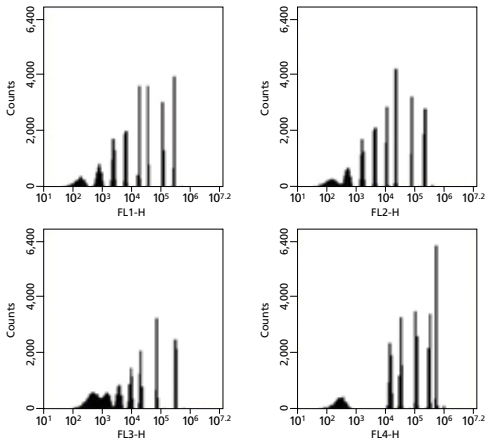
BD Accuri C6 Eawag Water Quality Template (zip file)
2012 BD Template_Eawag_24 tube rack.c6t file
2012 BD Template_Eawag_96 well plate.c6t file
Eawag Water Quality Template ReadMe.doc file

The template and product information sheet are available on the BD Biosciences website.

Instrument Setup: Validation Beads

BD Accuri™ Spherotech Validation Beads contain a mixture of fluorochromes that are spectrally similar to many of the fluorochromes used in flow cytometry. Due to the unique design of the BD Accuri C6, there is no need to use the beads to align the system or to adjust voltages. Instead, you can use the beads to validate the linearity, sensitivity, and resolution of the cytometer detectors at the beginning of each workday. Two BD Accuri C6 software templates make daily validation easy and convenient.

Validating Performance of the BD Accuri C6



Analysis of BD Accuri Spherotech Validation Beads (Cat. Nos. 653144 and 653145) on the BD Accuri C6.
8-Peak beads were used to validate performance of the FL1, FL2, and FL3 detectors, while 6-Peak beads were used to validate the FL4 detector. Software templates were used to streamline acquisition and analysis.

BD Accuri™ Spherotech 8-peak Validation Beads (Cat. No. 653144) are used to validate performance of the FL1 (FITC), FL2 (PE), and FL3 (PE-Cy™5) detectors.

BD Accuri™ Spherotech 6-peak Validation Beads (Cat. No. 653145) are used to validate performance of the FL4 (APC) detector.

For detailed procedural and troubleshooting steps to use in validating the BD Accuri C6, see Chapter 2 of the BD Accuri™ C6 Software User Guide, available at bdbiosciences.com.

Description	Particle size, µm	Size	Cat. No.
BD Accuri Spherotech Validation Beads (8 peaks)	3.0–3.4	4 mL	653144
BD Accuri Spherotech Validation Beads (6 peaks)	3.0–3.4	2 x 4 mL	653145

The templates and the *BD Accuri™ C6 Software User Guide* are available on the BD Biosciences website.

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