

BD Accuri[™] C6 Cytometer



Flow cytometry within reach™

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The BD Accuri[™] C6 is a personal flow cytometer that brings cell analysis within reach by being easy to use, simple to maintain, and affordable.

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 cytometer make these powerful capabilities more accessible to a new generation of flow cytometry users.

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. Many BD Accuri C6 cytometer users 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. A wide dynamic range of over 7 full decades ensures that all data is available all of the time. Information obtained from the BD Accuri C6 can be re-analyzed at any time if gating or compensation changes are required, or to accommodate new research.

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

Pre-optimized detectors, minimized setup time

The system is equipped with a blue and red laser, two light scatter detectors, and four fluorescence detectors with optical filters optimized for the detection of FITC, PE, PerCP, and APC. A compact optical design, fixed alignment, and preoptimized detector settings make the system easier to use.

Optional filters and the Selectable Laser Module expand the available fluorochrome combinations. During manufacture, laser and optical alignments are set and locked down. The result is that each BD Accuri C6 cytometer is manufactured with standardized fluorescence performance so that users do not need to adjust detector voltages.



Data is digitally collected over a wide dynamic range of 7.2 decades (16 million channels of digital data), making all data available to users as needed. Gating strategies and fluorescence compensation values can be set before, during, or after data collection. After data is collected, the BD Accuri™ C6 software Zoom function allows visualization of data at any scale, so that users can precisely set gates and regions.

Should updates in the values be required later, or if optimization is needed, simply change the settings and re-analyze the data. This flexibility also allows data to be re-examined to accommodate new research findings.

The system has been put through intense testing to ensure that the design can withstand rugged conditions. Provided the system is anchored, it can run samples even if the benchtop is in motion, for example, onboard a ship.



T-cell phenotyping, 4-color analysis

Thawed human peripheral blood mononuclear cells (PBMCs) were stained with directly labeled anti-CD45RA FITC, CD4 PE, CD8 PE-Cy[™]7, and CD3 APC in PBS + 1 mg/mL of BSA, covered, on ice, for 30 minutes. Cells were acquired and gated on lymphocytes to identify CD3⁺CD8⁺ cytotoxic (blue) and CD3⁺CD4⁺ helper (red) T-cell populations.

After FITC staining, subpopulations of CD45RA⁺ and CD45RA⁻ cytotoxic and helper T cells were identified.



Apoptosis detection

The BD Accuri C6 can perform most flow cytometric apoptosis assays, including Annexin V, caspase activation, PARP cleavage, mitochondrial membrane potential ($\Delta\Psi$ m), and DNA fragmentation. (A) K562 cells (human chronic myelogenous leukemia) were treated with CCCP to induce apoptosis, then stained with JC-1 according to the BDTM MitoScreen (JC-1) protocol (Cat. No. 551302). The cells were washed and collected on the BD Accuri C6. CCCP treatment (C) resulted in a shift in $\Delta\Psi$ m (red to green) vs untreated cells (B), indicating apoptosis.



Sample flexibility with optional walkaway sample loading

A unique low-pressure pumping system drives the fluidics. A sheath-focused core enables event rates of up to 10,000 events per second and a sample concentration of over 5×10^6 cells per mL. In addition, the system derives sample volume and can calculate absolute counts or sample concentration per microliter.



The non-pressurized system supports any brand of 12 x 75-mm (or smaller) sample tubes, including microcentrifuge tubes and tubes made of polypropylene or polystyrene. The BD Accuri C6 cytometer simplifies system maintenance with automatic cleaning cycles on instrument shutdown. The system can employ laboratory-grade water for sheath fluid, reducing operating costs.

For walkaway convenience, the optional BD CSampler[™] accessory offers reliable and easy-to-use automation. The system supports 48- and 96-well plates and deepwell plates, and is also supplied with a 24-tube rack for standard 12 x 75-mm tubes. They are processed directly in the BD Accuri C6 cytometer, saving time. The BD CSampler adds minimal footprint to the BD Accuri C6, about three feet square for the pair, keeping the benchtop free for other uses.

To streamline sample processing, the BD CSampler allows multiple collection settings to be applied to plate or tube runs. To process a sample immediately, a run can be paused using the Interrupt function. When the priority operation is complete, the original plate can be returned to the BD CSampler to resume the original run. Easy-to-read software messages keep users informed of system status.

Gap-free analysis of intracellular calcium

A The three left-hand cytograms show calcium data obtained on a Beckman Coulter CyAn[™] ADP using the "stop-flow" method, showing time gaps when control and test compounds were added.

B The three right-hand cytograms show data obtained on a BD Accuri C6, adding the same compounds in open Eppendorf tubes without interrupting sample acquisition. No time gaps were observed; otherwise, both methods obtained comparable data.

Data from Vines A, McBean GJ, Blanco-Fernández A. A flow cytometric method for continuous measurement of intracellular Ca2⁺ concentration. *Cytometry Part A*. 2010;77:1091-1097; reproduced courtesy of the authors.

A: Beckman Coulter Cyan ADP





Applications in kinetic analysis of cellular responses Changes to intracellular calcium (Ca2⁺) levels can occur rapidly, in some cases within nanoseconds of stimulation, and obtaining accurate data is a significant research challenge. To add test compounds to the cell suspension, a "stop-flow" method is often used in which sampling is paused, the sample tube opened, the agonist added, and the tube resealed. This technique leaves a gap in data collection that may miss essential changes in Ca2⁺ levels.

The BD Accuri C6 employs non-pressurized peristaltic pumps in an open fluidics system. Open tubes allow convenient addition of test compounds to the cell suspension without interrupting sampling. This "continuous-flow" method enables non-stop analysis of calcium flux and other kinetic cellular responses, such as pH, reactive oxygen and nitrogen species, mitochondrial membrane potential, and nanoparticle uptake.



Intuitive software—master in minutes

BD Accuri C6 software has an intuitive user interface that was developed based on hundreds of hours observing researchers using flow cytometers.

As a result, most novice flow cytometry users find it so easy to use, that they can collect and analyze data in less than an hour. Software options and instrument controls are clearly visible from the software's tabbed interface which enables access to the collection, analysis, and statistics functions.

Data is acquired from the Collect tab. Users can create new plots, or copy and re-use plots from this tab. The software supports a full range of selection regions including rectangular, polygon, quadrant, horizontal, and vertical markers.

The Analyze tab displays plots and samples in any combination. In the Analyze tab, users can create color histogram overlays, print multiple plots, and compare samples. Use the Zoom tool to magnify areas of data, instead of voltage adjustments to set the channel range viewed, to better visualize results. Sample data can be customized in the Statistics tab. Data is displayed in a master table, and statistics can be easily copied and pasted into spreadsheets to facilitate reporting. To simplify creating presentations, plots can be imported into Microsoft® Office applications using drag and drop. BD Accuri C6 software supports live gating, event coloring, export of publication-quality, vector-scalable graphics, and batch analysis, for review or modification of multiple samples for the automatic creation of Microsoft PowerPoint® and Excel files.

BD Accuri C6 software files can be exported in FCS 3.0 format for seamless importing of user data into flow cytometry analysis programs including FCS Express and FlowJo[™].



Collect tab



Frozen fixed H9 embryonic stem cells (hESCs) were stained with antibodies from the BD Stemflow[™] Human and Mouse Pluripotent Stem Cell Analysis Kit (Cat. No. 560477) and analyzed on the BD Accuri C6. Compared to isotype controls (not shown), the cells expressed high levels of positive pluripotency markers SSEA-4 and Oct3/4, and low levels of the negative marker SSEA-1.





Statistics tab

Analyze tab

Batch Analysis tab

Sample figure creation using BD Accuri C6 software

The human embryonal carcinoma (EC) cell line 2102Ep can serve as a reference standard for human embryonic and induced pluripotent stem cells because it expresses most of the same markers. After staining with antibodies to stem cell markers, 2102Ep EC cells were collected and analyzed on a BD Accuri C6. Overlays of single-parameter histograms were drawn with BD Accuri C6 software. Plots were dragged and dropped into presentation software, where labels were added.



APPLICATIONS

Easy to learn and use fits a wide range of applications



Creating and using software templates

To make the BD Accuri C6 cytometer even easier to use, software templates are available for BD reagent kits and cocktails. Templates contain a predefined workspace for quick and easy setup and analysis. Markers, regions, gates, parameter names, and sample names are predefined for fast setup.

Templates are available for popular applications such as immunophenotyping, apoptosis, cell cycle, microbial counting, and intracellular cytokines. Users can also develop custom templates for frequently run assays, saving time. Simply open the template, enter settings, and click Run to start immediately collecting data.

Discriminating intact from damaged bacteria using the Eawag water quality template

A standard flow cytometric staining protocol and a corresponding BD Accuri C6 software analysis template are used to discriminate bacteria from debris in drinking water samples. When a sample is stained with the DNA dye SYBR® Green I, all bacteria appear within the template's single, fixed gate, while noise and debris are excluded. 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. The template is available at bdbiosciences.com.

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





Efficient gene expression analysis

Screening thousands of cells for reporter gene expression levels is fundamental to understanding how genes are regulated inside the cell. Advances in flow cytometry and a full spectrum of fluorescent proteins now available allow biomedical researchers to more quickly, easily, and affordably leverage this technology in gene expression analysis.

Fluorescent proteins have come a long way since the original application of Green Fluorescent Protein (GFP) for the detection of gene expression. Fluorescent proteins now span the entire spectrum from short violet to long red, and can be used to study a wide variety of cellular phenomena.

The BD Accuri C6 simplifies the detection of fluorescently tagged genes incorporated into cells. It can also measure the effects of gene knockdown when RNA interference (RNAi) is used to reduce gene expression. With multiparameter flow cytometry, one fluorescent antibody can be used to measure the intracellular level of the transfected siRNA target protein, while others can measure the cells' functional responses such as up- and downregulation of surface markers or intracellular protein phosphorylation.





Detection of GFP expression in bacteria

Two *E. coli* cultures, one wild type and the other transfected with a constitutive GFP-expressing plasmid, mixed in a 1:1 ratio.

Data courtesy of Tim F. Cooper, Department of Biology and Biological Chemistry, University of Houston, Houston, TX.

Special order products for unique needs

The BD special order program allows customers to purchase instruments configured to fit precise research and assay needs. This innovative program is tailored to the special needs of research at the leading edge of biomedical discovery, and offers a wide range of choices to help researchers create the ultimate customized instrument for their requirements.



To support the evolving needs of researchers, the BD Accuri C6 cytometer can be custom configured by the BD special order team with a wide choice of innovative lasers. This expanded level of flexibility and choice helps make the special order BD Accuri C6 fit a wider choice of research application requirements.

Your BD Sales Representative is your point of contact to discuss a special order product for your lab. Together with an engineer from the special order team, they will follow a structured process to define, design, and manufacture an instrument that exactly matches your requirements. Strict manufacturing controls are in place to ensure high-quality outcomes.

Analysis of DNA content and ploidy in plants

Arabidopsis thaliana root tissues were chopped with a fresh razor blade, stained with PI, and acquired on the BD Accuri C6. On an FL2 vs FL3 plot, nuclear events cluster in a narrow diagonal region (A, B). Gating on these nuclear events, FL2 fluorescence (C) shows clear peaks corresponding to cell ploidy.

Data courtesy of David W. Galbraith, School of Plant Sciences and Bio5 Institute for Collaborative Bioresearch, University of Arizona, Tucson, AZ, USA.





Speed analysis of marine and freshwater samples Environmental research in marine and freshwater ecosystems is predominantly focused on the microbiomes of those aquatic environments. Of critical concern to environmental research is the primary productivity of phytoplankton and the distribution of phytoplankton and cyanobacteria species responsible for harmful algal blooms.

The BD Accuri C6 can help speed sample analysis for biologists studying marine and freshwater ecosystems. The system is transportable enough to travel aboard ship and can handle particles as small as $0.5 \mu m$, quickly delivering multiparametric measurements of particle size and autofluorescence in both cultures and environmental samples.



FL4 ex: 640; em: 675 ±12.5 nm

Analysis of aquatic microorganisms with the BD Accuri C6

In water samples from four sites in Saginaw Bay, Michigan, plots of phycocyanin fluorescence vs chlorophyll fluorescence were used to separate fluorescent phytoplankton into cyanobacteria (region P6) and other fluorescent phytoplankton (P7). Microorganism counts obtained by direct volume on the BD Accuri C6 showed that sites 1 and 2, close to the nutrient-rich mouth of the Saginaw River, had at least six times more cyanobacteria than the other two locations.

Data courtesy of Juli Dyble Bressie, National Oceanographic and Atmospheric Administration, Seattle, WA, USA.



FL4 ex: 640; em: 675 ±12.5 nm

S E R V I C E S

Services and support

BD Biosciences is fully committed to the success and satisfaction of its customers and offers a range of options for BD Accuri support.



Fast, easy installation

The BD Accuri C6 cytometer can be customer installed within just an hour of taking it out of the box. A step-by-step quick start guide and online video simplify installation.

Preventative maintenance

Preventative maintenance procedures should be performed every two months to change the sheath filter, pump tubing, and fluidic filters, and to clean the SIP.

Technical application support

Our technical application support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, technical application specialists are well equipped to address customer needs in both instrument and application support.

Training

If desired, optional hands-on training is available on the BD Accuri C6 cytometer. The training combines flow cytometry theory and practical skills to operate the BD Accuri C6 cytometer.

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