BD FACSCanto II

A proven platform for maximum reliability and the highest quality results
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Built on more than 25 years of BD experience and leadership in flow cytometry and multicolor analysis, the BD FACSCanto™ II system is an easy-to-use benchtop analyzer that delivers proven performance, accuracy, and high quality results. The BD FACSCanto II provides the ultimate in flexibility and can be configured with two or three lasers to detect up to eight colors.

The BD FACSCanto II features many innovations. At the heart of the cytometer, for example, the fluidics system features a fixed alignment flow cell to minimize startup time and improve reproducibility. The optical system features a patented design that maximizes signal detection and increases sensitivity and resolution for each color in a multicolor assay. These capabilities and many other innovations make the BD FACSCanto II one of the most powerful and versatile benchtop analyzers available for both clinical and research applications.

The BD FACSCanto II is designed to address the needs of today’s busy clinical lab. It provides a high degree of automation and quality control to reduce hands-on time for technicians and improve reliability of results. The newly designed BD FACS™ Loader features single-tube instrument setup and testing as well as walkaway sample introduction to help operators rapidly adopt routine clinical applications freeing technician time for more important activities. To further simplify operation, BD FACSCanto™ clinical software automates setup, compensation analysis, and quality control for predefined clinical applications.

For the research lab, the BD FACSCanto II system has the versatility to meet demanding research requirements. Sample introduction can be accomplished in a single tube or via the BD™ High Throughput Sampler (HTS),* a microtiter plate loader well suited for 96- and 384-well plates that can accommodate high throughput research applications. BD FACSDiva™ software efficiently controls the setup, acquisition, and analysis of flow cytometry data.

The BD FACSCanto II is supported by a broad portfolio of reagents. A full complement of highly qualified BD technical and application support personnel is available to help streamline work and maintain optimal instrument performance.

* For Research Use Only.
In the fluidics system, the sample travels up the sample injection tube, and hydrodynamic focusing within the flow cell forces particles into a single-file stream where laser light intercepts the stream at the sample interrogation point. The unique flow cell design permits particles to flow through the center of the flow cell. Increasing the sample pressure increases the core diameter and the flow rate.

A fluidics cart holds large fluid tanks necessary to operate and maintain the instrument. For sample acquisition, positive-pressure pumps in the fluidics cart send sheath fluid past a 0.2-µm filter to a pressurized interior reservoir inside the instrument called the plenum. The plenum maintains a nearly constant fluid level and dampens pump pulsation using a new dynamic feedback pressure control system designed to regulate pressure. As a result, sheath flow rate does not vary with the level of fluid in the sheath cubitainer, and the reservoir automatically removes small air bubbles from the sheath supply.

Daily routine procedures, such as startup, shutdown, and cleaning routine cycles, are automated as a result of fluidic integration with BD FACS Canto clinical software or BD FACSDiva software. BD FACS™ Shutdown solution prevents salt crystal buildup in fluidics lines and is supplemented with a preservative to prevent bacterial growth. During the instrument shutdown procedure, the BD FACS Shutdown solution replaces sheath fluid in all sample and sheath fluid lines.

The BD FACSCanto II fluidics system is designed to streamline work, save time, and improve performance.

**Saves time, improves reproducibility**

**High Performance, Innovative System**

**Hydrodynamic focusing**

A high flow rate is generally used for measurements such as immunophenotyping, for which data is less resolved but is acquired more quickly. A lower flow rate is generally used in applications for which optimal resolution and sensitivity are critical.
LED alerts

LEDs are located in the front door to monitor each acquisition parameter. Each LED blinks when the signal level reaches a prescribed threshold.
Maximize multicolor performance

**Innovative Optical Design Delivers Highest Sensitivity**

The innovative designs for both the excitation and collection optics reduce excitation losses, yielding more information from each sample.

The optics of the BD FACSCanto II system consist of an excitation source with up to three lasers, a blue (488-nm, air-cooled, 20-mW solid state), a red (633-nm, 17-mW HeNe) and a violet (405-nm, 30-mW solid state). Laser excitation optics illuminate cells in the sample and collection optics direct light scatter and fluorescence signals through spectral filters to the detectors.

**Excitation**

The excitation optics consist of multiple fixed wavelength lasers, fiber optics up to the beam-shaping prisms, and achromatic focusing lenses that produce spatially separated beam spots in the flow cell. Each lens focuses the laser light into the gel-coupled cuvette flow cell. Since the optical pathway and the sample core stream are fixed, alignment is fixed from day to day and from experiment to experiment with no need for user intervention.

**Emission**

The emission signals are transmitted from the flow cell to the detector arrays, an octagon for the blue and a trigon each for the red and the violet laser signals. The octagon contains five PMTs and detects light from the 488-nm blue laser. A PMT in the octagon collects side scatter signals. The trigons contain two PMTs each and detect light from the 633-nm red and the 405-nm violet lasers.

**Collection Optics**

The octagon and trigon are BD patented detector arrays that use serial light reflections to guide signals to their target detectors, resulting in highly efficient light collection and providing maximum signal retention at the detector level.

This BD serial reflective design further enhances instrument sensitivity by collecting the dimmest emission signals first, moving from the longest wavelengths (typically PE-Cy7™) to the shortest (FITC).
Emission spectra of commonly used fluorochromes

The BD FACS Canto II system is designed for these fluorophore combinations.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Laser</th>
<th>Excitation Laser Line (nm)</th>
<th>Fluorescence Channel</th>
<th>Fluorochromes provided by BD Biosciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD FACS Canto II flow cytometry system</td>
<td>Solid State (L1)</td>
<td>488</td>
<td>Green</td>
<td>FITC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
<td>PE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Orange</td>
<td>PE-Texas Red®</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red</td>
<td>PerCP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Infrared</td>
<td>PE-Cy7</td>
</tr>
<tr>
<td>HeNe (L2)</td>
<td>Red</td>
<td>633</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Far Red</td>
<td>633</td>
<td></td>
<td>Alexa Fluor® 700®</td>
</tr>
<tr>
<td></td>
<td>InfraRed</td>
<td>633</td>
<td></td>
<td>APC-Cy7</td>
</tr>
<tr>
<td>Violet (L3)</td>
<td>Green</td>
<td>405</td>
<td>AmCyan®</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blue</td>
<td>405</td>
<td></td>
<td>BD Horizon™ V450</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pacific Blue™</td>
</tr>
</tbody>
</table>
Productivity support

Options and Related Tools to Support Lab Requirements

Sample introduction productivity can be gained with the optional BD FACS Loader or the BD High Throughput Sampler (HTS).

BD High Throughput Sampler (HTS)*
Designed for research use, the optional BD High Throughput Sampler (HTS) provides fully automated and rapid sample acquisition that can speed through a microtiter plate in less than 15 minutes, with less than 1% sample carryover. In high-throughput mode, fast acquisition speed is achieved by synchronizing two high-precision pumps for sample mixing, sample injection, and probe washing. Standard-throughput mode can be selected for acquisition of larger sample volumes. The HTS supports standard 96 and 384-well plates. It is operated using BD FACSDiva software, allowing users to define customized delivery protocols, mixing, wash, and analysis parameters.

* For Research Use Only.

Barcode Reader
The optional BD FACSCanto hand-held barcode reader reads the two-dimensional barcode labels and it is used to read the compensation matrix of the BD™ 7-color setup beads reagent into BD FACSCanto clinical software.
BD FACS Loader

The BD FACS Loader is an instrument option that allows walkaway sample introduction to further improve productivity. The BD FACS Loader carousel accommodates up to 40 12x75-mm tubes and automatically loads them on the BD FACSCanto II system without operator intervention. Mounted directly on the cytometer, the device includes a drive system, a tube lifter mechanism, and sensors on the sliding drawer. Two sliding doors safely enclose the drawer to protect technicians from moving parts during operation.

The BD FACS Loader utilizes compressed air to allow a more reliable tube load as well as an intelligent tube guide mechanism that automatically sends an alert if a tube is not properly positioned for loading.

A unique ID and optically read label are printed on each carousel for easy carousel identification. The BD FACS Loader is operated through both BD FACSCanto clinical and BD FACSDiva software. The BD FACS Loader is compatible with the BD FACS™ Sample Prep Assistant (SPA) and the BD FACS™ Lyse Wash (LWA) Assistant.

Sample Preparation Support

BD Biosciences offers two BD FACSCanto complementary instruments to help busy laboratories increase speed, reduce variability, and streamline sample preparation.

The BD FACSCanto Sample Preparation Assistant supports walkaway lyse-no-wash sample automation processing. The BD FACSDiva Lyse/Wash Assistant (LWA) lyses, mixes, washes, and resuspends samples in PBS. Pre-programmed as well as custom protocols are available.
Sample prep, setup, and analysis

A Turnkey System for Clinical Use

Designed to address the needs of today’s busy clinical lab, the BD FACSCanto II has a proven track record of reducing hands-on time and improving reliability of results. A high degree of automation and quality control helps save time, reduce cost, and improve reproducibility of results.

Cytometer settings are tracked over time by Levey-Jennings graphs to monitor cytometer performance. Adjustments are made automatically to ensure consistent data integrity and reproducible results from day to day and experiment to experiment. Integrated quality control features further support data integrity by notifying operators if an assay fails predefined standards.

BD FACSCanto clinical software includes specific application modules optimized for use with specific IVD reagent kits. The modules feature automated gating, calculations, and report generation to deliver a consistent, reproducible, and standardized analysis.

As a leading provider of tools for flow cytometry, BD Biosciences is committed to offering comprehensive, innovative software solutions, including third-party solutions that support application specific customer requirements.

For example, optional Laboratory Information System (LIS) software easily connects the BD FACSCanto II with an existing customer Laboratory Information System to enable direct, bi-directional transfer of data. The software simplifies laboratory workflow by customizing data reporting, securing validation, and transferring data to reduce manual transcription. The solution automates the process from request to reporting to help reduce errors, improve data quality and laboratory productivity.

Monitoring HIV—Identification and enumeration of lymphocyte subsets

The BD Multitest™ 6-color TBNK kit is the first and only 6-color In Vitro Diagnostic application to provide a complete immune panel in a single tube, saving valuable time and resources for sample processing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percent</th>
<th>Value/AbsCnt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bead Events</td>
<td>1544</td>
<td></td>
</tr>
<tr>
<td>CD3+CD16+56/CD45/CD4/CD19/CD8</td>
<td>66.76</td>
<td>1171.51</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>14.41</td>
<td>252.78</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>52.51</td>
<td>921.30</td>
</tr>
<tr>
<td>CD3+CD4-CD8-</td>
<td>0.62</td>
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<tr>
<td>CD16+CD56+</td>
<td>16.80</td>
<td>294.82</td>
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<tr>
<td>CD19+</td>
<td>15.84</td>
<td>278.01</td>
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<tr>
<td>CD45-</td>
<td>1754.68</td>
<td></td>
</tr>
<tr>
<td>%/8 Ratio</td>
<td>3.64</td>
<td></td>
</tr>
</tbody>
</table>

Total Events: 10068

Parameter

| Lymph Events | 2714 |
| Bead Events | 1544 |
| CD3+ | 66.76 | 1171.51 |
| CD3+CD8+ | 14.41 | 252.78 |
| CD3+CD4+ | 52.51 | 921.30 |
| CD3+CD4-CD8- | 0.62 | 10.99 |
| CD16+CD56+ | 16.80 | 294.82 |
| CD19+ | 15.84 | 278.01 |
| CD45- | 1754.68 |
| %/8 Ratio | 3.64 |

Total Events: 10068
Detection of HLA-B27 Antigen

The BD HLA-B27 module for the BD FACSCanto II system provides a complete system for rapid detection of HLA-B27 antigen, which is clinically relevant to the evaluation of seronegative spondyloarthropathies.

HLA-B27

<table>
<thead>
<tr>
<th>Gated Events</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preset HLA-B27 Marker</td>
<td>145</td>
</tr>
<tr>
<td>Sample HLA-B27 Median</td>
<td>166</td>
</tr>
</tbody>
</table>

Conclusion: HLA-B27 positive sample
Versatile, easy-to-use

A Powerful Platform for Research Use

The BD FACSCanto II system brings BD flow cytometry and multicolor analysis to a new level to help researchers meet complex challenges and advance their research.

BD FACSDiva Software for Setup, Acquisition, and Analysis

BD FACSDiva software efficiently controls the setup, acquisition, and analysis of flow cytometry data from the BD FACSCanto II workstation. BD FACSDiva operating software is common across many BD cell analyzers and cell sorters, including BD™ LSR and BD FACSaria™ systems, giving researchers application flexibility.

BD FACSDiva software enables researchers to preview and record data from multiple samples with an automated acquisition process. Acquisition templates, user-definable experiment layouts, and simple compensation procedures also managed by the software further facilitate acquisition.

For analysis, the software includes powerful features such as hierarchical snap-to gating, a variety of plots, and batch analysis. Recorded data can be analyzed by creating plots, gates, population hierarchies, and statistical views on a BD FACSDiva global worksheet. The global worksheet can be used to analyze multiple sample tubes from the same experiment, to save time. User-definable batch analysis and automated capabilities such as gate resizing, pausing between data files, exporting statistics, and printing before proceeding to the next data file are additional BD FACSDiva features.

Lysed whole human blood

Eight-color immunophenotyping panel using FITC, PE, PerCP-Cy™5.5, PE-Cy™7, APC, APC-Cy7, AmCyan, and Pacific Blue™ fluorophores.
Cytometer Setup and Tracking

BD™ Cytometer Setup and Tracking (CS&T) beads provide, in a single vial reagent format, automated determination of BD FACSCanto II configuration baselines and monitoring of performance over time, using BD FACSDiva software.

BD™ CompBeads Compensation Particle Sets are designed to optimize compensation settings in multicolor flow cytometry. Particularly helpful when using tandem dye conjugates, these particle sets simplify establishing compensation corrections for spectral overlap for any combination of fluorochrome-labeled antibodies.

BD FACSDiva Worksheet

Lysed whole human blood sample in an 8-color panel using V450, AmCyan, FITC, PE, PerCP-Cy5.5, PE-Cy7, APC and APC-H7 fluorophores.
Committed to Customer Success

BD Biosciences is fully committed to the success and satisfaction of its customers. Supporting flow cytometry applications for over 35 years, BD training, support, and field service teams are dedicated to helping customers achieve optimal instrument performance, ease of use, and streamlined workflow. With unmatched flow cytometry experience, this world-class service organization is available to help with your BD FACSCanto II product installation, future upgrades, and application support.

Training
Hands-on training is included with each BD FACSCanto II product. Training courses are held at BD training centers worldwide. BD flow cytometry training courses combine theory and practice to provide participants with the skills and experience they need to take full advantage of the capabilities of their BD FACSCanto II system.

Technical Application Support
BD Biosciences technical applications support specialists are available to provide field- or phone-based assistance and advice. Expert in all aspects of flow cytometry, BD technical application specialists are well equipped to address customer needs in both instruments and applications support.

Field Service
When instrument installation or service is required, a BD Biosciences Technical Field Service Engineer can be dispatched to the customer site. BD Biosciences field service engineers are located across the world. On-site service and maintenance agreements are available to provide long-term support for BD FACSCanto II systems.
BD flow cytometers are Class I (1) laser products.

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