ThermoFisher SCIENTIFIC

Bigfoot Spectral Cell Sorter

Time savings, safety, precision, and flexibility—all in one instrument

invitrogen

Discover the cutting-edge Bigfoot Spectral Cell Sorter

The Invitrogen™ Bigfoot™ Spectral Cell Sorter with Sasquatch Software (SQS) provides the innovation, ease of use, and high-speed cell sorting you need to meet the needs of your lab today and into the future.

Key features:

1. Convenient storage is provided for adapters, spare parts, and tubes to help reduce cluttered lab space.

No external support components are needed. Say goodbye to external water baths, vacuum pumps, and compressors.

2. Hot-swappable, bulk-fluid bottles allow for a full shift of operation or for continuous operation by changing the fluid during a sort. Optional kits are available for connecting to house deionized (DI) water and waste to reduce the need to fill and empty bulk-fluid tanks.

Onboard cleaner, decontamination, and sheath bottles allow for automated system rinsing and cleaning without manual intervention.

3. With a custom-designed, integrated biosafety enclosure and aerosol management system (AMS), the Bigfoot Spectral Cell Sorter provides safety and protection in a compact footprint without compromising high-parameter sorter performance.

4. Multi-tube input paired with 18-way virtual sorting and integrated temperature control gives flexibility for all sorting applications.

Six input positions—the Bigfoot Spectral Cell Sorter enables sampling from 1.5, 5, and 15 mL tubes with automatic tube-type sensing and built-in crash detection

Integrated wash station and on-board calibration beads—the integrated wash station reduces carryover and on-board calibration beads allow programmable, automated start-up and calibration

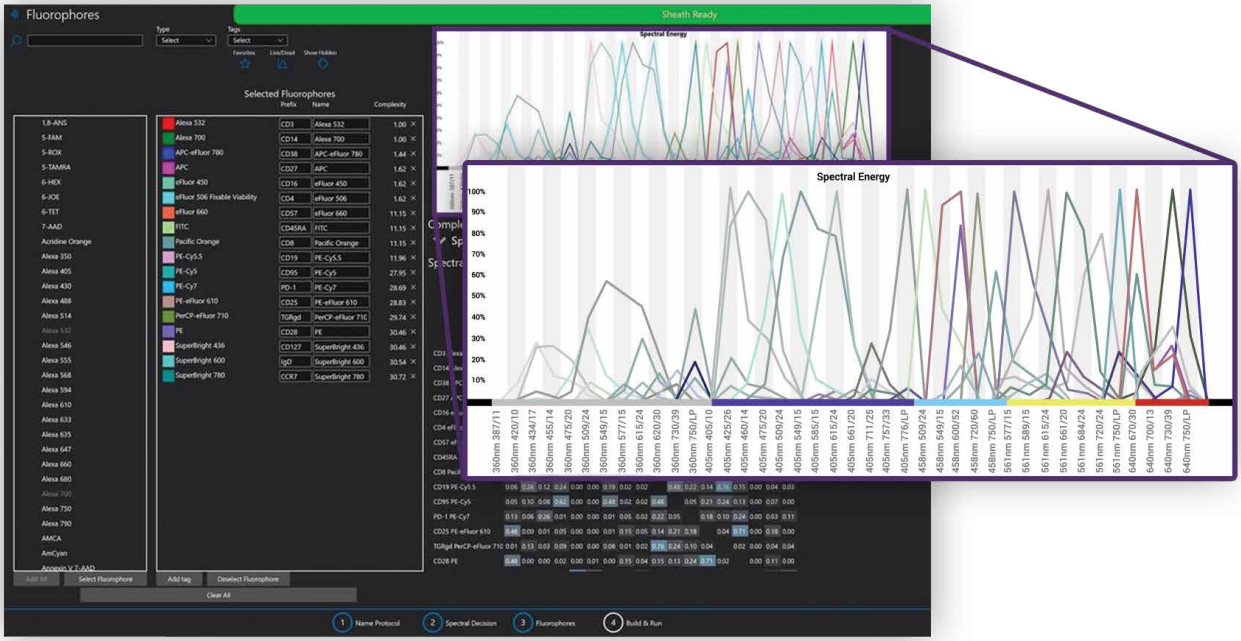
Built-in agitation and temperature control—using the built-in agitation and temperature control (4–37°C), the Bigfoot Spectral Cell Sorter maintains the integrity of your samples from start to finish

5. Custom electronics, designed from the ground up specifically for cell sorting, enable >100,000 events per second (EPS) acquisition and >70,000 EPS sorting, providing unmatched performance.

6. The Bigfoot Spectral Cell Sorter can be configured with up to 9 lasers and 60 detectors, providing the versatility for both standard fluorescence detection and spectral unmixing; multiple scatter options allow simultaneous standard and small-particle detection, multi-laser scatter detection, and/or polarization.

7. SQS provides quick start-up, automated calibration, and accurate quality control (QC) combined with an experiment designer, intuitive interface, and efficient shutdown, making the system easy to use while reducing downtime. Remote access capability, system health information, and email notifications save time and streamline your workflow.

8. Adjustable monitor and keyboard arms provide ergonomically optimized flexibility over multiple heights and positions.



Live spectral unmixing for sorting and analysis

The Bigfoot instrument allows both spectral sorting and spectral analysis on cell populations.

- **Configurable**—the high-end configurations of the Bigfoot Spectral Cell Sorter take advantage of the large number of lasers and detectors and allow spectral unmixing for both analysis and sorting in real time
- **Simple**—the experiment wizard assists the user in running controls, identifying potential issues, and applying the unmixing algorithms to produce high-quality data
- **Flexible**—the Bigfoot Spectral Cell Sorter allows both acquisition and sorting on spectrally unmixed data or conventionally compensated data, streamlining the workflow from panel development to high-speed sorting

High throughput

High speed

Configurable, quick, and calibrated, the high throughput of the Bigfoot Spectral Cell Sorter is in a league of its own: up to 10x faster than the competition.

- **Adaptable**—the Bigfoot system has user-configurable sort output holders for 1.5, 5, 15, and 50 mL tubes; microwell plates of up to 1,536 wells; microscope slides; and even 10x™ chips with integrated temperature control (4–37°C), providing maximum versatility
- **Virtual sorting**—with standard six-way sorting and virtual 18-way sorting, the Bigfoot Spectral Cell Sorter can be used to separate multiple populations—from a single sample or different samples—for walk-away sorting
- **Calibration**—with built-in stream calibration and drop delay as well as media detection, sort setup is simplified for users
- **High throughput**—with sort rates of >70,000 EPS, sorting is fast and configurable; from four-way sorting into 96-well plates, multi-way sorting into 384-well plates, or straight-down sorting into 1,536-well plates, the speed and recovery of the Bigfoot instrument may exceed your expectations
- **Integrated**—from built-in media detection cameras to volume tracking of sorted samples, the integrated features of the Bigfoot Spectral Cell Sorter reduce common errors with cell sorting
- **Jet-in air**—the jet-in-air sensing of the Bigfoot instrument is gentle with fragile cells, which enables the usage of a wide range of nozzle tip sizes while taking advantage of high sort speed and yield

Fluidics

Stable

- **Five-axis automated stream alignment**—ease of use is improved and short- to long-term variability reduced with automated five-axis stream alignment and QC
- **Precise droplet monitoring**—integrated control systems enable optimal droplet formation persistence and position stabilization over time, supporting accurate drop delay maintenance through the day
- **Simplified tip swapping**—designed with ease of use in mind, the nozzle storage station and swap-tip wizard simplify the workflow and help reduce user errors during setup
- **Reduced dead volume**—built-in bubble detection notifies the user and automatically stops sample, reducing dead volume
- **70–150 µm nozzle tips**—supports 70, 100, 120, and 150 µm nozzle tips with varying sheath pressures
- **Hot-swappable sheath tanks**—provide for continuous multi-hour sort runs without interruption

An innovation in dye development

A new paradigm for multicolor flow cytometry

Invitrogen™ NovaFluor™ dyes offer:

- Unique spectral signatures
- Narrow emission
- Minimal cross-laser excitation
- Decreased spillover spread for higher resolution
- Compatibility with today's most powerful instruments

Find out more at thermofisher.com/novafluor-dyes

Integrated aerosol containment

The Bigfoot Spectral Cell Sorter containment system and AMS are designed to be fully integrated parts of the cell sorter. Sample-related subsystems are segregated inside the contained area for optimal safety, sanitation, and performance. Sealed optical windows surround the nozzle, defining the barrier between the inside and outside of the contained area. This separation allows lasers, excitation optics, and scatter objective lenses to remain outside the contaminated zone yet close to the interrogation point, which maintains the superior performance of a jet-in-air sorter. All other subsystems, such as detection, electronics, and fluidics, are also outside the containment area. This allows better service access and temperature regulation as compared to other cell sorters.

Although not an actual biosafety cabinet (BSC), the Bigfoot Spectral Cell Sorter provides personnel and product protection similar to a Class II BSC. Test procedures and criteria laid out within NSF 49 and EN 12469 standards can be utilized to demonstrate performance.

Both NSF 49 and EN 12469 standards require certification while the instrument's hood is empty, which is not the normal use case for this application of biocontainment. The Bigfoot Spectral Cell Sorter is a hybrid Class II enclosure and AMS, able to be certified while in operation to meet the safety and airflow requirements of these standards and cell sorting guidelines. This means the containment system:

- Maintains an average air velocity of 100 ft/min (NSF 49) or >79 ft/min (EN 12469) through the work access opening
- Provides high-efficiency particulate air (HEPA)–filtered downflow air that is mixed with the downflow and inflow air
- Exhausts HEPA-filtered air into either the laboratory or, via an optional canopy connection, through an external exhaust system
- Holds all biologically contaminated ducts and plenums under negative pressure

The sliding sash on the Bigfoot system's integrated biosafety enclosure allows users access to the internal workspace, including the nozzle and sample lines, while maintaining consistent airflow into the instrument.

Electronics

Powerful

With custom electronics, firmware, and software designed specifically for high-performance sorting, the Bigfoot Spectral Cell Sorter has power and flexibility.

- **Accurate**—proprietary electronics simultaneously collect high dynamic range data for measured peak, area, and width for every channel to accurately characterize your sample
- **High-end performance**—the massively parallel, pipelined architecture eliminates hard aborts and allows complex, high-color experiments with up to a 60 x 60 compensation matrix, or spectral unmixing without limiting instrument performance
- **Zero dead time**—dynamic window extension supports full data collection for every sample
- **Automatic laser delay**—without user interaction, the electronics automatically configure the optimal laser delay for different nozzle sizes and pressures
- **Sort logic**—flexible configuration allows for independent sort logic setups built from 64 total bivariate gates of up to 512 x 512 resolution, along with multiple modes for purity, enrichment, and drop envelopes

Optics

Consistent path lengths, stable optical filter layouts, and highly sensitive photomultiplier tube (PMT) detectors optimize detection across the entire spectrum.

Flexible

The optical platform of the Bigfoot system has flexible laser options and optimized filter sets.

- **Flexible**—the Bigfoot Spectral Cell Sorter offers free space excitation of up to nine lasers into seven pinholes ranging from 349 nm to 785 nm, allowing flexible wavelength selection for your multicolor experiments
- **Stable**—integrated beam shaping and short path lengths maintain optical stability day to day
- **Configurable**—with up to 60 detectors, the Bigfoot instrument can adapt to your multicolor applications while still allowing optical filter changes for future needs

Spectral and conventional flow cytometry panel builder

Design your panel using the Invitrogen™ Flow Cytometry Panel Builder, offering:

- A quick and intuitive workflow
- Incorporation of antibodies
- Fluorophore selection built on spectral visualization of all fluorophores per laser

Find out more at thermofisher.com/flow-cytometry

Application data

23-color immunophenotyping data

An immunophenotyping panel is a useful tool for monitoring disease and therapy progress. It is used to divide a cell sample into subsets and determine what percentage of the total leukocyte pool each subset represents. In the past, parallel panels would have to be run on the same cell sample to successfully phenotype T regulatory cells, natural killer (NK) cells, NKT cells, monocytes, macrophages, granulocytes, B cells, and T cells. The more parameters that can be analyzed in a single sample, the further the cells can be divided into helper, memory, activated, senescent, and functional cell subsets. This data set was obtained with PBMCs that were isolated by Ficoll™ polymer gradient separation from whole blood obtained from a healthy donor.

The Bigfoot Spectral Cell Sorter was used with seven lasers in the following configuration: 349 nm/405 nm/445 nm/488 nm/561 nm/640 nm/785 nm. The 445 nm laser in this configuration is unique in that it can excite Brilliant Ultra Violet™ 496 and Brilliant Violet™ 480 dyes for added spectral dimension, and the 785 nm laser can be useful for discriminating between live and dead cells as well as direct excitation of APC/Fire™ 810 dye (Table 1). There is a PMT detector for each channel, and the filter configuration for each laser enables rapid and accurate unmixing during cell sorting. Because of these features and the jet-in-air sensing process to interrogate the stream as it exits the nozzle, the flow cytometry panel must be optimized for the Bigfoot Spectral Cell Sorter as opposed to an instrument that employs avalanche photodiodes or cuvette-based interrogation of cells in the stream.

Table 1. Composition of the 23-color immunophenotyping panel.

Dye	Marker	Dye	Marker
Brilliant Ultra Violet 395	CD14	Super Bright 702	CD127
Brilliant Ultra Violet 496	CD16	Super Bright 780	CD20
Brilliant Ultra Violet 563	CD8	FITC	HLA-DR
Brilliant Ultra Violet 615	PD-1	PE	CD25
Brilliant Ultra Violet 661	CD38	PE/Dazzle 594	CD197
Brilliant Ultra Violet 737	CD4	PE-Cyanine5	CD56
Super Bright 436	CD45RA	PE-Cyanine7	CD196
Pacific Blue	CD123	Allophycocyanin (APC)	CD27
Brilliant Violet 480	CD185	NovaFluor Red 710	CD19
Brilliant Violet 510	IgM	APC-eFluor 780	CD3
Brilliant Violet 570	CD8	APC/Fire 810	CD15
Super Bright 600	IgD		

Isolating singlets from doublets

The first step is isolating singlets from doublets using forward scatter A (FSC-A) and forward scatter H (FSC-H) plots (Figure 1). The backbone of our immunophenotyping panel consisted of the CD3 marker for T cells, the CD19 marker for B cells, the CD56 marker for NK cells, and the CD14 marker for monocytes. However, to ensure the subset populations are as pure as possible, CD11c, CD20, and HLA-DR markers can be used for additional back-gating. The CD11c, CD20, CD56, and CD14 markers can be removed for T cell gating. The CD56 and CD14 markers can be removed for B cell gating, and the CD3, CD56, and CD20 markers can be removed for monocyte gating. If more discriminatory NK cell gating is desired, starting with a CD3 and CD56 gating strategy will help you distinguish NK cells from NKT cells. Additional T cell, B cell, and monocyte markers can then be removed from the analysis.

Selecting primary-tier markers

The CD4 and CD8 markers can also be considered primary-tier analytical markers for $CD3^+$ T cell analysis that excludes B cell, monocyte, and NK markers (Figure 2). Primary-tier markers should be paired with fluorophores in the dim range of the index staining spectrum for the Bigfoot Spectral Cell Sorter if the instrument is less sensitive for a particular wavelength. Fluorescence minus one (FMO) controls should be used to verify the accuracy of population gating. This is because the expanding range of spreading error can become problematic with high-level multicolor combinations and have a negative impact on population resolution. For example, $CD4^+$ regulatory T cells are also $CD25^+$ and have a low-expression or negative CD127 cell surface marker phenotype. Memory T cell subsets exhibit transitional CD45RA and CD197 expression patterns. Analysis of either population would benefit from the use of bright fluorophores that have no significant degree of spectral spillover. Naive T cells are $CD45RA^+$ and $CD197^+$. Memory T cell subsets are $CD45RA^-$ and $CD197^{+/-}$, while effector T cell subsets are $CD45RA^+$ and $CD197^-$. Additional helper T cell subsets can be deduced from the memory T cell subsets.

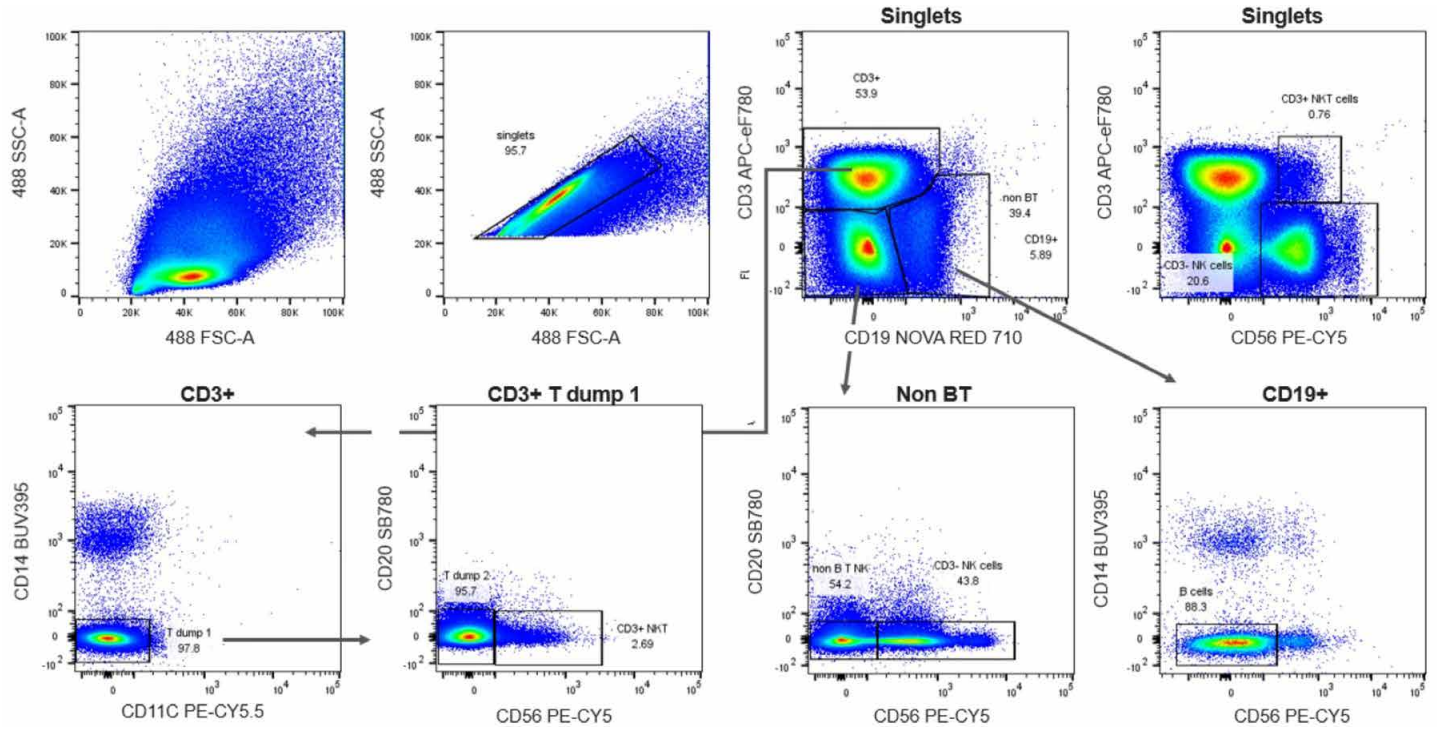


Figure 1. Gating for lymphocytes in the human PBMC population. The backbone panel included Invitrogen™ eBioscience™ CD3 APC-eFluor™ 780, CD19 NovaFluor Red™ 710, CD56 PE-Cyanine5, CD14 Brilliant Ultra Violet™ 395, and CD20 Super Bright™ 780 antibody conjugates to delineate the monocyte, NK cell, T cell, and B cell subsets.

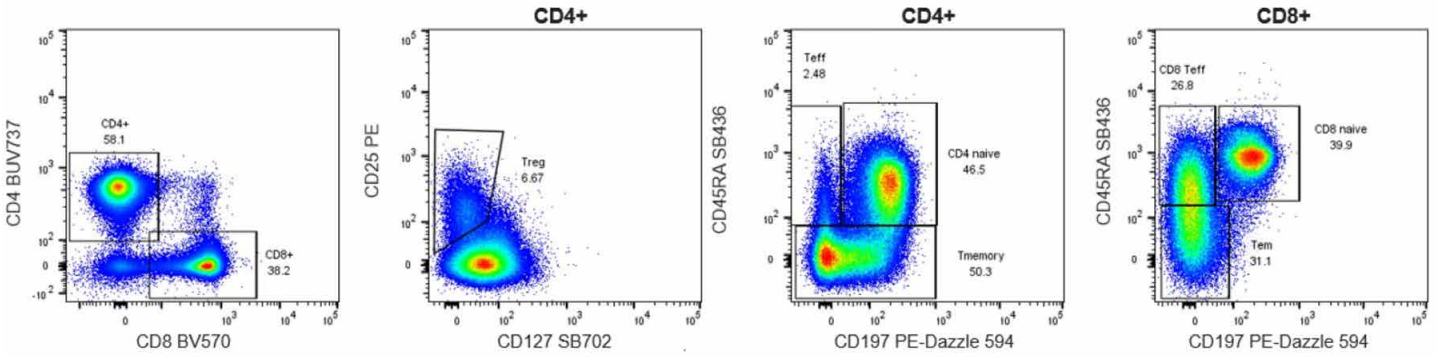


Figure 2. Gating for CD3⁺ T cells. Invitrogen™ eBioscience™ CD197 PE/Dazzle™ 594, CD45RA Super Bright™ 436, CD25 PE, and CD127 Super Bright™ 702 antibody conjugates for transitional markers were used to delineate a second tier of CD3⁺ T cell subsets that included regulatory and memory T cells.

Phenotyping of T helper cell subsets

The performance of the Bigfoot Spectral Cell Sorter does not appear to be affected whether the unmixing controls are prepared with compensation beads or cells. Invitrogen™ UltraComp eBeads™ Plus Compensation Beads were used as unmixing controls for this experiment. The default double wash setting was selected to minimize carryover between the controls. If your samples or fluorophores are particularly sticky, increase the duration of the wash steps.

T helper cell subsets consist of functionally and phenotypically distinct memory T cells. Th1 and Th2 cells are functionally important for fighting microbial and parasitic infections, respectively, and are characterized by CD183, CD194, and CD197 expression. The Th9 and Th22 subsets are functionally important in allergic reactions and intestinal homeostasis, and they are characterized by CD196 and CCR10 expression.

T follicular helper (Tfh) cells are instrumental for B cell function and are characterized by CD185 expression. Th17 cells are important for host protection against extracellular bacteria and fungi, and they are characterized by CD194 expression. However, detection of intracellular cytokines and transcription factors would be necessary to establish a definitive functional phenotype.

T cell subset activity

Analysis of transitional activation, senescence, and proliferation markers can benefit from the use of bright and spectrally distinct fluorophores. The data in Figure 3 show a range of immature (positive) and mature (negative) CD38 expression by CD4+ cells. When CD38 is plotted against HLA-DR, the presence of CD4+ CD38+ cells that exhibit MHC class II expression indicates the presence of an activated T cell subset.

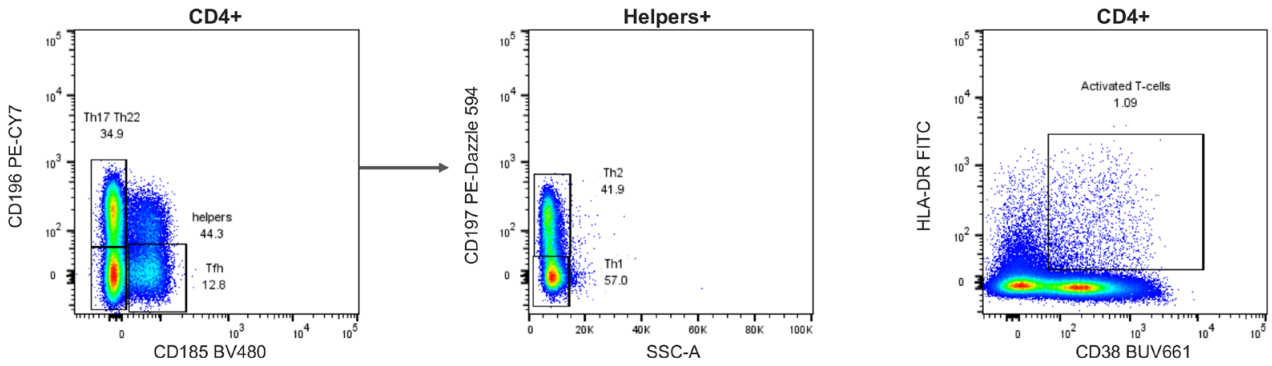


Figure 3. Gating for CD4+ T helper cell subsets. The second-tier markers CD196, CD185, and CD197 on CD4+ T helper cell subsets were detected with Invitrogen™ eBioscience™ CD196 PE-Cyanine7, CD185 Brilliant Violet™ 480, and CD197 PE/Dazzle™ 594 antibody conjugates. Invitrogen™ HLA-DR FITC and Invitrogen™ eBioscience™ CD38 Brilliant Ultra Violet™ 661 antibody conjugates were used to delineate activated T cells.

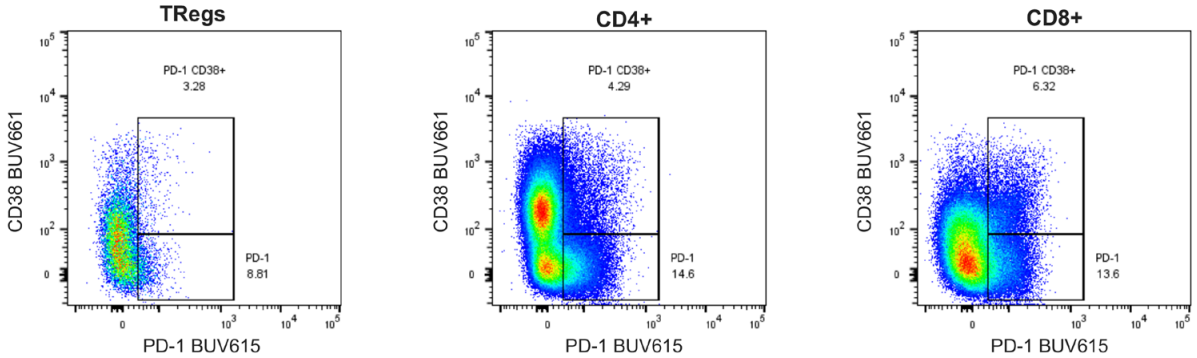


Figure 4. Analysis of PD-1 expression. Invitrogen™ eBioscience™ CD38 Brilliant Ultra Violet™ 661 and PD-1 Brilliant Ultra Violet™ 615 antibody conjugates were used to detect third-tier expression markers with variable expression on regulatory, CD4+, and CD8+ T cells. Antibody staining was performed using Invitrogen™ eBioscience™ Super Bright™ Complete Staining Buffer, and FMO controls were used as gating controls.

Checkpoint markers as a measure of immune function

Programmed cell death protein 1 (PD-1) has an important role in immune regulation. PD-1 functions as an apoptosis regulator to maintain immune balance and inhibit autoimmunity. It can promote apoptosis of antigen-specific T cells and inhibit apoptosis of regulatory T cells. PD-1 is an important immune checkpoint protein with therapeutic indications. Normal non-activated $CD4^+$ and $CD8^+$ T cells exhibit low expression of PD-1 and can be difficult to resolve. Poor resolution can also be compounded by spreading error from spectral spillover. We recommend using a bright fluorophore with limited spectral spillover like Brilliant Ultra Violet™ 661 or Brilliant Ultra Violet™ 615 (Figure 4).

B cell subset phenotyping

B cell subsets are extremely biologically diverse. Even CD20, which is a common marker for basic B cell phenotyping, is not always expressed on certain subsets in the B cell lineage like pre- and pro-B cells and plasma cells. CD19, CD20, CD27, CD38, IgD, IgM, CD10, and HLA-DR are basic markers for B cell subsets. Delineation with this combination of markers based on cell maturity, transitional state, activation, and class switching is possible (Figure 5). Of particular interest might be class switching between naive $CD27^+$ cells that produce IgD and mature $CD38^+$ cells that produce IgM. Each subset is functionally important in the life cycle of B cells.

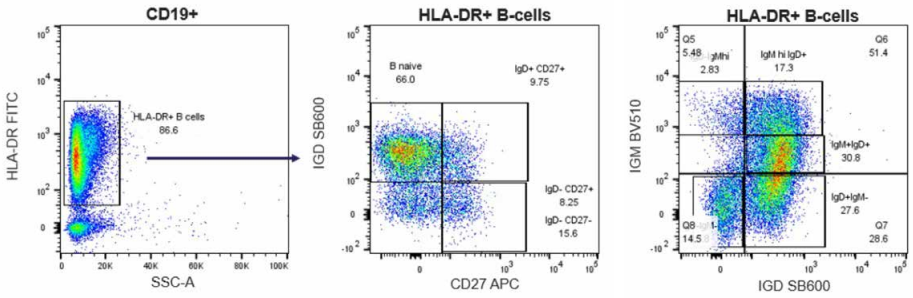


Figure 5. Gating for B cells. Common phenotypic markers for B cell subsets were detected using an HLA-DR FITC antibody and Invitrogen™ eBioscience™ IgD Super Bright™ 600, CD27 APC, and IgM Brilliant Violet™ 510 antibody conjugates.

The non-B, non-T cell population could be further resolved using the CD15 marker to exclude any contaminating granulocytes and the CD56 marker to differentiate NK cells (Figure 6). NK cells have functional subsets that are defined by CD16 expression. The non-B, non-T, and non-granulocyte population can be refined using HLA-DR, which is an MHC class II marker that is expressed on antigen-presenting cells like monocytes. Classical and non-classical monocyte subsets can be defined by their CD14 and CD16 expression patterns.

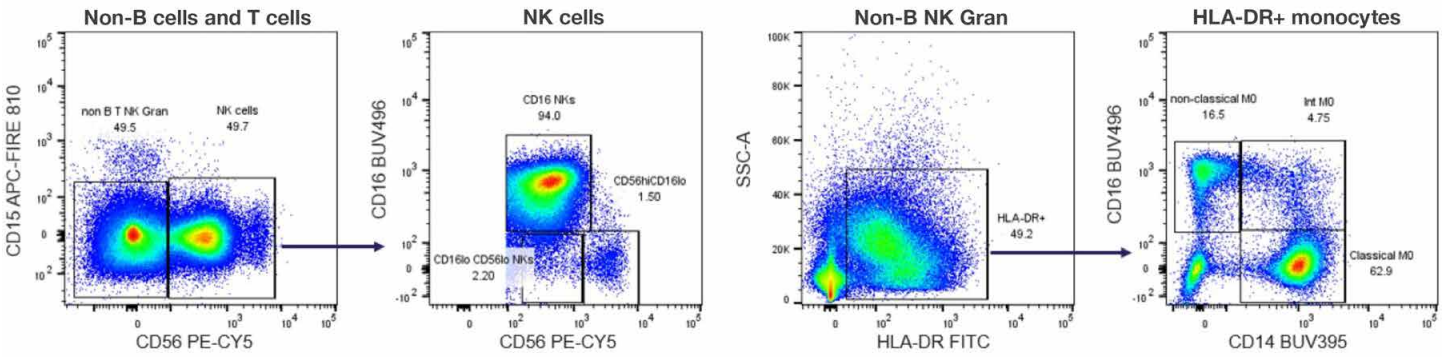


Figure 6. Gating for NK cells and monocytes. A CD15 APC/Fire 810 antibody conjugate was used to exclude contaminating granulocytes from the analysis. NK cells were differentiated using an Invitrogen™ eBioscience™ CD56 PE-Cyanine5 antibody conjugate, based on high or low CD56 expression, as well as an Invitrogen™ eBioscience™ CD16 Brilliant Ultra Violet™ 496 antibody conjugate. An HLA-DR FITC antibody was used to identify antigen-presenting cells that could be further resolved into classical, nonclassical, and intermediate monocytes based on transitional expression of CD16 and CD14.

For the complete data set, visit **thermofisher.com/bigfootdata**

AB Assurance Service Plan

Preferred coverage for peace of mind

Our technical services, field engineering, and training teams are fully committed to aiding in your success using the Bigfoot Spectral Cell Sorter for your research. Instrument service plans, consulting, and training programs are designed to help ensure instrument performance, team readiness, and overall optimal research outcomes using the system.

	AB Assurance
Response time	2 business days*
Planned maintenance	✓
Access to technical support (Monday–Friday, standard business hours)	✓
Parts, labor, and travel	✓
Qualification service	Available as add-on
Field application scientist (FAS) consultation	Available as add-on

* Availability limited in some geographic areas.

Service contracts for the Bigfoot Spectral Cell Sorter

Description	Cat. No.
AB ASSURANCE, BIGFOOT 9 L	ZG11SCBIGFOOT9L
AB ASSURANCE, BIGFOOT 7 L	ZG11SCBIGFOOT7L
AB ASSURANCE, BIGFOOT 6 L	ZG11SCBIGFOOT6L
AB ASSURANCE, BIGFOOT 5 L	ZG11SCBIGFOOT5L
AB ASSURANCE, BIGFOOT 4 L	ZG11SCBIGFOOT4L

Configurations

All configurations of the Bigfoot Spectral Cell Sorter offer two or more forward- and side-scatter channels in addition to the fluorescence channels shown in the tables.

Configurations offering both spectral analysis and conventional compensation

Number of lasers	Number of fluorescence detection channels for included lasers									Total detection channels	Cat. No.
	UV (349 nm)	Violet (405 nm)	Blue-violet (445 nm)	Blue (488 nm)	Green (532 nm)	Yellow-green (561 nm)	Red (594 nm)	Far red (640 nm)	Near-IR (785 nm)		
5	12	12		7		12		5		53	PL00302
6	12	12		7		12		5	3	56	PL00301
6	12	12	4	7		12		5		57	PL00300
7	12	12	4	7		12		5	3	60	PL00299
9	12	12	7		12		4	4	3	60	PL00285

Configurations offering only conventional compensation

Number of lasers	Number of fluorescence detection channels for included lasers									Total detection channels	Cat. No.
	UV (349 nm)	Violet (405 nm)	Blue-violet (445 nm)	Blue (488 nm)	Green (532 nm)	Yellow-green (561 nm)	Red (594 nm)	Far red (640 nm)	Near-IR (785 nm)		
4		7		7		7		4		27	PL00304
5	7	7		5		7		4		35	PL00303

Learn more at **thermofisher.com/bigfoot**