ThermoFisher SCIENTIFIC

Bigfoot Spectral Cell Sorter

Speed, precision, flexibility, and safety—all in one instrument

invitrogen

Sample handling

Discover the cutting-edge Bigfoot Spectral Cell Sorter

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

Key features:

1. 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.

2. Convenient storage is provided for adapters, spare parts, and tubes to help declutter lab space.

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

3. Hot-swappable, bulk-fluid bottles allow for a full shift of operation or for continuous operation by changing the fluid during a sort.

Onboard cleaner, decontamination, and sheath fluid bottles allow for automated system rinsing and cleaning.

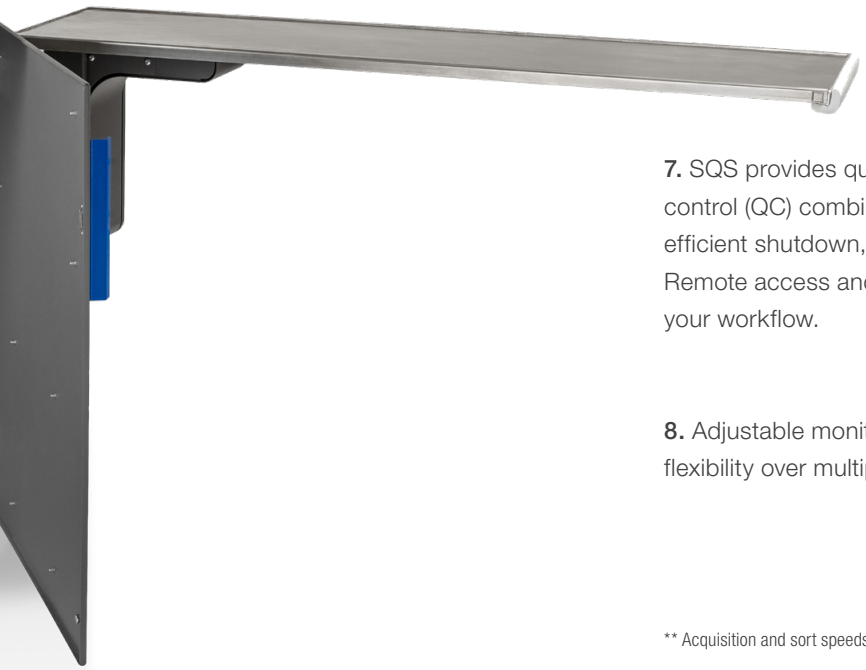
4. With a custom-designed, integrated biosafety enclosure and aerosol management system (AMS), the Bigfoot Spectral Cell Sorter provides safety and protection in a compact footprint without compromising high-parameter sorter performance.

* 532 nm and 594 nm lasers are not used in spectral unmixing experiments.

5. Multi-tube input paired with 18-way virtual sorting and integrated temperature control gives flexibility for many 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 onboard calibration beads**—the integrated wash station reduces carryover and onboard calibration beads enable programmable, semi-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 helps maintain the integrity of your samples from start to finish

6. 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 exceptional performance.



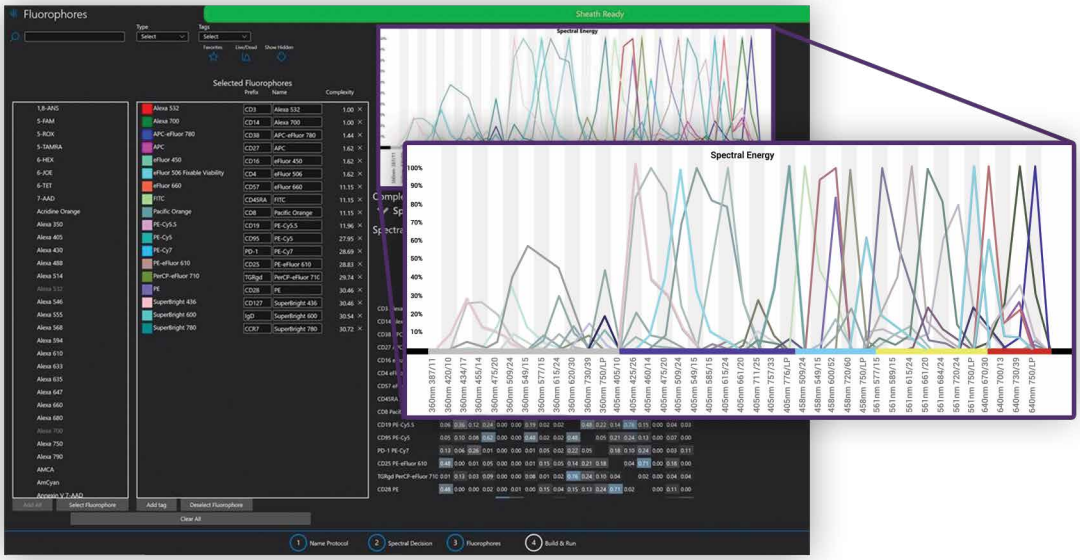
7. SQS provides quick start-up, automated calibration, and accurate quality control (QC) combined with an experiment designer, intuitive interface, and efficient shutdown, making the system easy to use while reducing downtime. Remote access and system health information help save time and streamline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

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

** Acquisition and sort speeds are application-dependent.

Spectral unmixing

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
- **Easy to operate**—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

"The tech support experience is probably the best I've encountered in any of my work. The instrument itself is a pleasure to work with, and it's fun to watch people new to flow in my lab take to it quickly and naturally. I'm really proud of the science we're doing here—working in brain cancer, autoimmunity, leukemia, Alzheimer's, and HIV—and so glad that it's powered by the Bigfoot Spectral Cell Sorter and your team. I am so happy with the experience."

—Pratip Chattopadhyay, CEO, Talon Biomarkers

High throughput
High speed

Quick and configurable, the Bigfoot Spectral Cell Sorter offers high throughput that's in a league of its own.

- **Adaptability**—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 6-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, sort setup is simplified for users
- **High throughput**—with sort rates of >70,000 EPS, sorting is fast and configurable; from 4-way sorting into 96-well or 384-well plates or straight-down sorting into 1,536-well plates, the speed and recovery of the Bigfoot instrument outperforms other cell sorters
- **Integration**—with built-in cameras and volume tracking of sorted samples, the integrated features of the Bigfoot Spectral Cell Sorter help 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

High-throughput plate sorting

Plate sorting is accomplished by movement of a stage that holds the plate, positioning it directly under a sort stream that will deliver a cell into each well. Because of the requirement for precise targeting, the plate deposition mechanism requires careful calibration and alignment. The Bigfoot Spectral Cell Sorter is a cutting-edge, high-parameter instrument that features a multitude of cell sorting advancements. Innovations in plate deposition enable unprecedented accuracy, recovery, and speed down to single-cell sorting. These improvements include built-in stream calibration and drop delay, built-in media imaging that permits accurate plate setup and verification, highly robust hardware for precise single-droplet targeting with minimal adjustment, and a straight-down sorting option for maximum deposition accuracy. In addition, the sort output hardware facilitates maximum flexibility, permitting deposition into plates with up to 1,536 wells and even nonstandard devices like 10x Genomics chips. Finally, and most impressively, the Bigfoot Spectral Cell Sorter is capable of 4-way sorting into 96-well and 384-well plates. This multiway plate sorting feature is unavailable on any other cell sorter; it enables **11-second sorting into 96-well plates, or 20-second sorting into a 384-well plate**, far surpassing other currently available hardware.

Figure 1. Test pattern with alignment targets of four-way plate sorting mode. (A) 96-well plates and **(B)** 384-well plates.

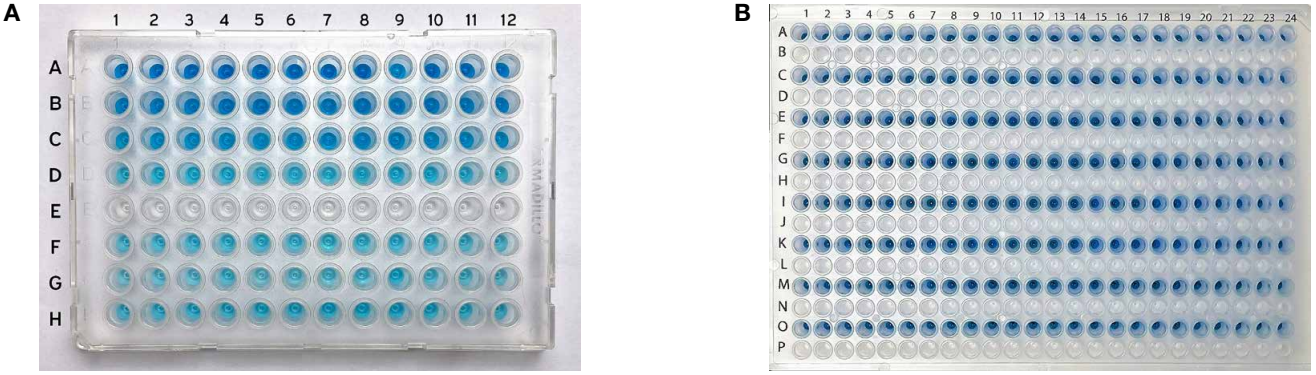


Figure 2. Accurate droplet deposition. Images show 96-well and 384-well PCR plates following multi-droplet event sorting suspended in HRP solution in a 96-well and a 384-well cell culture–treated plate. **(A)** In a 96-well plate, wells in rows A–D received 4, 3, 2, and 1 droplet, respectively, as evidenced by blue gradient from strong to pale. Wells in row E received no droplets (confirmed by no color), while rows F–H received 1 droplet per well. **(B)** In a 384-well plate, odd rows (starting with A) received one droplet resulting in a blue color, while even rows (starting with B) received no droplets and were colorless.

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 a swap-tip wizard help guide users 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 fluid tanks**—provide for continuous multi-hour sort runs without interruption

Biosafety
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 exceptional 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 compared to other cell sorters.

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.

The biosafety enclosure on the Bigfoot Spectral Cell Sorter provides personnel and product protection similar to a Class II biosafety cabinet (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

Electronics
Powerful

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

- **Accuracy**—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
Flexible

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

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 9 lasers into 7 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

High-parameter immune cell sorting
Sample staining

An immunophenotyping panel is a useful tool for monitoring immune responses to external and internal stimuli. It is used to divide a cell sample into subsets and determine what percentage of the total leukocyte pool each subset represents.

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. Here, we use a 28-color mouse immunophenotyping panel to demonstrate the spectral unmixing and sorting capability of the Bigfoot Spectral Cell Sorter to resolve fluorescent signals accurately and precisely, sort six ways simultaneously with great efficiency, and produce highly pure sorted samples. This broad 28-color immunophenotyping panel identifies immunologically relevant populations present in lymphoid organs of general interest to the scientific community and was created following basic panel-building precepts.

Spleen tissues from specific pathogen-free (SPF) BALB/c mice were processed into single-cell suspension samples, stained, and fixed for acquisition on a six-laser Bigfoot Spectral Cell Sorter. The instrument was configured with 349 nm, 405 nm, 445 nm, 488 nm, 561 nm, and 640 nm lasers, fitted with a 100 µm nozzle, and samples were run at 30 psi in purity sort mode. Table 1 lists the full immunophenotyping panel used for sample staining.

Table 1. 28-color immunophenotyping panel.

Laser+detector	Filter	Fluorophore	Antigen	Clone
349 UV-1	387/11	Brilliant Ultra Violet 395	CD25	PC61
349 UV-6	507/19	Brilliant Ultra Violet 496	CD19	eBio1D3
349 UV-8	575/15	Brilliant Ultra Violet 563	CD44	IM7
349 UV-9	615/24	Brilliant Ultra Violet 615	CD11c	HL3
349 UV-10	670/30	Brilliant Ultra Violet 661	CD93	AA4.1
349 UV-12	750/LP	Brilliant Ultra Violet 805	B220	RA3-6B2
405 V-1	420/10	Brilliant Violet 421	Siglec H	551
405 V-3	455/14	eFluor 450	CD21	eBio4E3
405 V-5	507/19	Brilliant Violet 510	CCR2	475301
405 V-7	575/15	Brilliant Violet 570	CD8	53-6.7
405 V-8	615/24	Super Bright 600	I-A/I-E	M5/114.15.2
405 V-9	661/20	Brilliant Violet 650	Ly6G	1A8
405 V-10	710/20	Super Bright 702	IgD	11-26c
405 V-11	747/33	Brilliant Violet 750	CD4	GK1.5
405 V-12	770/LP	Super Bright 780	CD11b	M1/70
445 VB-1	465/22	Brilliant Violet 480	CD62L	MEL-14
488 B-1	507/19	FITC	TER-119	TER-119
488 B-3	583/30	LIVE/DEAD Olive	Dead cells	
488 B-6	720/60	PCPeF710	CD1d	1B1
488 B-7	750/LP	RealBlue 780	CD24	M1/69
561 Y-1	575/15	PE	CD127	A7R34
561-Y-3	605/15	PEeF610	IgM	II/41
561 Y-5	661/20	PE-Cy5	CD3	17A2
561 Y-7	700/13	PE-Cy5.5	CD49b	DX5
561 Y-9	760/50	PE-Cy7	CD23	B3B4
637 R-1	670/30	APC	CD335	29A1.4
637 R-2	700/13	NovaFluor Red 700	CD45	30-F11
637 R-5	770/LP	APCeF780	Ly6C	HK1.4

Gating strategy

Before identifying populations of interest for sorting and analysis, it is crucial to exclude artifacts of sample and instrument processing from the data. The first step in this gating strategy is to isolate singlets from doublets using side scatter area (SSC-A) versus side scatter height (SSC-H) parameters. Next, cell and tissue debris are further excluded by scatter gating, forward scatter area (FSC-A) versus SSC-A, followed by the exclusion of dead cells that are stained with Invitrogen™ LIVE/DEAD™ Fixable Olive viability dye. The TER-119 marker is used to exclude remaining erythroid cells after lysis. All CD45-pos cells that are also TER-119-neg are selected to continue gating.

Next, neutrophils are identified based on the positive expression of both Ly6G and Ly6C, and gating analysis is continued on the Ly6G-neg cells. These Ly6G-neg cells are then separated based on their expression of Siglec-H and Ly6C for the identification of cellular subsets as shown in Figure 3.

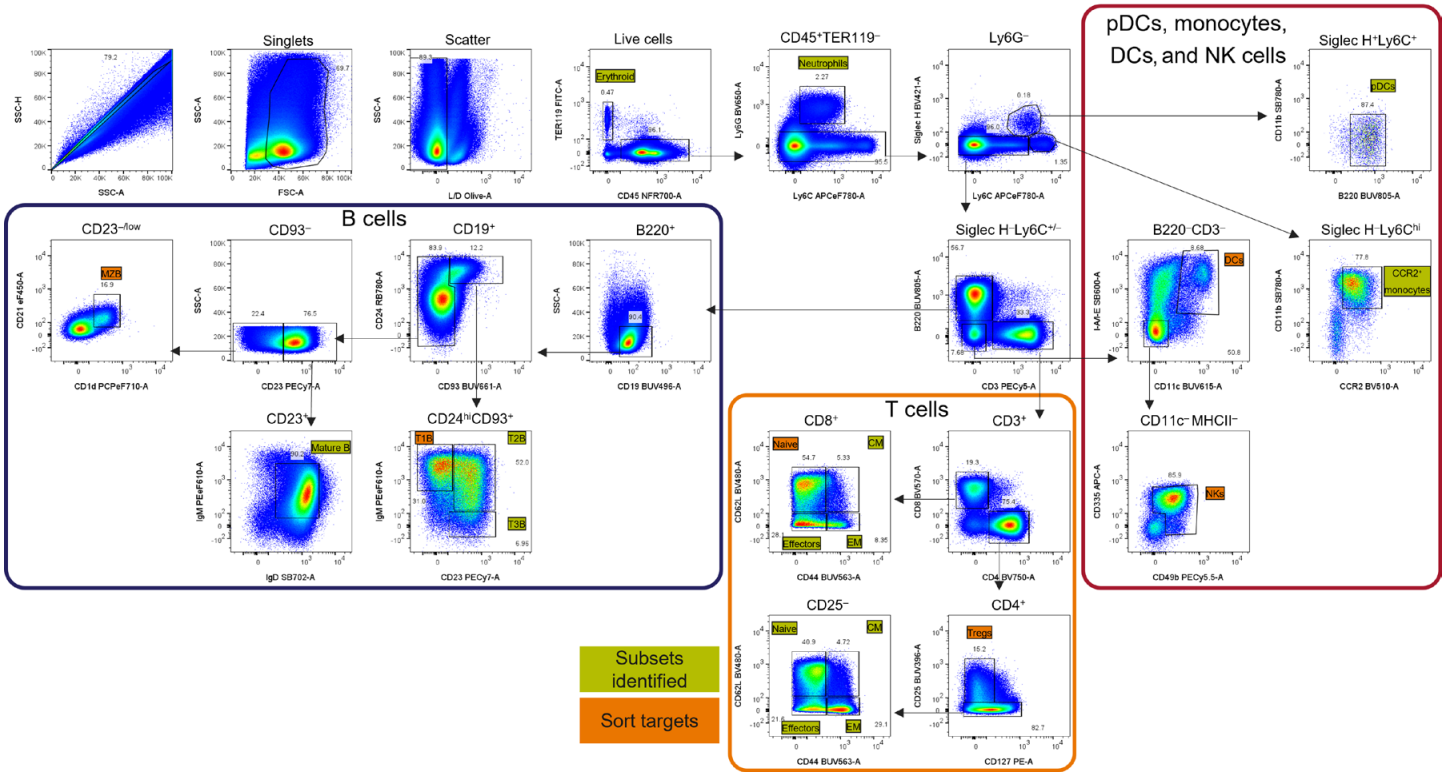


Figure 3. Cell gating for subset identification and sorting for the spleen sample. Sequential gate progression starts at the top and moves from left to right. When more than one population is identified in the same plot, arrows are used to delineate gate progression for the different subpopulations. Green shapes represent the final gating strategy for identified subsets, which include live erythroid cells, neutrophils, pDCs, CCR2+ monocytes, TCM cells, TEM cells, TEff cells, T2B cells, T3B cells, and mature IgM+ B cells. Orange shapes represent the six sort targets, which include DCs, NK cells, Treg cells, CD8+ naive T cells, T1B cells, and MZB cells.

Note: pDC = plasmacytoid dendritic cells, TCM = central memory T cells, TEM = effector memory T cells, TEff = T effector cells, T2B = translational 2 B cells, DCs = dendritic cells, NK = natural killer cells, Treg = regulatory T cells, T1B = translational 1 B cells, and MZB = marginal zone B cells.

Sorting efficiency, recovery, and purity

Efficiency is defined as the reported number of cells sorted by the cell sorter as a percentage of the number of target cells present in the sample. We evaluated sorting efficiency across three separate experiments with the same target populations. The sort numbers varied within the three independent experiments due to the amount of sample available for staining, while the frequency of the sort targets within the sample was consistent (MZB cells = 1.5%, CD8+ naive T cells = 2.5%, NK cells = 2.7%, Treg cells = 3.4%, T1B cells = 1.9%, and DCs = 0.38%). In each sample, the flow rate was adjusted as needed to maintain an event rate of 2,200–2,800 events per second during sorting. Figure 4A shows the efficiency results across these three experiments and highlights the consistency of this parameter.

Recovery refers to the proportion of desired cells in the sorted sample compared to the number the instrument indicates as sorted events. Recovery is an effective measure of sort performance since it considers the drop delay calculation and provides the actual yield of the isolation. To calculate recovery after sorting, the total volume of sample was verified for each sorted tube by subtracting the weight of the empty tubes before the sort from the weight of the tubes after the sort. Following volume measurement, recovery was assessed by acquiring the sorted samples on the Invitrogen™ Attune™ NxT Flow Cytometer. The samples were gated on scatter and the cell concentration of each sample (events/µL) recorded. Adjusted sorted cell numbers were obtained by multiplying the cell concentration by the sample volume. Lastly, the number of target cells in the sorted tube was divided by the number of target cells the instrument indicated it sorted. The calculated recovery percentages are displayed in Figure 4B.

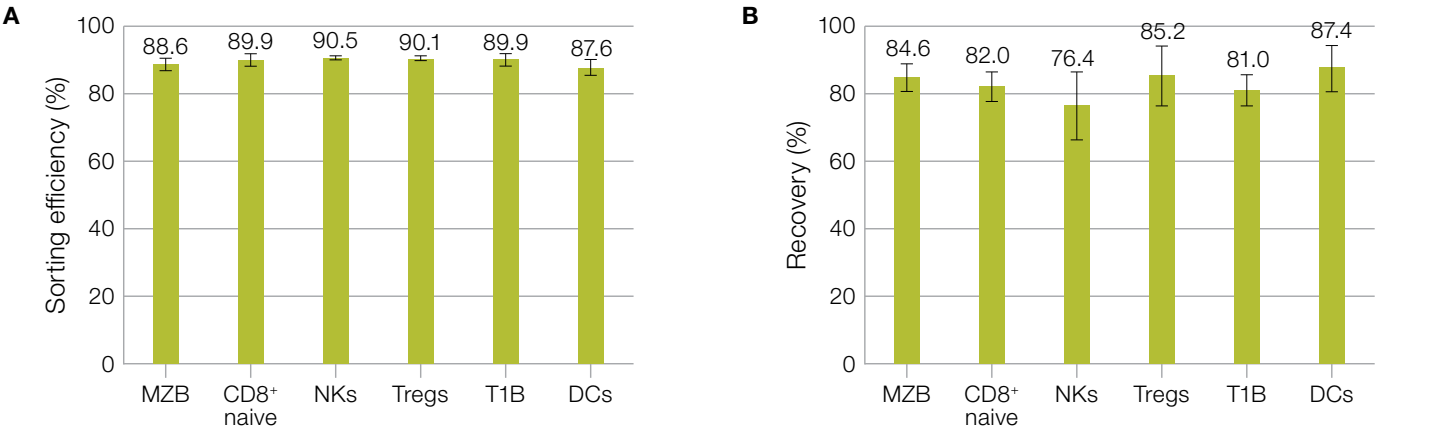


Figure 4. Sorting efficiency and recovered cell percentages for mouse spleen cells.*

(A) Sorting efficiency. Samples were acquired at a constant flow rate to maintain a speed of 2,200–2,800 events per second while sorting. Sorting priority index logic was based on the target population frequency on the spleen sample, with the less-abundant events receiving the highest priority. DCs were less abundant in the spleen sample and were therefore placed in the right-3 position with the highest priority, followed by the MZB placed in the left-3 position. The T1B were placed in right-2, CD8+ naïve in left-2, Tregs right-1, and NKs left-1.

(B) Percentage of sorted cells that can be recovered for downstream analysis/applications. Recovery was assessed by acquiring the sorted samples on an Attune NxT Flow Cytometer, gating the sample on scatter and retrieving the cell concentration of the sorted samples (events/µL). Sample volume was obtained by subtracting the weight of the empty tubes before sort from the weight of the tubes after sort. Adjusted sorted cell numbers were obtained by multiplying cell concentration to sample volume. The number of target cells in each sorted tube was divided by the number of target cells the Bigfoot instrument showed as sorted for each tube. The result is the calculated recovery percentage for each tube.

* Data are represented as mean ± standard deviation for three independent experiments.
Note: MZB = marginal zone B cells, NKs = natural killer cells, Tregs = regulatory T cells, T1B = transitional 1 B cells, and DCs = dendritic cells.

Purity is defined as the target cells found as a percentage of the total cell number in the sorted tube. Determination of post-sort purity requires a flow cytometric analysis of the collected sample, which was performed on the Bigfoot instrument (Figure 5).

The Bigfoot Spectral Cell Sorter can resolve high-dimensional data by unmixing the spectral signatures of overlapping dyes. This allows greater panel expansion and consequently increases the amount of information that can be gathered from each sample. We have demonstrated that this 28-color panel can be used to identify up to 20 different populations from one sample. From those populations, the Bigfoot instrument can sort six ways simultaneously, with high efficiency and purity, including several rare subsets from three tissue types.

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

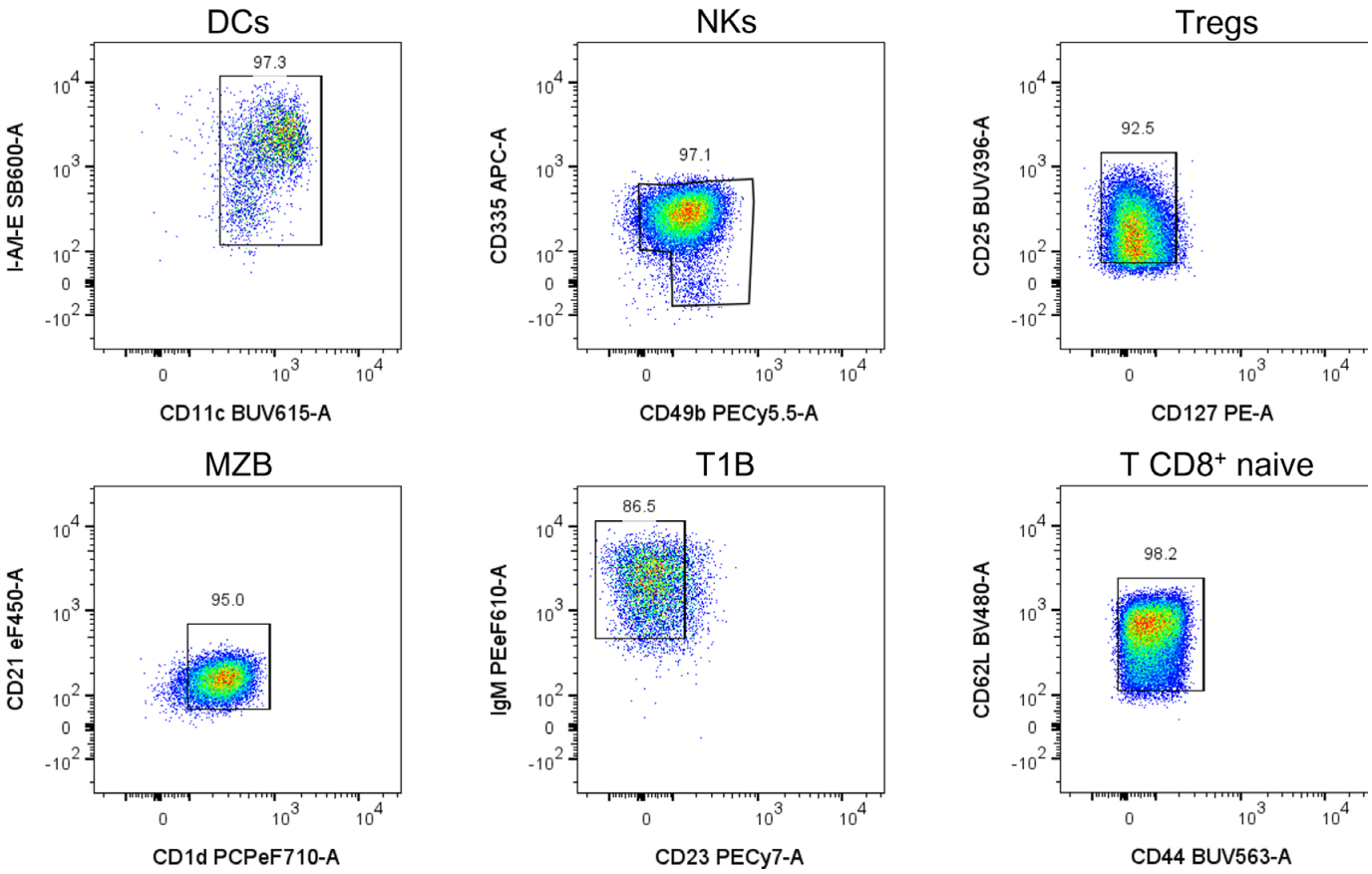


Figure 5. Purity check of sorted spleen cells. After sorting and assessing total sort numbers, the samples were rerun on the Bigfoot instrument for purity assessment. The gates were not adjusted for the post-sort purity assessment.

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.

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 fluorescence detection channels for included lasers											
Number of lasers	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)	Scatter detectors	Total detection channels	Cat. No.
5	12	12		7		12		5		5	53	PL00302
6	12	12		7		12		5	3	5	56	PL00301
6	12	12	4	7		12		5		5	57	PL00300
7	12	12	4	7		12		5	3	5	60	PL00299
9	12	12	4	7		12		5	3	5	60	PL00285

** 532 nm and 594 nm lasers are not used for spectral analysis, but all fluorescence detectors are used.

Configurations offering only conventional compensation

	Number of fluorescence detection channels for included lasers											
Number of lasers	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)	Scatter detectors	Total detection channels	Cat. No.
4		7		7		7		4		2	27	PL00304
5	7	7		5		7		4		5	35	PL00303
4		7		7		7		4		3	28	PL00370

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