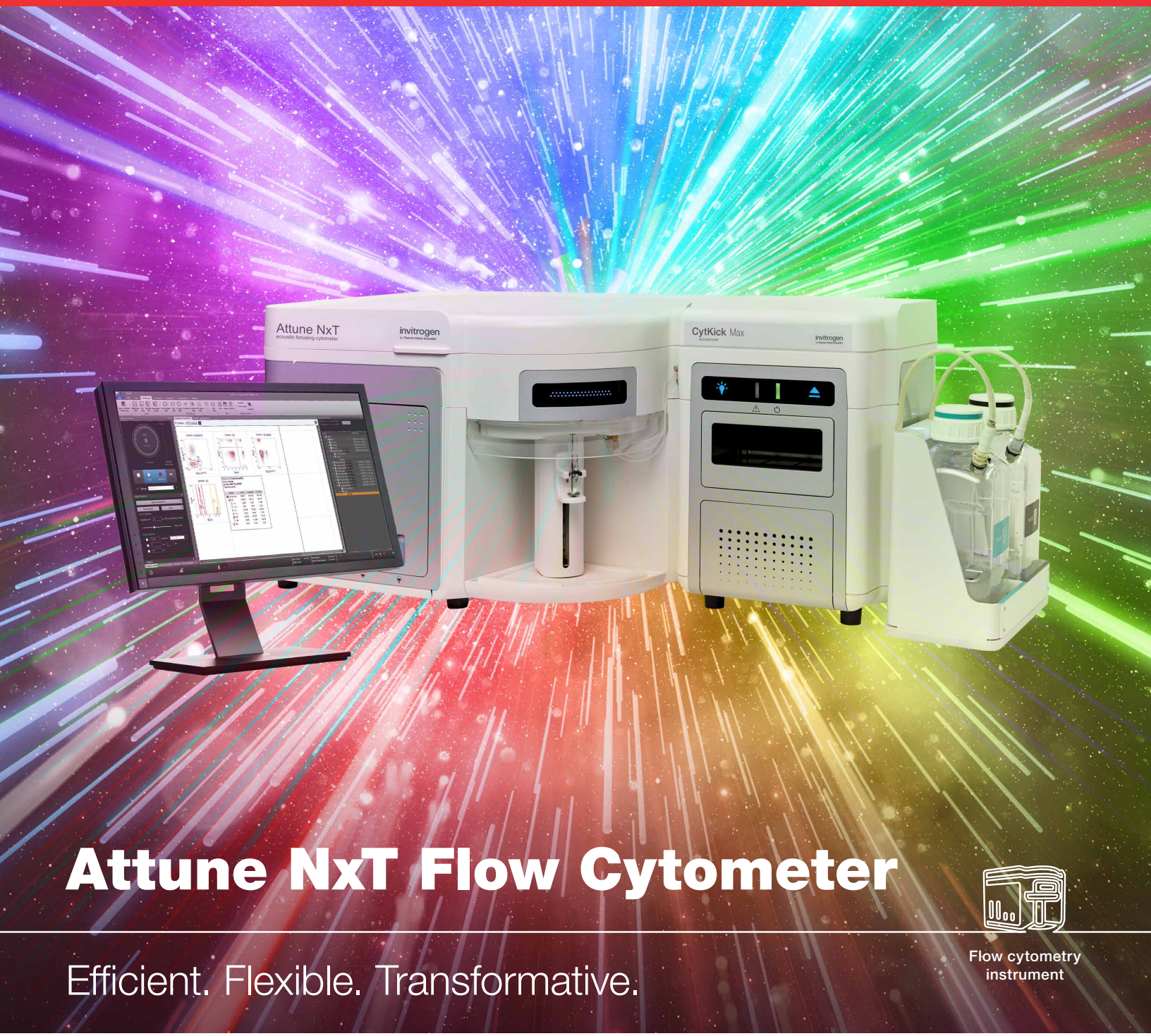
invitrogen



Attune NxT Flow Cytometer

Flow cytometry instrument

Efficient. Flexible. Transformative.

Attune NxT Flow Cytometer, CytKick Autosamplers, 21 CFR Part 11 compliance software, flow antibody conjugates and reagents

ThermoFisher SCIENTIFIC

Building a legacy of advancing flow cytometry technology

Our suite of comprehensive flow cytometry solutions, including the Invitrogen™ Attune™ NxT Flow Cytometer, Invitrogen™ CytKick™ family of autosamplers and automation options, together with the Invitrogen™ cell health reagent portfolio and Invitrogen™ eBioscience™ antibody conjugates, help drive discovery of new biological insights for many applications.

Find out more at **thermofisher.com/attune**

Designed for efficiency, speed, and accuracy

Technology

With acoustic-assisted hydrodynamic focusing, the Attune NxT Flow Cytometer (Figure 1) avoids compromise between data quality and higher sample rates by uncoupling cell alignment from sheath flow. Acoustic-assisted hydrodynamic focusing precisely aligns cells using ultrasonic radiation pressure (>2 MHz) to transport particles into the center of the sample stream. This prefocused stream is then injected into the sheath stream, resulting in a narrow particle stream and uniform laser illumination, regardless of the sample input rate (Figure 2).

The instrument's speed specifications include:

- Sample input flow rate ranges from 12.5 to 1,000 µL/min
- Data acquisition speed up to 35,000 events/second with 34 parameters
- Maximum electronic speed is 65,000 events/second with all parameters

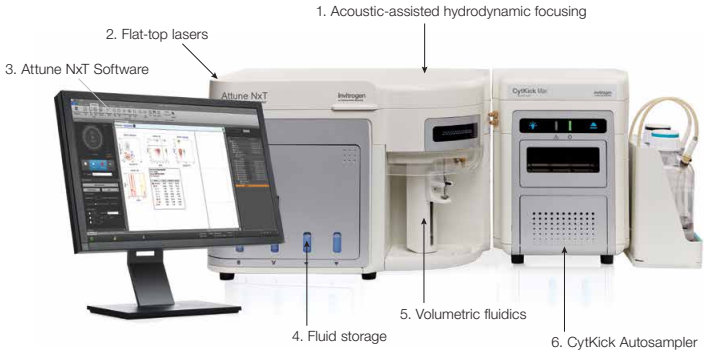


Figure 1. The Attune NxT Flow Cytometer components. (1) Patented acoustic-assisted hydrodynamic fluidics increase sample input speed while maintaining data integrity. **(2)** Flat-top lasers deliver more even application of light to each cell. **(3)** Invitrogen™ Attune™ NxT Software designed to guide users through complex flow cytometry experiments. **(4)** Fluid storage designed for minimal waste. **(5)** Volumetric fluidics provide cell counting and a resistance to clogging. **(6)** The CytKick Autosampler provides easy one-click transition from tube to plate.

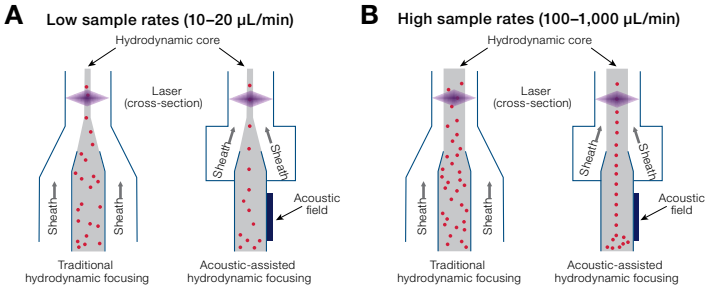


Figure 2. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A) In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. **(B)** In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.

Benefits

- Greater reproducibility and consistency in data
- Maintain consistent concentration results across all flow rates (Figure 3)
- Process very dilute or concentrated samples while maintaining low coefficients of variation (CVs) (Figure 4)

Data

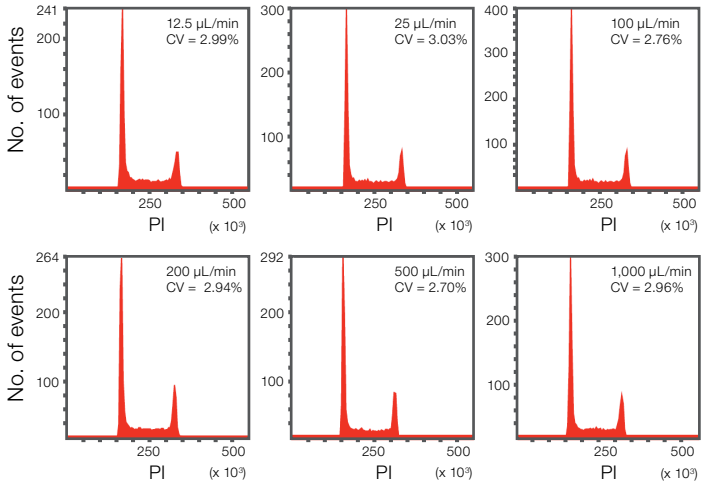


Figure 3. Minimal data variation at high sample rates with the Attune NxT Flow Cytometer. Jurkat cells were fixed and stained with propidium iodide (PI), treated with RNase, and analyzed at a concentration of 1 x 10^6 cells/mL at different sample rates. The left peak in all graphs reflects cells in G_0/G_1 phase, while the right peak reflects cells in G_2/M phase. Regardless of sample rate, the width of the G_0/G_1 and G_2/M peaks, and the CVs remain consistent, even at the highest sample rate of 1,000 µL/min.

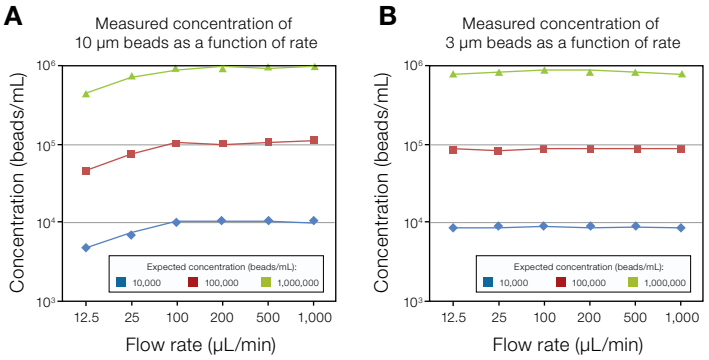


Figure 4. Data demonstrating the measured vs. expected concentration as a function of flow rate. (A) Measured concentration of 10 µm beads as a function of rate. Larger particles (e.g., 10 µm) show consistent results across the flow rate range 100–1,000 µL/min. **(B)** Measured concentration of 3 µm beads as a function of rate. Smaller particles (e.g., 0.2–3 µm) show consistent concentration results across all flow rates for the three concentrations of beads/mL tested.

"I can't believe how quick and easy it is to collect a large body of empirical evidence on the Attune NxT Flow Cytometer to fully support our hypothesis."

— Jordi Petriz, PhD
Group Leader, Functional Cytomics Group;
Josep Carreras Leukaemia Research Institute,
Barcelona, Spain

"What I like most about the Attune NxT Flow Cytometer is its accuracy and its speed of processing. I could list a range of other things that I like about it because we are building up that list as we discover more aspects of its functions, but the core things are speed and accuracy that are not available with hydrodynamic flow cytometers."

— Tim Inglis, BM, DM, PhD, FRCPath, FRCPA, DTM&H
School of Medicine, University of Western Australia,
PathWest Laboratory Medicine

Smooth flow delivery for accurate counts

The Attune NxT Flow Cytometer delivers samples into the instrument with minimal variation (Figure 5). Smoother delivery of samples provides more confidence when presenting cell counting data.

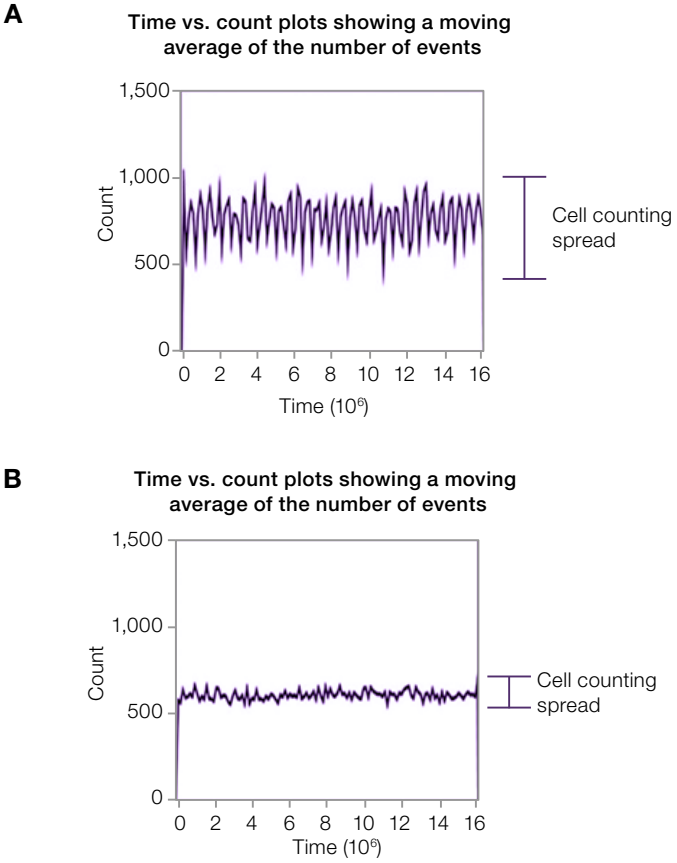
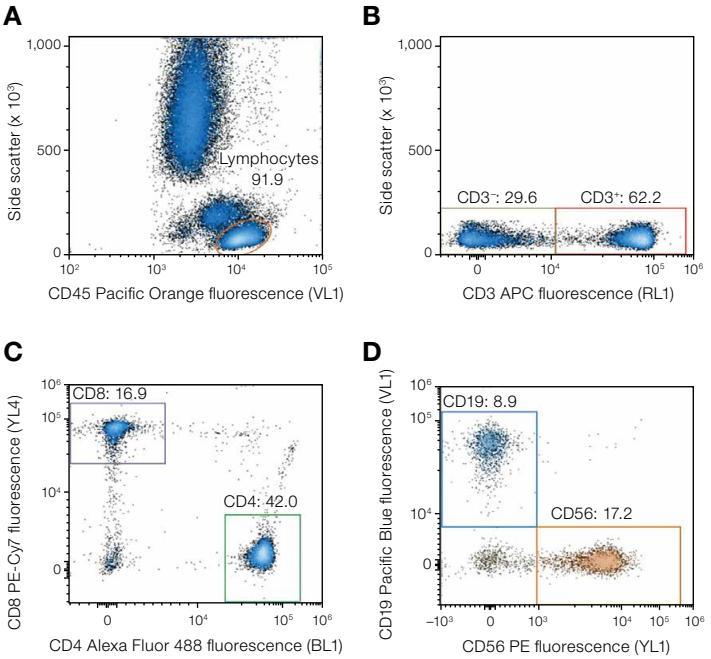


Figure 5. Time vs. count plot to obtain a moving average of the number of events showing up at a given time. (A) Data from a flow cytometer with a peristaltic pump, showing fluid pulsation ±33% of the average count and a total spread of 66% of the average count. **(B)** Time vs. count data from Attune NxT Flow Cytometer with a non-peristaltic pump, showing fluid pulsation of ±5%.

Technology

Samples on the Attune NxT Flow Cytometer are delivered by a positive-displacement syringe pump for volumetric analysis meaning that all events are automatically counted, and particle counts or concentrations can be viewed with the simple click of a button. Figure 6 shows the scatter plots and cell concentrations for all lymphocyte subpopulations.

Data



Name	Count	%Total	%Gated	Concentration
All Events	66,906	100.00	100.00	297.4
Lymphs	20,683	30.91	30.91	91.9
CD3⁺	13,990	20.91	67.64	62.2
CD4	9,439	14.11	67.47	42.0
CD8	3,811	5.70	27.24	16.9
CD3⁻	6,655	9.95	32.18	29.6
CD19	2,001	2.99	30.07	8.9
CD56	3,869	5.78	58.14	17.2

Figure 6. Lymphocyte subset analysis. A 100 µL aliquot of normal human whole blood was labeled with fluorophore-conjugated antibodies against CD surface markers, followed by red blood cell (RBC) lysis using 2 mL of Invitrogen™ High-Yield Lyse Fixative-Free Lysing Solution (Cat. No. HYL250), resulting in a 1:21 dilution of the blood. **(A)** Lymphocytes are identified on a precedence density plot of CD45 vs. side scatter with an oval gate around the lymphocyte ($CD45^+$) population. **(B)** Cells in the lymphocyte gate are displayed on a precedence density plot of CD3 vs. side scatter. Rectangle gates surround the $CD3^+$ T cell and $CD3^-$ B and natural killer (NK) cell populations. **(C)** Cells in the $CD3^+$ gate are then displayed on a precedence density plot of CD4 vs. CD8 to quantify $CD4^+$ helper T cells ($CD4^+ CD3^+ CD45^+$) and $CD8^+$ cytotoxic T cells ($CD8^+ CD3^+ CD45^+$). **(D)** $CD3^-$ cells are displayed on a precedence density plot of CD56 vs. CD19 to distinguish $CD56^+$ NK cells from $CD19^+$ B cells. The statistics table shows the gating and measured concentrations (cells/µL).

Benefits

- Syringe easily removed for cleaning or replacement
- Consistent cell concentration results across all flow rates (Figure 7)
- Precise counts without the need for expensive beads

> "We have yet to clog the machine with our debris-rich primary tumor samples. Of course, the acoustic technology greatly facilitates the identification of small populations, like cancer stem cells, increasing our capacity to detect and quantify these rare events with high efficiency and reliability."
>
> – Bruno Sainz Jr., PhD
> Autónoma University of Madrid, School of Medicine

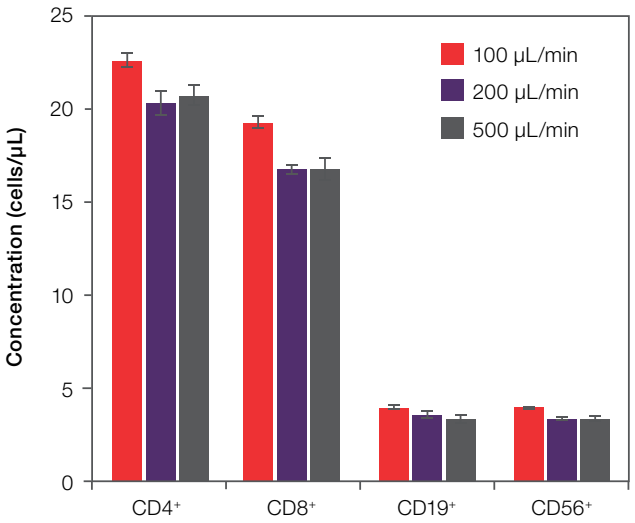


Figure 7. Replicate samples collected at three flow rates on the Attune NxT Flow Cytometer. Cell concentrations were measured using three different flow rates: 100, 200, and 500 µL/min. The Attune NxT Flow Cytometer provides similar concentration measurements for each lymphocyte subpopulation, regardless of the flow rate. Each bar represents the mean cells/µL ±standard deviation of three samples run at each indicated flow rate for each population.

Reduce clogging from difficult samples

Your research samples are precious, as they are often difficult to produce. The Attune NxT Flow Cytometer is less prone to clogging, allowing challenging samples such as cardiomyocytes, heterogeneous blood cells, and cancer cells to flow with confidence.

Technology

Engineered to actively resist clogging, a syringe-driven system (Figure 8) and larger flow cell help prevent the loss of precious sample such as cancer stem cells from primary pancreatic tumors (Figure 9), and is drastically less susceptible to clogs. The Attune NxT Flow Cytometer employs a non-pressurized system that mechanically decreases the occurrence of clogging.

Benefits

- Easy flow of difficult samples such as large or sticky cells
- Sample recovery feature built into software
- Comparatively lower fluid consumption (~1.8 L/day)

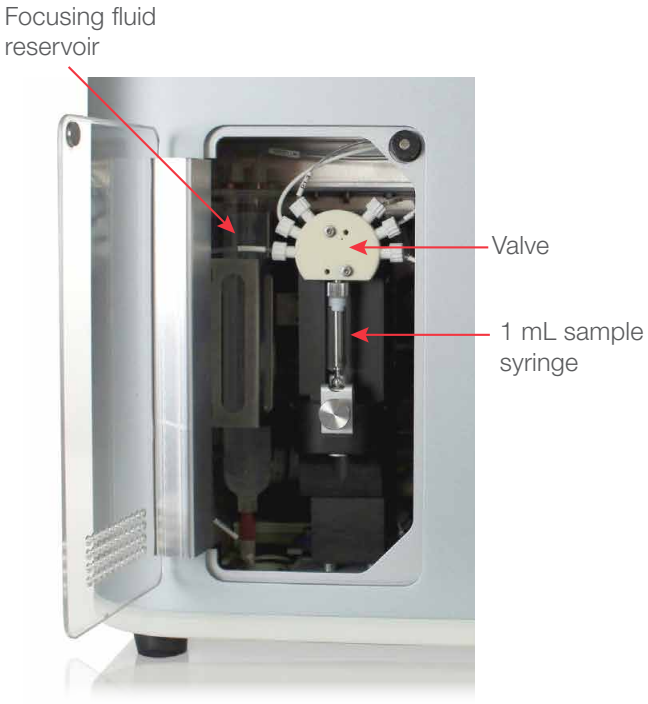


Figure 8. Positive-displacement syringe pump. Syringe easily removed for cleaning or replacement.

denotes environmentally friendly feature

Detect and quantify rare events with high efficiency and reliability

Data

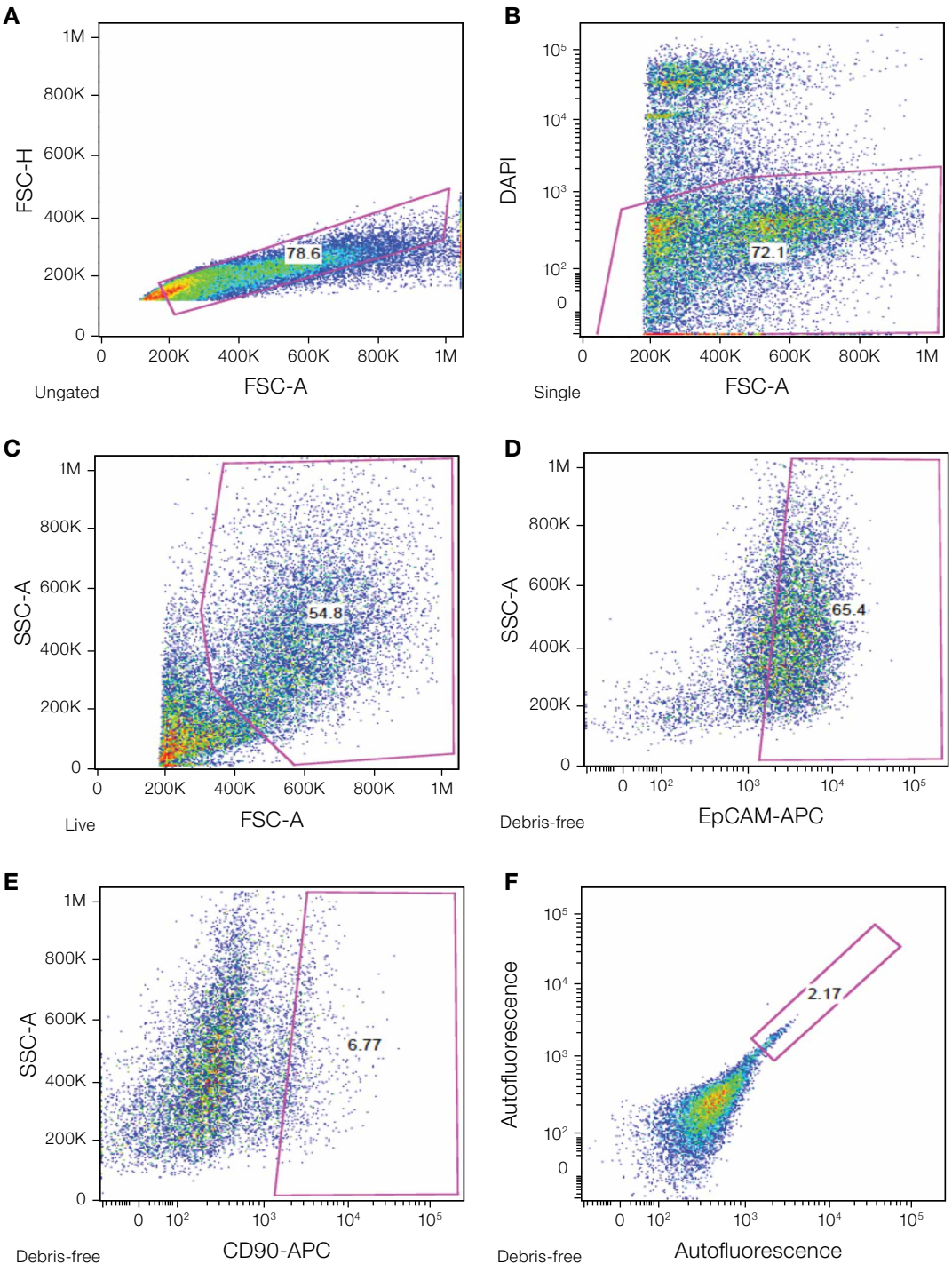


Figure 9. The Attune NxT Flow Cytometer detects autofluorescent and CD90⁺ rare cancer stem cells from primary pancreatic tumors without clogging. Tumors were minced and enzymatically digested with collagenase, followed by an overnight incubation with 30 µM riboflavin in RPMI medium with 10% FBS. Cells were then blocked with flebogamma and stained with anti-EpCAM or anti-CD90 antibodies. **(A–C)** Single, live, and debris-free cell gating strategy. **(D)** EpCAM⁺, **(E)** CD90⁺, and **(F)** autofluorescence-positive cells within the tumor population. Data courtesy of Bruno Sainz Jr., PhD.

Application highlight

Improved data resulting from less trauma to cells

Acoustic focusing allows the Attune NxT Flow Cytometer to deliver a no-wash, no-lyse protocol (Figure 10) to minimize cell loss, significantly reduce time, and simplify sample preparation.

> "Multiplexing and compensation are much easier and extremely efficient with the Attune NxT Flow Cytometer."
>
> — Bruno Sainz Jr., PhD
> Autónoma University of Madrid, School of Medicine

Benefits

- Improve lab safety with reduced sample handling with no-wash protocol
- Completely cut out time-consuming centrifugation steps
- Save countless hours running dilute samples and reduce reagent costs
- Eliminate cell loss due to wash steps or RBC removal procedure
- Ideal for limited sample volumes and for functional live-cell assays when using the Attune NxT No-Wash No-Lyse Filter Kit (Cat. No. 100022776) (Figure 11)

Protocol

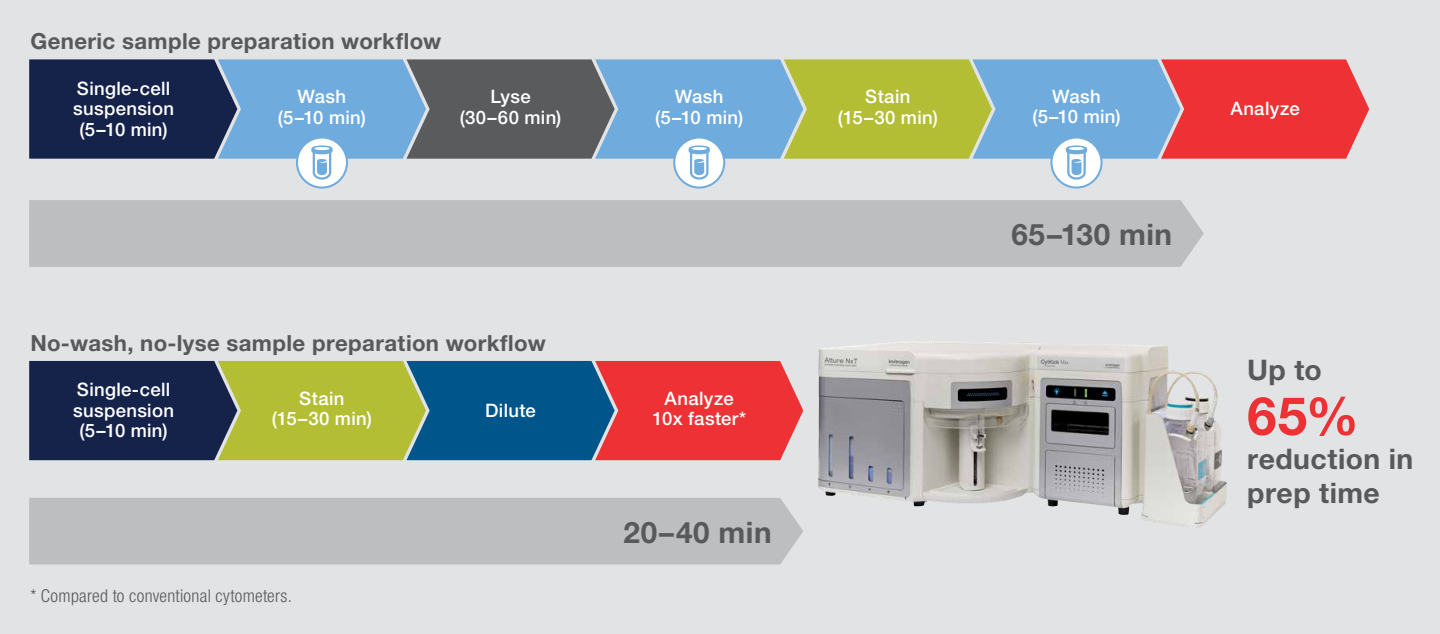


Figure 10. No-wash, no-lyse sample preparation workflow.

No lyse, no wash, with no compensation

Data

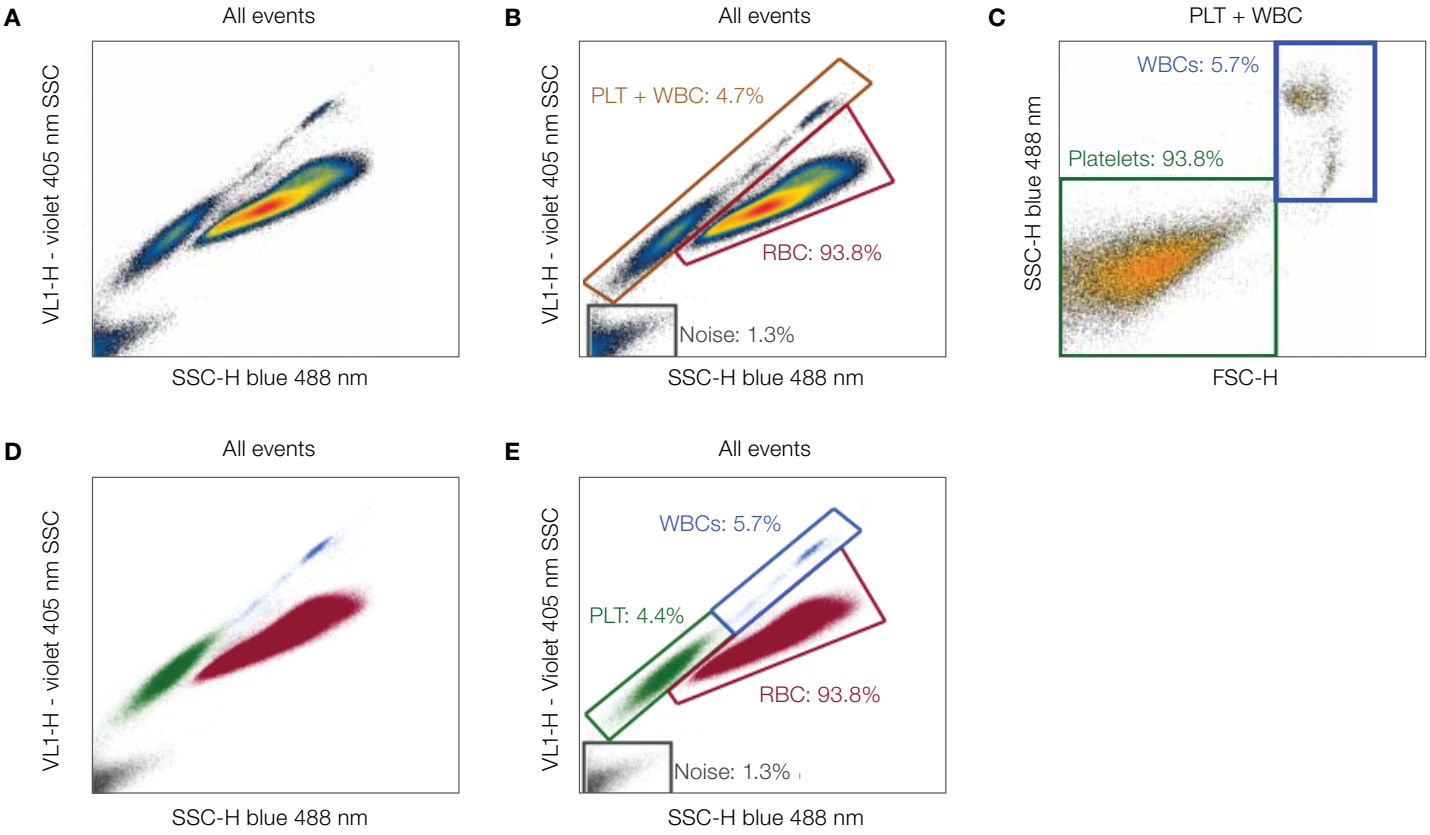


Figure 11. Forward scatter (FSC) and side scatter (SSC) analysis with blue (488 nm) and violet (405 nm) lasers on intact whole blood (no-lyse, no-wash). (A, B) RBCs, white blood cells (WBCs), and platelets are separated on the basis of light scatter only by using a combination of blue and violet laser SSC analysis. Hemoglobin in RBCs readily absorbs light at 405 nm, shifting the RBC population to the right by reducing the SSC for RBCs in the violet laser channel relative to leukocytes and platelets. Dual FSC and SSC threshold is set low enough to show instrument noise, ensuring the full platelet population is visualized. **(C)** Using the gate that includes WBCs and platelets, a standard plot of FSC vs. 488 nm SSC can be used to distinguish the platelet population from the WBCs with regions created around the two populations. **(D)** Using color-backgating on plot **(A)**, the RBC population is colored red, the platelet population is colored green, and the WBC population is colored blue, while the noise is black. The three main WBC populations of lymphocytes, monocytes, and granulocytes can be distinguished. **(E)** Placing regions around the RBC, WBC, and platelet populations show the dominant cell type in whole blood is the RBC, while the WBCs and platelets are relatively rare events.

Precision optical performance

Minimize instrument downtime with the Attune NxT Flow Cytometer's optical system. The lasers are designed to last the life span of a flow cytometer and provide a wider area of light intensity.

Technology

The Attune NxT Flow Cytometer uses flat-top lasers with an intensity profile that allows a much wider window of alignment (Figure 12). This innovative design helps ensure precise fixed alignment of 4 spatially separated solid-state lasers onto the sample stream (Figure 13), minimizing the effects of changes in fluidics or optics. The stability of the optical system leads to increased data consistency over time, superior performance, and first-class reliability.

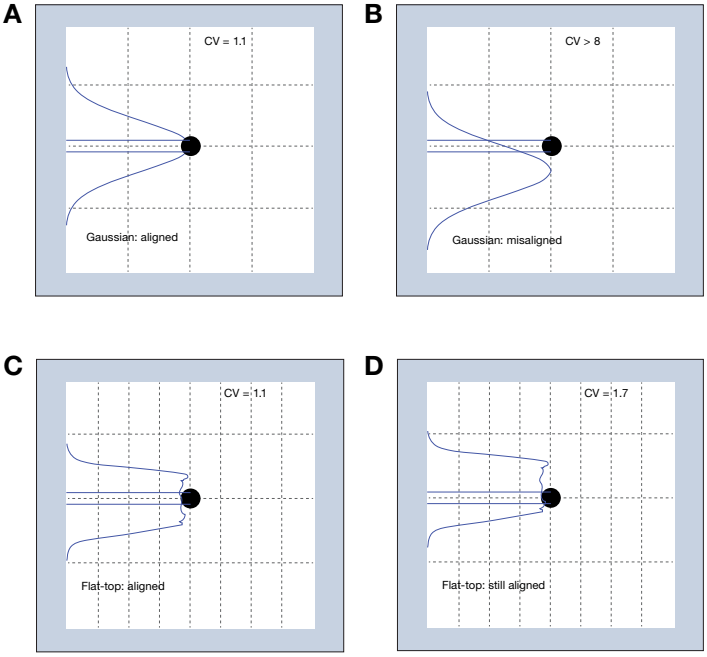


Figure 12. Emission profiles of lasers used in flow cytometers. **(A)** Gaussian laser profile with proper alignment, **(B)** Gaussian laser profile with misalignment, **(C)** flat-top laser profile with proper alignment, and **(D)** flat-top laser profile still in proper alignment.

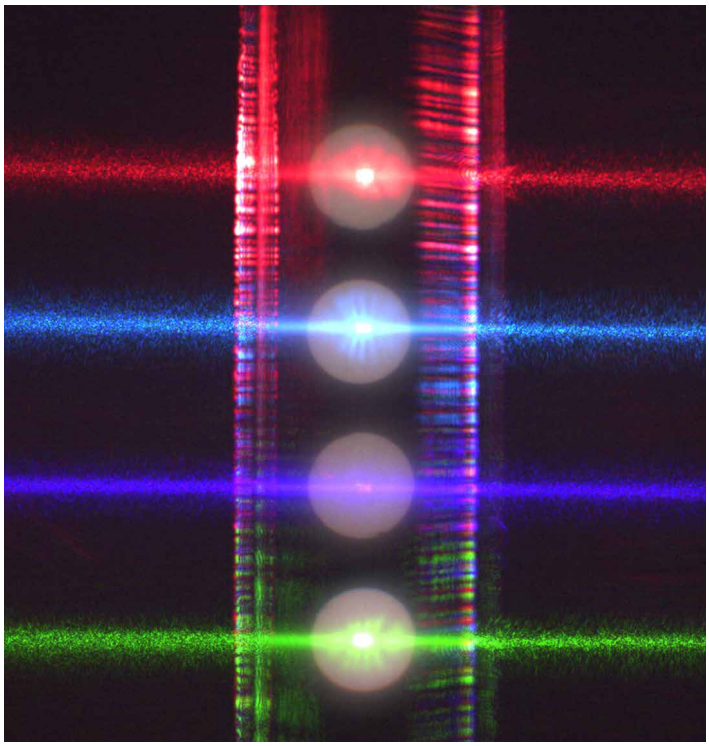


Figure 13. The Attune NxT Flow Cytometer can be configured with up to 4 spatially separated lasers.

"Having evaluated the instrument over several months, I would say the Attune NxT Flow Cytometer fits the superior category of flow cytometers."

– JP Robinson, PhD
Purdue University

Benefits

- No warm-up delay
- Simmer mode: automatic shutoff prolongs laser usage lifetime up to 10x
- Lasers are only turned on when acquiring samples, increasing the lifetime of the laser

CytKick Autosamplers

Walk-away automation

Choose the option that best suits your throughput and experimental needs. Invitrogen™ CytKick™ and CytKick™ Max Autosampler models offer walk-away automation that is seamlessly integrated with your Attune NxT Flow Cytometer. For increased productivity and added choice, both autosamplers offer:

- **Broad compatibility**—compatible with many different plate formats, including 96-well, 384-well, and deep-well plates on all models
- **Intelligent probe design**—helps minimize clogging
- **Automated cleaning**—performs automated cleaning when the instrument is shutting down
- **Consistent data**—designed to provide minimal variation regardless of sampling method (tube vs. plate) and collection rate
- **Mixing by aspiration**—mixing the sample by aspiration instead of shaking helps to ensure homogeneity of the sample and maintains cell viability
- **Plate and tube compatibility with one-click transition**—no disassembly, no additional QC, and no reboot required for conversion between plates and tubes

For the highest throughput and the broadest flexibility options, choose the CytKick Max Autosampler:

- **Expanded sample vessel compatibility**—accommodates 1.5 mL and 2 mL microcentrifuge tube racks (up to 24-tube racks per vessel)
- **Passive cooling**—available for 96-well U-bottom plates and microcentrifuge tube racks
- **Short acquisition time**—22 min for a 96-well plate (Boost mode, using one rinse and one mix, and full analysis of a 20 µL sample)

"We looked at several metrics and compared the [CytKick] Autosampler to other 96-well plate readers. The autosampler proved to have very good stability and very low carryover. We were most impressed by the way that the autosampler took advantage of the Attune NxT Flow Cytometer's fluidics and high-volume throughput. Without compromising stability or precision, the autosampler was able to run plates much faster than any other plate reader."

– EM Meyer
University of Pittsburgh Cancer Institute

"The dual tube-to-plate operation—instant change from tubes to plates is really an excellent feature."

– JP Robinson, PhD
Purdue University

Find out more at **thermofisher.com/cytkickmax**

Data

A Tube

B Plate

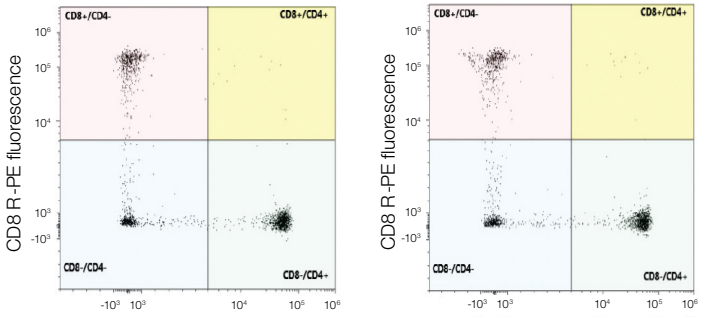
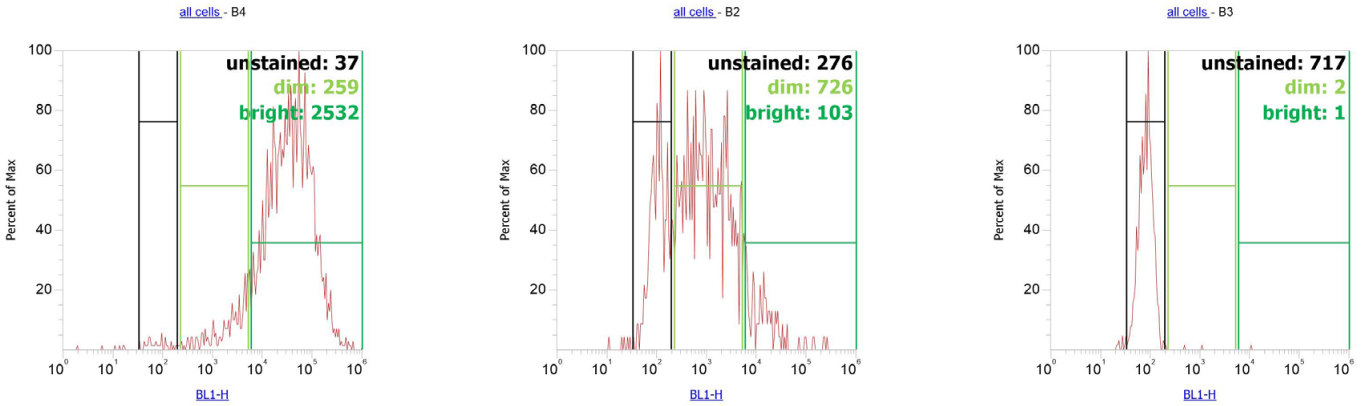


Figure 14. Consistent results are achievable regardless of sampling method. Whole blood lysed with ammonium chloride was labeled with Invitrogen™ mouse anti–human CD45 Pacific Orange™, mouse anti–human CD4 FITC, and mouse anti–human CD8 R-PE antibody conjugates. Labeled samples were analyzed on a blue and violet laser–configured Attune NxT Flow Cytometer equipped with a 488 nm laser for fluorescence excitation of FITC (530 BP) and R-PE (574/24 BP), and a 405 nm laser for Pacific Orange dye (603/48 LP). Identical samples, including compensation controls, were analyzed using either **(A)** tube mode or **(B)** plate mode with a standard collection rate of 200 µL/min. Lymphocytes were gated using a CD45 vs. side scatter plot and analyzed for expression of CD4 and CD8 antigens. Minimal variation was observed between analysis in a tube alone and on a plate running on the CytKick Autosampler.



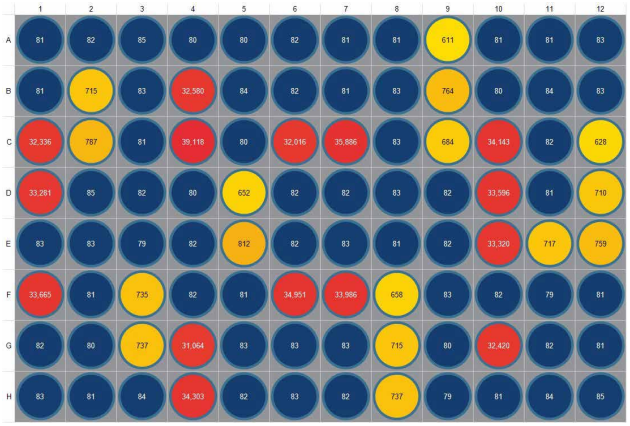


Figure 15. CytKick Autosampler heat map function identifies variation within a parameter across a 96-well plate. U2OS and HeLa cells were transduced with Invitrogen™ BacMam GFP Transduction Control (BacMam 2.0) (Cat. No. B10383) overnight at 37°C. The BacMam 2.0 baculovirus transduction system works well with U2OS cells, resulting in bright GFP expression, while HeLa cells exhibit dim GFP expression. Transduced and unstained cells were trypsinized and resuspended in PBS at 2.5 x 10^5 cells/mL, and 100 µL of the cell suspension was added per well to various wells of a 96-well round bottom plate resulting in wells with either bright GFP-expressing cells, dim GFP-expressing cells, or unstained cells. The plate was collected on the Attune NxT Flow Cytometer using 488 nm excitation and 530/30 nm emission with the CytKick Max Autosampler in high-throughput mode (20 µL collection volume, 1,000 µL/min, Boost enabled, 1 mix, 1 rinse) in under 22 minutes. Histograms show the median fluorescence intensity of representative wells containing unstained, dim GFP-expressing, and bright GFP-expressing cells, while the heat map shows the median fluorescence intensity for the entire plate where unstained wells are blue, dim wells are yellow, and bright wells are red.

Flexibility to create a practical instrument

Detect the full range of fluorescence

The Attune NxT Flow Cytometer accommodates up to 14 color panels. The filter and laser are configurable and field upgradeable, giving the freedom to upgrade up to 4 lasers and 16 detection channels (Tables 1 and 2).

Table 1. The Attune NxT Flow Cytometer system configurations.

Lasers	Laser configuration	Cat. No.	Violet 405 nm	Blue 488 nm	Yellow 561 nm	Green 532 nm	Red 637 nm	Total detection channels*
1	Blue	A24864	Available as upgrade	4	Available as upgrade	Available as upgrade	Available as upgrade	6
2	Blue/green	A28995	Available as upgrade	3	–	4	Available as upgrade	9
	Blue/yellow	A24861	Available as upgrade	3	4	–	Available as upgrade	9
	Blue/red	A24863	Available as upgrade	4	Available as upgrade	Available as upgrade	3	9
	Blue/violet	A24862	4	4	Available as upgrade	Available as upgrade	Available as upgrade	10
	Blue/violet 6	A29002	6	3	Available as upgrade	–	Available as upgrade	11
3	Blue/green/red	A28997	Available as upgrade	3	–	4	3	12
	Blue/red/yellow	A28993	Available as upgrade	3	4	–	3	12
	Blue/green/violet	A28999	4	3	–	4	Available as upgrade	13
	Blue/violet/ yellow	A24859	4	3	4	–	Available as upgrade	13
	Blue/red/violet	A24860	4	4	Available as upgrade	Available as upgrade	3	13
	Blue/red/violet 6	A29003	6	3	Available as upgrade	–	3	14
4	Blue/red/violet /green	A29001	4	3	–	4	3	16
	Blue/red/yellow /violet	A24858	4	3	4	–	3	16
	Blue/red/yellow/ violet 6	A29004	6	2	3	–	3	16

* Includes forward scatter (FSC) and side scatter (SSC).

Up to **16** parameters

Up to **4** lasers

Up to **14** colors

Benefits

- Field upgradeability to accommodate expanding needs
- Use more lasers for expanded multicolor panel design options
- Use fewer reagents

Table 2. The Attune NxT Flow Cytometer filter configurations.

Cat. No.	A24864	A28995	A24861	A24863	A24862	A29002	A28997	A24860	A28999	A28993	A24859	A29003	A29004	A29001	A24858
Detectors	4	7	7	7	8	9	10	10	11	10	11	12	14	14	14
Channel	Emission filter (nm)														
BL1	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30
BL2	574/26	590/40	590/40	574/26	574/26	574/26	590/40	574/26	590/40	574/26	590/40	574/26	695/40	590/40	590/40
BL3	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40		695/40	695/40
BL4	780/60			780/60	780/60			780/60							
GL1		575/36					575/36		575/36					575/36	
GL2		620/15					620/15		620/15					620/15	
GL3		695/40					695/40		695/40					695/40	
GL4		780/60					780/60		780/60					780/60	
YL1			585/16							585/16	585/16		585/16		585/16
YL2			620/15							620/15	620/15		620/15		620/15
YL3			695/40							695/40	695/40		780/60		695/40
YL4			780/60							780/60	780/60				780/60
RL1				670/14			670/14	670/14		670/14		670/14	670/14	670/14	670/14
RL2				720/30			720/30	720/30		720/30		720/30	720/30	720/30	720/30
RL3				780/60			780/60	780/60		780/60		780/60	780/60	780/60	780/60
VL1					440/50	450/40		440/50	440/50		440/50	450/40	450/40	440/50	440/50
VL2					512/25	525/50		512/25	512/25		512/25	525/50	525/50	512/25	512/25
VL3					603/48	610/20		603/48	603/48		603/48	610/20	610/20	603/48	603/48
VL4					710/50	660/20		710/50	710/50		710/50	660/20	660/20	710/50	710/50
VL5						710/50						710/50	710/50		
VL6						780/60						780/60	780/60		

Expand the range of performance for your violet laser

The Attune NxT Flow Cytometer is easily upgradeable to 6-channel detection for the violet (405 nm) laser (Table 3). The Attune NxT Flow Cytometer with violet 6-channel configuration is designed to accommodate a wide variety of experimental conditions. Combined with the Invitrogen™ Super Bright and other appropriate dyes, the system provides expanded choices for panel design (Table 4). See available Super Bright dyes at **thermofisher.com/superbright**

Benefits

- Modular expansion options for growth when needed, not before
- Facilitate application development with fewer restrictions on fluorochrome detectors
- Enhanced capability to perform a variety of applications on a single instrument

Download the poster at **thermofisher.com/attune-14C**

Technology

Table 3. Attune NxT Flow Cytometer configuration using 6 fluorescence detectors for the violet laser.

Laser	Fluorescence detectors		
	2-laser	3-laser	4-laser
Violet, 405 nm	6	6	6
Blue, 488 nm	3	3	2
Yellow, 561 nm	NA	NA	3
Red, 637 nm	NA	3	3
Total fluorescence detectors available	9	12	14
Total parameters per configuration*	11	14	16

* Includes FSC and SSC.

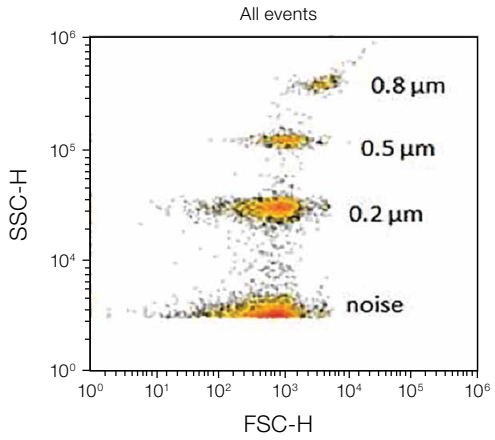


Figure 16. FSC and SSC discrimination of 0.2 µm, 0.5 µm, and 0.8 µm particles using the Submicron Bead Calibration Kit from Bangs Laboratory.

Table 4. Fluorophore guidelines for the 6 fluorescence detectors off the violet laser in the Attune NxT Flow Cytometer.

Detector	Bandpass (nm)	Fluorophores*
VL1	450/40	Super Bright 436, eFluor 450, LIVE/DEAD™ Fixable Violet, Vybrant™ DyeCycle™ Violet, SYTOX™ Blue, CellTrace™ Violet, VioBlue™, Brilliant Violet™ 421, Pacific Blue™, BD Horizon™ V450
VL2	525/50	eFluor 506, LIVE/DEAD™ Fixable Aqua, CFP, VioGreen™, Brilliant Violet™ 510, Pacific Green™, BD Horizon™ V500
VL3	610/20	Super Bright 600, LIVE/DEAD™ Fixable Yellow, Qdot™ 605, Pacific Orange™, Brilliant Violet™ 605
VL4	660/20	Super Bright 645, Brilliant Violet™ 650
VL5	710/50	Super Bright 702, Qdot™ 700, Brilliant Violet™ 711
VL6	780/60	Super Bright 780, Brilliant Violet™ 786

* List is not inclusive of all available fluorophores.

Attune NxT Small Particle Side-Scatter Filter for small-particle detection

The Invitrogen™ Attune™ NxT Small Particle Side-Scatter Filter is for use with the Attune NxT Flow Cytometer. It is designed to increase the dynamic range of the side-scatter detection without sacrificing resolution of the detector. The use of this 488/10 nm side-scatter filter combined with ultrafiltering of the focusing fluid has been found to dramatically decrease background noise and enable discrimination of 100 nm particles (Figure 17).

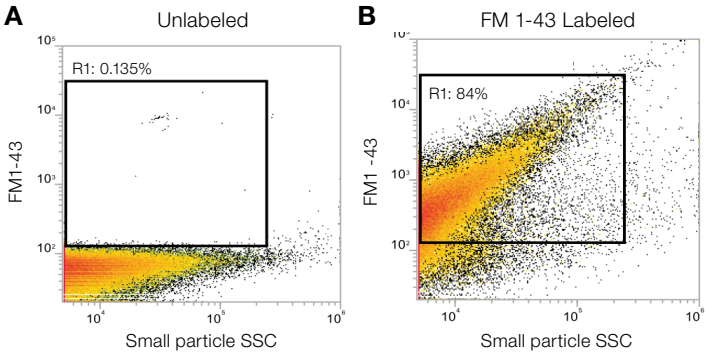


Figure 17. Exosomes were acquired on the Attune NxT Flow Cytometer using the Attune NxT Small Particle Side-Scatter Filter for SSC detection. Plot A shows unlabeled particles. Plot B shows particles labeled with the membrane probe FM™ 1-43 dye (Cat. No. T35356), detected using 488 nm excitation and 695/40 nm bandpass emission. Data from Steve McClellan, Mitchell Cancer Institute, University of South Alabama.

Data

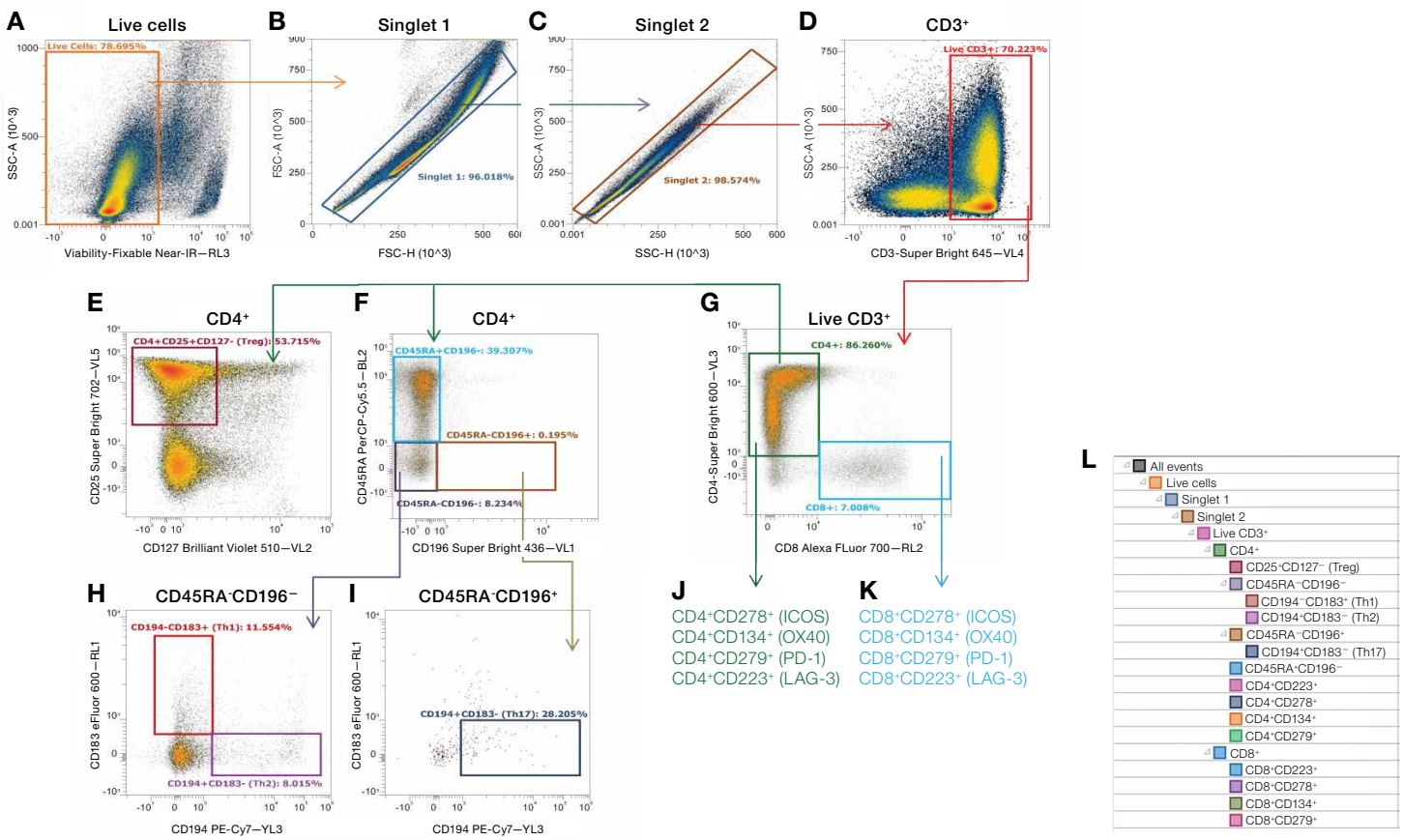


Figure 18. T lymphocyte immunophenotyping: 14-color flow cytometry panel design using the Attune NxT violet 6 channel option and Super Bright fluorescent dyes gating strategy. (A) A region is placed around live peripheral blood mononuclear cells (PBMCs) as identified by the Invitrogen™ LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit. **(B, C)** Live cells are analyzed through sequential singlet gating. A region is then placed on the **(D)** $CD3^+$ population for gating on **(G)** $CD4^+$ and $CD8^+$ populations. The $CD4^+$ population is used to gate on **(E)** CD127 vs. CD25, for **(F)** CD45RA vs. CD196, and **(J)** CD278, CD134, CD279, and CD223 populations. The $CD45RA^-/CD196^-$ population from **(F)** is gated on **(H)** CD183 vs. CD194. The $CD45RA^-/CD196^+$ population from **(F)** is gated on **(I)** CD183 vs. CD194. The $CD8^+$ population from **(G)** is used for gating **(K)** CD278, CD134, CD279, and CD223 populations. **(L)** The entire gating strategy is displayed in hierarchical format using the Attune NxT violet 6 channel option and v2.6 software for easy visualization.

Attune NxT 21 CFR Part 11 Compliance Software

Regulatory-compliant electronic records and signatures

Invitrogen™ Attune™ NxT 21 CFR Part 11 Compliance Software provides users with secure software that authenticates user log-in information, logs any unauthorized attempts to access system software, and notifies user of any data that has been tampered with. The software also provides the user with a full audit trail—electronic records and electronic signatures that are trustworthy, reliable, and equivalent to paper records.

- **Electronic signatures**—ensures reliability, authenticity, and validity, signatures associated with a record cannot be excised, copied, or falsified
- **Auditing**—provides full audit trails with user actions recorded
- **Security**—authenticates user's log-in information, logs any unauthorized attempts, identification of data tampering, and verifies each user's permission
- **Streamline workflow**—permits e-signature function for data verification that can be transitioned to GMP production and quality control

Find out more at **thermofisher.com/attune21cfrpart11**

Table 5. Attune NxT 21 CFR Part 11 Compliance Software ordering information.

Lasers	Laser configuration	Violet, 405 nm	Blue, 488 nm	Yellow, 561 nm	Green, 532 nm	Red, 637 nm	Total detection channels	Cat. No.
1	Blue	Available as upgrade	4	Available as upgrade	Available as upgrade	Available as upgrade	6	A24864CFR
2	Blue/green	Available as upgrade	3		4	Available as upgrade	9	A28995CFR
	Blue/yellow	Available as upgrade	3	4		Available as upgrade	9	A24861CFR
	Blue/red	Available as upgrade	4	Available as upgrade	Available as upgrade	3	9	A24863CFR
	Blue/violet	4	4	Available as upgrade	Available as upgrade	Available as upgrade	10	A24862CFR
	Blue/violet 6	6	3	Available as upgrade		Available as upgrade	11	A29002CFR
3	Blue/green/red	Available as upgrade	3		4	3	12	A28997CFR
	Blue/red/yellow	Available as upgrade	3	4		3	12	A28993CFR
	Blue/green/violet	4	3		4	Available as upgrade	13	A28999CFR
	Blue/violet/yellow	4	3	4		Available as upgrade	13	A24859CFR
	Blue/red/violet	4	4	Available as upgrade	Available as upgrade	3	13	A24860CFR
	Blue/red/violet 6	6	3	Available as upgrade		3	14	A29003CFR
4	Blue/red/violet/green	4	3		4	3	16	A29001CFR
	Blue/red/yellow/violet	4	3	4		3	16	A24858CFR
	Blue/red/yellow/violet 6	6	2	3		3	16	A29004CFR

Attune NxT system software

Figure 19. Intuitive, user-friendly software interface with familiar workflow.

Compensation tools

- Both negative and unstained gating parameters are available
- On-plot compensation adjustment
- Modification of compensation to add or remove parameters as needed after compensation is set up
- Set up and collect compensation controls directly from a plate

User management

- Levey-Jennings and "Performance History" reports of baseline and performance tests to monitor trends
- Ability to create and manage multiple user accounts
- System access based on user-account privileges

Learn more about Attune NxT software at
thermofisher.com/attune-cytometer-software

Flow cytometry reagents

Accelerate your science with a comprehensive suite of solutions for the analysis of cells and their function with Invitrogen™ eBioscience™ flow cytometry antibodies and Invitrogen™ cell health reagents.

Antibodies—build and expand your panels using our extensive portfolio of antibodies, conjugated to over 30 types of fluorophores including traditional, eFluor™, Alexa Fluor™, and Super Bright violet excitable polymer dyes

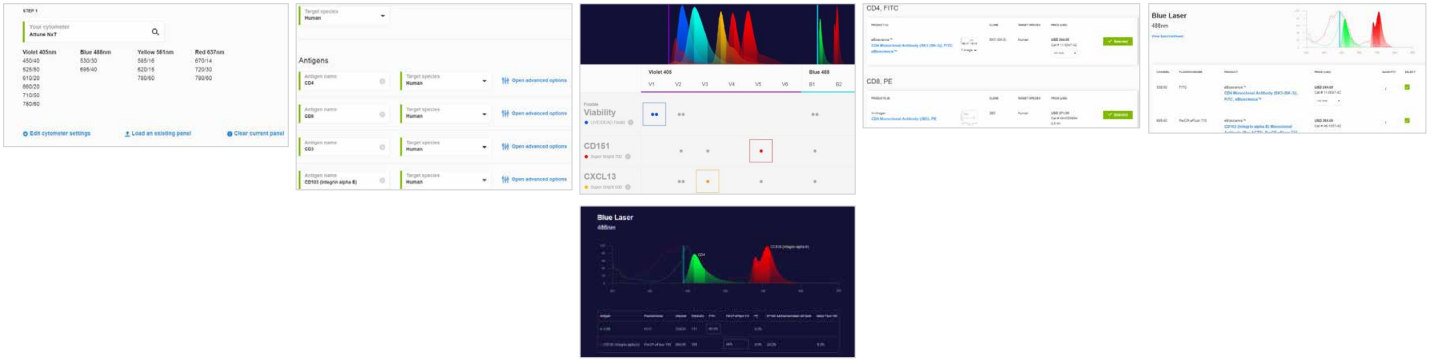
Buffers—we offer a wide variety of buffers to suit your research needs, whether your experiment calls for extracellular, intracellular, and/or nuclear cell staining

Reagents—incorporate a comprehensive variety of cell function assays for studying viability, apoptosis, cell cycle, metabolism, and cell proliferation

Instrument and compensation—beads are essential to perform quantitative measurements on individual cells and other particles with high precision, speed, and accuracy, especially when performing flow cytometry using multiple channels, markers that are poorly expressed, or from limited sample; select from our wide range of easy-to-use beads for your experimental needs

Simplified panel design with a 5-step panel design strategy

Step 1: Cytometer | **Step 2:** Antigens | **Step 3:** Fluorophores | **Step 4:** Products | **Step 5:** Summary



Design your panel for the Attune NxT Flow Cytometer using the Invitrogen™ Flow Cytometry Panel Builder:

- A quick and easy-to-use web tool
- Allows incorporation of antibodies using the configuration of your Attune NxT Flow Cytometer
- Fluorophore selection built on spectral visualization of all fluorophores per laser

For more information, go to **thermofisher.com/flow-cytometry**

Robotic automation solutions

Orbitor RS2 Microplate Mover

Maximize operating capacity, mitigate human operator error, and enable rich, reproducible data with the Thermo Scientific™ Orbitor™ RS2 Microplate Mover as part of a comprehensive, multicomponent workcell for robotically automated flow cytometry.

Technology

The robotic mover offers active and passive protective safety features, demonstrated reliability, and flexible configuration options for arrangement and storage. Operation is managed by Thermo Scientific™ Momentum™ Scheduling Software, established with instrument drivers available for over 350 instruments. The easy-to-use dashboard facilitates dynamic scheduling for active prioritization, visualized progress, and plate tracing, making it suitable for novice to experienced users.

Benefit

- Robust performance, precise motion, and consistent performance
- Compatible with a diverse range of plate types
- Works with both lidded and unlidded plates

The Attune NxT Flow Cytometer configured for robotic automation with the Orbitor RS2 Microplate Mover.

Aftermarket care

Partner with a flow cytometry company invested in supporting you through a lifetime of research

Choose a service plan that is right for you—beyond repair to proactive care

- **Peace of mind**—during every stage of ownership: instrument install, repair, and maintenance
- **Flexible service options**—over 1,000 technical specialists delivering 30 years of experience servicing life sciences instrumentation
- **AB Assurance plan and extended warranty**—covers all costs associated with instrument repairs

invitrogen

Ordering information

Product*	Description	Cat. No.
Attune IQ/IPV	Attune Operation Qualification and Instrument Performance Qualification (IQ/IPV)	4465413
Attune IQ/OQ	Attune Installation Qualification and Operation Qualification (IQ/OQ)	4465445
Orbitor RS2 Microplate Mover	AB Protection Orbitor Robot NxT	ZG30SCORBROBNXT
Accessories		
Attune NxT External Fluid Supply		A28006
Attune NxT Software, single license		A25554
Attune NxT Software, 5 licenses		A24856
Attune NxT Software, 10 licenses		A24855
Attune NxT Software 21 CFR Part 11, single license		A47288
Attune NxT Software 21 CFR Part 11, server license, 5 users		A47289
Attune NxT Software 21 CFR Part 11, server license, 10 users		A47290
Orbitor RS2 Microplate Mover, Stacks		A33007
Orbitor RS2 Microplate Mover, Hotels		A33008
Orbitor RS2 Microplate Mover, Stacks/Hotels		A35220
Upgrades		
Attune NxT Yellow Laser Upgrade Kit		100022779
Attune NxT Red Laser Upgrade Kit		100022778
Attune NxT Green Laser Upgrade Kit		A32701
Attune NxT Violet 6 Conversion Kit, Blue Laser		A35428
Attune NxT Violet 6 Conversion Kit, Violet Laser		A36569
Attune NxT Violet 6 Conversion Kit, Red Laser		A36571
Attune NxT Violet 6 Conversion Kit, Yellow Laser		A36572
Attune NxT Fluorescent Protein Filter Kit—GFP, YFP, mCherry		100022775
Attune NxT No-Wash No-Lyse Filter Kit		100022776
Attune NxT Custom Filter Holder Kit		A27784
Attune NxT Small Particle Side-Scatter Filter		100083194
CytKick Autosampler		A42901
CytKick Max Autosampler		A42973
Reagents and consumables		
Attune Debubble Solution (1X), 50 mL		A10496
Attune Focusing Fluid (1X), 1 L		4488621
Attune Focusing Fluid (1X), 10 L		A24904
Attune Wash Solution, 250 mL		A24974
Attune Shutdown Solution (1X), 250 mL		A24975
Attune Performance Tracking Beads		4449754

Find out more at **thermofisher.com/attune**

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