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Attune NxT Autosampler

Easy operation for multiple-sample processing

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The Attune NxT Autosampler—an optional accessory for the Attune NxT Flow Cytometer, enables rapid processing of multiple samples

Key features

- Compatible with many different plate formats, including 96-well, 384-well, and deep-well plates
- Intelligent probe design minimizes clogging and carryover (<1%) (Table 1) and helps prevent damage to the instrument
- Mixes sample by aspiration to enable homogeneity of the sample and to maintain cell viability (Table 2)
- Performs automated cleaning when the instrument is shut down
- Helps maintain consistent data while easily switching between use of the autosampler and individual tubes (Figure 1)
- Consistent data across different collection rates

Software

Setting up an experiment with Invitrogen™ Attune™ NxT Software is simple. Using a virtual plate layout view, you can create compensation wells, define instrument settings, and identify samples. The software also allows you to define multiple experiments on a single plate and recover unused samples (for samples collected or acquired in tube mode only).

The intuitive software is easy to use and enables quick data analysis. For example, a heat map view allows rapid screening and confirmation of samples while the instrument is acquiring data (Figure 2).

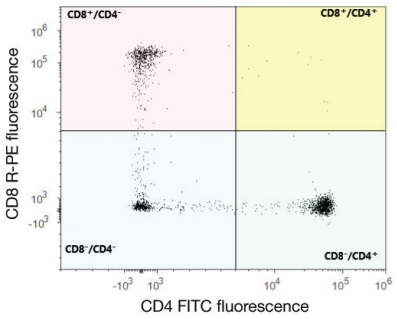
Table 1. Minimal carryover using the Invitrogen™ Attune™ NxT Autosampler. Jurkat cells at a concentration of 1 x 10^6 cells/mL were dispensed into a 96-well V-bottom plate and sampled using the Attune NxT Autosampler. Samples were analyzed on the Invitrogen™ Attune™ NxT Flow Cytometer using collection rates in standard mode (200 µL/min) and high-throughput mode (500 µL/min). Each sample was mixed once, and the Attune NxT Autosampler was washed 1–3 times prior to sampling the next well. Percent sample carryover was calculated.

	Number of washes and percent carryover		
Mode	1	2	3
Standard	0.01	0.01	0.01
High-throughput	0.02	0.02	0.02

Table 2. Gentle sample mixing using the Attune NxT Autosampler: increasing the number of mixing cycles does not adversely affect cell viability. Ammonium chloride–lysed whole blood (LWB) and NIH/3T3 (live/heat–treated) cells were stained with 2 µg/mL propidium iodide and loaded in triplicate into a 96-well V-bottom plate. Prior to acquisition, samples were mixed 0–5 times by the Attune NxT Autosampler, and then samples were analyzed using standard mode collection rates (100 µL/min for NIH/3T3, 200 µL/min for LWB) on the Attune NxT Flow Cytometer. Propidium iodide was excited using a 488 nm laser, and fluorescence emission was collected using a 640 nm longpass filter. Minimal variation was observed within each cell type, regardless of the number of mix cycles used prior to acquisition.

	Percentage of dead cells	
Number of mix cycles	LWB	NIH/3T3
0	0.75	34.10
1	0.78	32.83
2	0.74	33.52
3	0.74	32.75
4	0.74	33.26
5	0.75	31.58

A Tube



Tube statistics	
CD8⁺/CD4⁻	24.5%
CD8⁺/CD4⁺	0.6%
CD8⁻/CD4⁻	25.6%
CD8⁻/CD4⁺	49.4%

B Plate

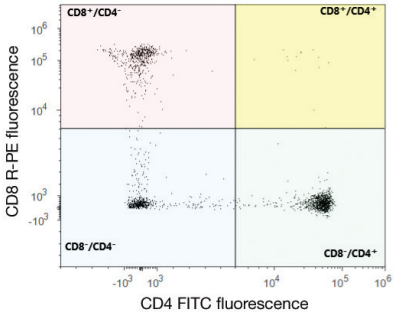
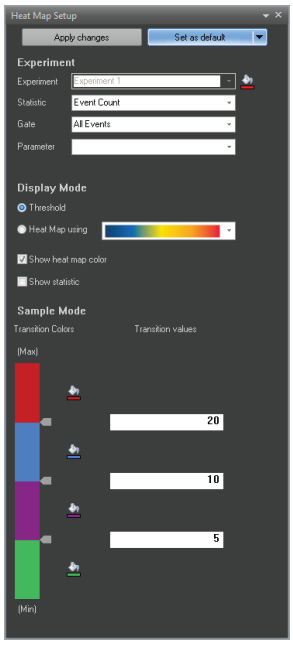


Plate statistics	
CD8⁺/CD4⁻	24.6%
CD8⁺/CD4⁺	0.6%
CD8⁻/CD4⁻	24.7%
CD8⁻/CD4⁺	50.1%

Figure 1. Consistent results are achievable regardless of sampling method. Whole blood lysed with ammonium chloride was labeled with Invitrogen™ mouse anti–human CD45 Pacific Orange™ (Cat. No. MHCD4530), mouse anti–human CD4 FITC (Cat. No. MHCD0401), and mouse anti–human CD8 R-PE (Cat. No. MHCD0804) antibody conjugates. Labeled samples were analyzed on a blue/violet-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 Attune NxT Autosampler.

A



B

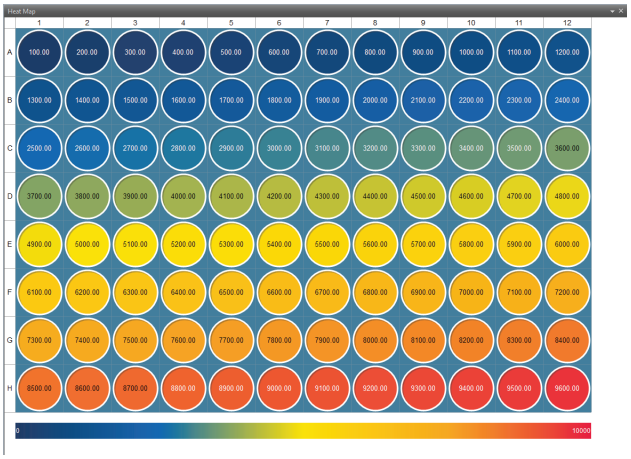


Figure 2. Consistent well-to-well results: the Attune NxT Autosampler heat map function identifies variation within a parameter across a 96-well plate. Live and heat–killed THP-1 cells were stained with 2 µg/mL propidium iodide, dispensed into a 96-well V-bottom plate, and run at a standard collection rate of 500 µL/min with 2 mix cycles per well and 2 rinse cycles between wells. Propidium iodide was excited using a 488 nm laser (640 LP). **(A)** On the heat map, a color gradient graphically represents the percentage of propidium iodide–positive cells (dead cells). Red-colored wells indicate 0% propidium iodide–positive cells (live cells) within the sample analyzed from that well; magenta-colored wells indicate a sample containing 100% propidium iodide–positive cells. **(B)** The values overlaid on each well in the heat map are the measured percentages of dead cells in the individual wells. Minimal variation is observed in propidium iodide fluorescence across each row of the entire plate, with a CV of 1.44% for the entire data set (96 wells).

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Attune NxT Autosampler product information

Physical characteristics
- Footprint (H x W x D): approximately 16 x 11 x 11 in. (40 x 29 x 29 cm)
- Weight: approximately 35 lb (16 kg)
- Operating temperature: 50–95°F (15–30°C)
- Operating humidity: <80% noncondensing
- Electrical requirements: 100–240 VAC, 50/60 Hz, <300 W

Space requirements
- Minimum width: 40 cm (15.8 in.). When attached to the Attune NxT Flow Cytometer, the total width is 167 cm (65.8 in.)
- Minimum depth: 58.5 cm (23.1 in.) provides 43.2 cm (17.1 in.) for the cytometer unit, a 10.2 cm (4 in.) ledge in front of the unit to place fluidics bottles, and 6.5 cm (2.5 in.) behind the unit for ventilation
- Minimum clear height: 74 cm (29 in.) above the mounting surface

Software/computer requirements
- Attune™ NxT Cytometric Software version 2.0 or higher
- Microsoft™ Windows™ 7 operating system

Compatible plate types
- 96 deep-well (flat, round, and V-bottom)
- 96-well standard depth (flat, round, and V-bottom)
- 384-well standard depth (flat, round, and V-bottom)
- 384 deep-well (flat, round, and V-bottom)

Processing time
- <45 minutes for 96-well plate using high-throughput mode
- <60 minutes for 96-well plate using standard mode, 2 wash cycles
- <180 minutes for 384-well plate using high-throughput mode
- <240 minutes for 384-well plate using standard mode, 2 wash cycles

Carryover
- <0.5%

Mixing cycles
- Each well mixed via full aspiration (not shaking)

Wash cycles
- User-defined number of wash cycles, dependent on plate-processing protocol and time to acquire plates

Minimum sample volume required
- 50 µL for 96-well plates

Minimum dead volume
- 30 µL

Fluidics requirements
- Onboard fluidics tanks: 800 mL total
- Capable of running four 96-well plates in standard mode with two washes per well

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

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