Application Note

Cell Analysis

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Application of NovoSampler Pro for Automatic Sample Loading and High-Throughput Analysis Using the NovoCyte Flow Cytometer

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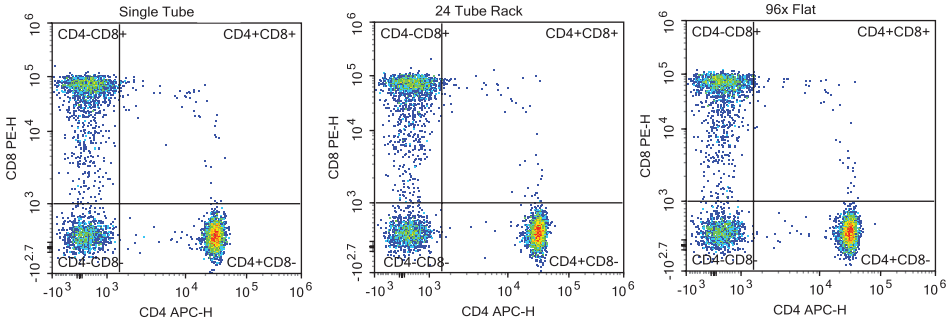
Introduction

Flow cytometry is widely used in cellular analysis, providing rapid analysis of multiple characteristics of many thousands (even millions) of single cells. With the continuous expansion of flow-based applications, there is an ever-increasing need for batch sampling and automatic processing. The Agilent NovoSampler Pro is an automatic sample loading system that can fulfill requirements for high-throughput and automated sample acquisition. The NovoSampler Pro seamlessly integrates with the Agilent NovoCyte flow cytometer, allowing easy operation and processing of multiple samples for high-performance data. It incorporates a vortex-based mixing mechanism, offering efficient mixing for various sample formats and maintaining homogeneity and integrity of biological samples. With its simple installation, easy operation, and exceptional performance, the NovoSampler Pro enhances flexibility and productivity for your flow cytometry experiments.

Materials and methods

The NovoSampler Pro delivers consistent data across different loading templates between the autosampler and individual tubes



Single tube

Gate	Absolute Count	% Lym
Lym	2,121	100.00
CD4−CD8+	667	31.34
CD4+CD8+	26.5	1.24
CD4−CD8−	437	20.41
CD4+CD8−	991	47.00

24-Tube Rack

Gate	Absolute Count	% Lym
Lym	2,095	100.00
CD4−CD8+	654	31.34
CD4+CD8+	25.2	1.22
CD4−CD8−	467	22.29
CD4+CD8−	949	45.15

96x Flat

Gate	Absolute Count	% Lym
Lym	2,162	100.00
CD4−CD8+	682	31.28
CD4+CD8+	26.5	1.13
CD4−CD8−	451	21.16
CD4+CD8−	1,002	46.44

Figure 1. Normal human peripheral blood stained with CD45/CD3/CD4/CD8 cocktail was loaded in to FACS tubes (single tube, 24-tube rack) or a 96-well flat-bottom plate (96x flat). Lymphocyte subpopulations were counted and analyzed after acquisition using the NovoSampler Pro autosampler or single tube. Statistics for each result are shown below the plots. Consistent results were obtained among the three methods of sample loading.

The NovoSampler Pro ensures thorough sample mixing with default parameter settings, while providing flexibility of custom parameter settings for different sample types

The NovoSampler Pro offers customized options for high-performance sampling. Users can adjust the vortexing speed, duration, and acceleration to optimize mixing efficiency, depending on sample type. We observed consistent counts for AccuCount Fluorescent Particles, Jurkat cells (not shown), and lysed PBMCs, with CVs of 1.9%, 3.1%, and 1.5% from 96-well plates on the NovoSampler Pro (Figure 2).

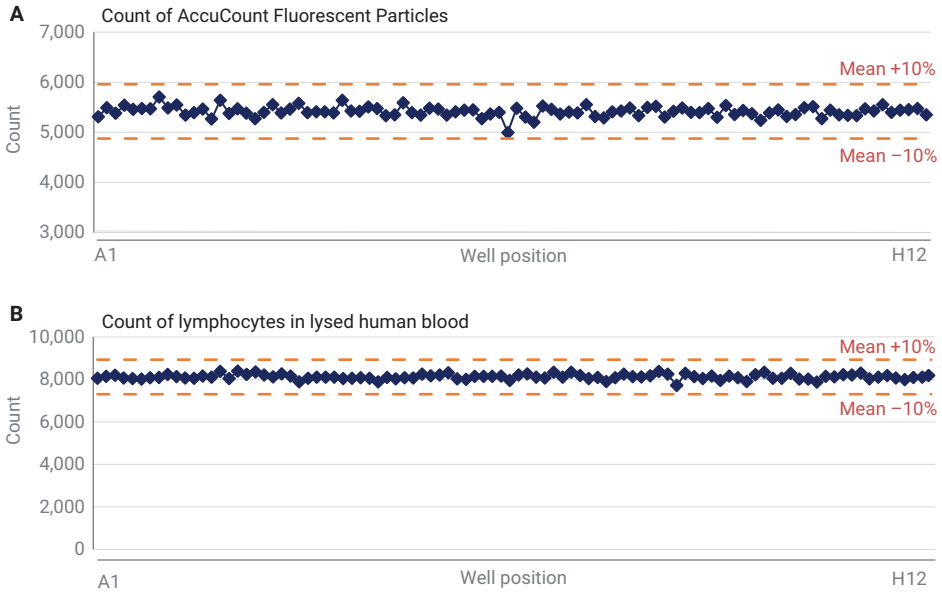


Figure 2. (A) AccuCount Fluorescent Particles (Spherotech, ACFP-50-5) diluted 10-fold with PBS containing 0.1% BSA was loaded onto a 96-well plate, and the plate was centrifuged at 1,000 rpm for 5 minutes to mimic the sample settling. Sample loading settings: 1,200 rpm, 15 seconds, stop condition of 50 µL, flow rate of 120 µL/min; (B) Normal human peripheral blood stained with CD45/CD3/CD4/CD8 cocktail. Sample loading settings: 1,200 rpm, 15 seconds, stop condition of 30 µL, sample flow rate of 66 µL /min.

The NovoSampler Pro ensures the homogeneity of a mixed sample and maintains the integrity of biological samples

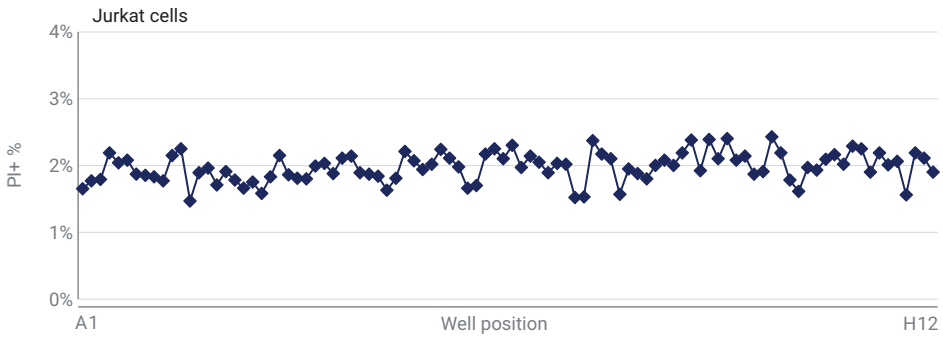


Figure 3. Cell viability of Jurkat cells stained with propidium iodide (PI) is maintained across the entire 96-well plate during mixing and sampling (Figure 3). Jurkat cells were suspended with PBS containing 0.2% BSA, stained with PI (2 µg/mL), and loaded onto a 96-well plate at 100 µL per well. Sample loading settings: default settings, stop condition of 30 µL, sample flow rate 66 µL/min.

NovoSampler Pro carryover is less than 0.1%

Table 1. The carryover of three automatic mixing groups were 0.021%, 0.014%, and 0.012%.

Sample	Count	Sample	Count	Sample	Count
ACBP 1-1	102,841	ACBP 2-1	100,370	ACBP 3-1	103,205
ACBP 1-2	99,320	ACBP 2-2	101,635	ACBP 3-2	104,101
ACBP 1-3	99,993	ACBP 2-3	103,289	ACBP 3-3	104,237
PBS 1-1	24	PBS 2-1	17	PBS 3-1	16
PBS 1-2	2	PBS 2-2	5	PBS 3-2	5
PBS 1-3	3	PBS 2-3	3	PBS 3-3	4
Carryover*	0.021%	Carryover*	0.014%	Carryover*	0.012%

* Carryover assay methods: The samples were laid out by three wells of Accucount Blank Particles (Spherotech, ACBP-50-10) and three wells of PBS at 150 µL per well. The samples were loaded using NovoSampler Pro, and the carryover was calculated using the acquired sample counts. Sample loading settings: automatic mixing settings, one mix and one wash per well, stop condition of 100 µL, sample flow rate 35 µL/min.

Results and discussion

The NovoSampler Pro seamlessly integrates with the NovoCyte flow cytometer, and supports automated analysis of a wide range of biological samples

1. **Immunophenotyping:** Immunophenotyping provides critical information of immunity abnormalities and plays an important role in mechanistic studies of diseases, such as cancer, autoimmune disorders, or immunodeficiency, and others. The NovoSampler Pro supports automated batch sample acquisition, ensures data reliability, and improves work efficiency (Tables 2 and 3).
2. **Dose-dependent effects of cell cycle inhibitors:** A common application of flow cytometry is cell-cycle analysis. It distinguishes cells in different phases of the cell cycle, aiding studies on drug efficacy and tumor cell differentiation. Etoposide, an anticancer drug, blocks metaphase while 5-fluorouracil (5-FU), a DNA/RNA synthesis inhibitor, inhibits thymidylate synthase and interferes with nucleotide synthesis. Jurkat cells treated with etoposide or 5-FU were automatically loaded using a NovoSampler Pro and analyzed on a NovoCyte flow cytometer. Etoposide and 5-FU inhibited the cell cycle of Jurkat cells in a dose-dependent manner, with etoposide blocking cells at G2/M phase (IC_{50} = 1.2 µM) and 5-FU at G0/G1 phase (IC_{50} = 82.7 µM) (Figure 4).

Cell counts (CD3+CD4+) and percentages (CD3+CD4+ over total CD45+) in human blood samples across entire 96-well plates

Table 2. Human peripheral blood samples in a 96-well plate were loaded automatically by an Agilent NovoSampler Pro. The CV of absolute CD3+CD4+ cell counting was 2.5%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	695	689	712	680	665	667	680	686	712	689	655	686
B	697	662	696	681	698	663	696	667	704	686	668	695
C	702	683	701	686	686	653	665	659	659	658	647	697
D	697	659	693	687	689	688	675	682	708	676	702	662
E	667	687	702	655	662	699	710	694	667	698	675	659
F	681	671	689	681	677	683	661	656	677	690	685	682
G	676	692	693	649	674	688	707	672	682	691	700	692
H	714	685	652	653	683	678	716	679	670	708	685	713

Table 3. Human peripheral blood samples in a 96-well plate were loaded automatically by an Agilent NovoSampler Pro. The %CV of CD3+CD4+ subpopulation over total CD45+ cells as 1.9%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	25.4%	24.6%	25.0%	24.9%	24.8%	23.6%	24.4%	25.2%	25.0%	24.8%	24.1%	25.1%
B	25.2%	24.0%	24.9%	24.7%	25.4%	24.4%	24.8%	24.3%	25.1%	25.0%	24.6%	25.3%
C	25.2%	24.7%	24.7%	24.9%	25.2%	24.0%	24.2%	24.5%	25.1%	24.5%	24.2%	25.1%
D	25.2%	24.1%	24.9%	24.9%	25.1%	24.9%	24.6%	24.9%	25.2%	24.2%	25.4%	24.2%
E	24.4%	24.8%	25.4%	24.0%	24.2%	25.3%	25.1%	24.8%	24.1%	24.7%	24.5%	24.3%
F	25.0%	24.4%	24.5%	24.9%	24.2%	24.7%	24.0%	23.7%	24.8%	25.2%	24.5%	24.8%
G	24.3%	24.3%	25.0%	23.7%	24.3%	24.9%	25.0%	24.4%	24.6%	25.2%	25.1%	25.1%
H	25.9%	25.0%	24.2%	23.9%	24.5%	25.1%	25.8%	24.5%	24.7%	25.1%	24.3%	25.7%

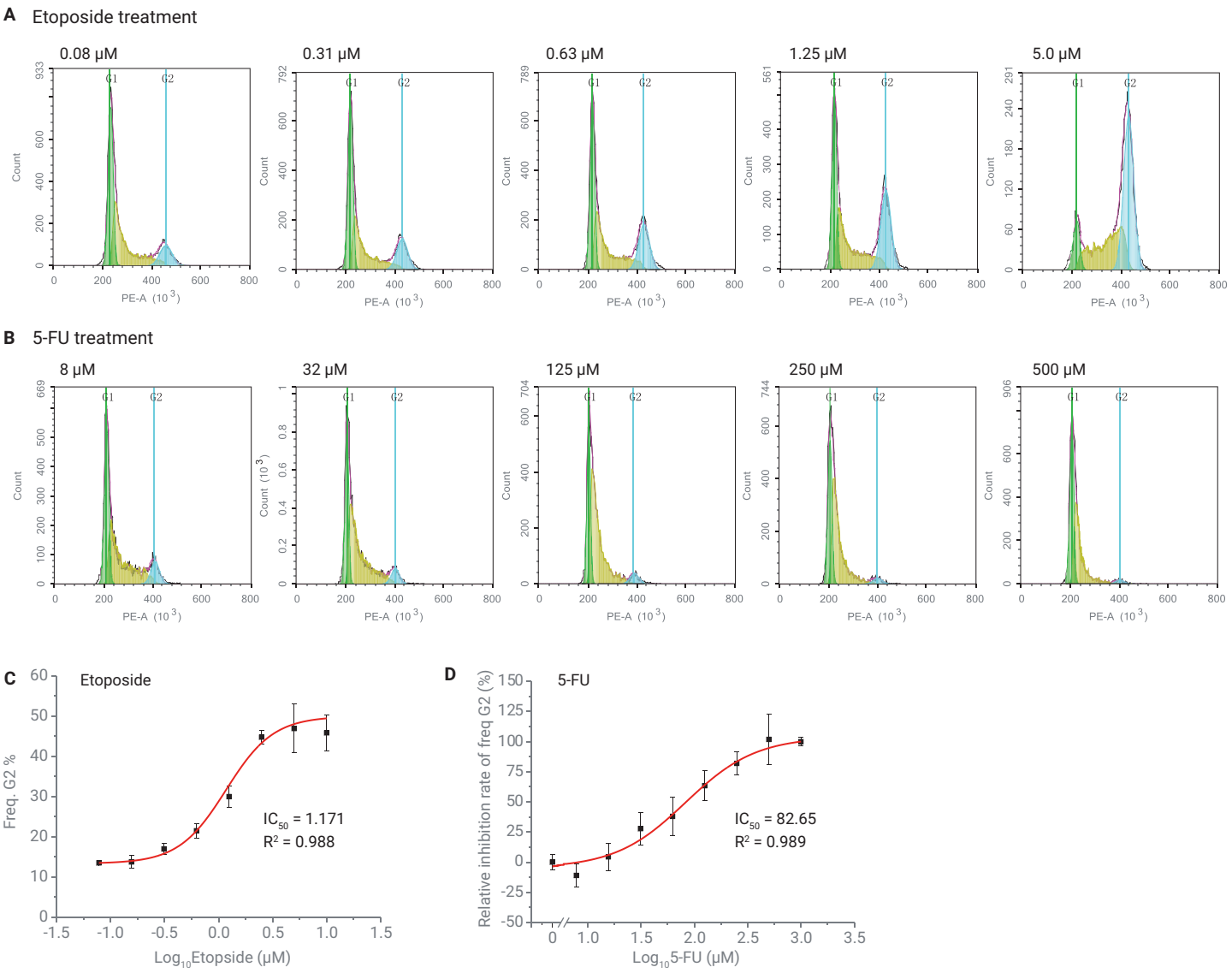


Figure 4. Dose-dependent effects of etoposide and 5-FU on Jurkat cells. Jurkat cells were seeded in a 96-well plate and treated with etoposide or 5-FU at indicated concentrations for 24 hours. The cells were fixed and stained with PI. The samples were loaded automatically by an Agilent NovoSampler Pro and analyzed on an Agilent NovoCyte flow cytometer using the automated cell cycle analysis module within the included NovoExpress software. (A) Cell cycle analysis plots of etoposide; (B) Cell cycle analysis plots of 5-FU; (C) Dose-dependent effects of etoposide on blocking Jurkat cells at G2/M phase; (D) Dose-dependent effects of 5-FU on blocking Jurkat cells at G0/G1 phase.

Conclusion

The NovoSampler Pro delivers consistent, reliable sampling data, and integrates with all models of the NovoCyte flow cytometer and NovoExpress software to provide fully automated sample acquisition using a wide variety of formats.

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