applied biosystems® by life technologies™

Microbiological applications

Using the the Attune® Acoustic Focusing Cytometer

Flow cytometry has been widely used in microbiology research, including detection and quantification of viable and nonculturable organisms [1], analysis of host-microbe interactions [2], analysis of microbial cell cycle [3], and detailed spatial and temporal analysis of microbial metabolism in different environments [4]. From simple to complex, coupled with the innovative technology of the Attune® Acoustic Focusing Cytometer, Life Technologies offers a complete solution for cytometric analysis of microbial physiology.

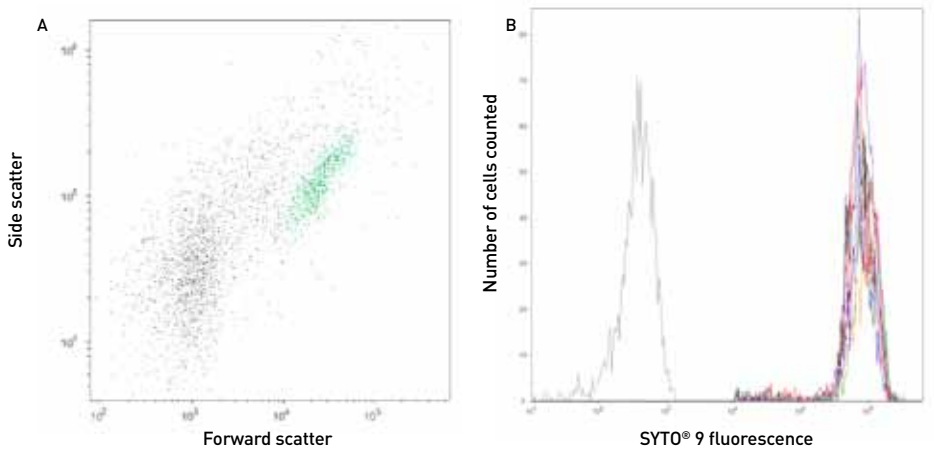

Figure 1. Consistent fluorescent detection at flow rates from 25 µL/min to 1,000 µL/min. S. aureus cells were stained with SYTO® 9 (Cat. No. S34854) and analyzed on the Attune® Acoustic Focusing Cytometer using 488 nm excitation and the 530/30 bandpass filter (BL1) to collect SYTO® 9 fluorescence emission. (**A**) Typical scatter observed using a BL1 fluorescence threshold. S. aureus cells are shown in green and have a greater forward scatter signal than electronic noise/debris. (**B**) Fluorescence histogram overlay indicating SYTO® 9 fluorescence of the S. aureus population identified in (**A**), collected at Sensitive 25 µL/min (red), Sensitive 100 µL/min (blue), Standard 25 µL/min (green), Standard 100 µL/min (black), Standard 200 µL/min (purple), Standard 500 µL/min (burgundy), and Standard 1,000 µL/min (orange) collection rates. Unstained cells are shown in grey, collected at Standard 25 µL/min. Little variation is observed across all collection rates.

Sensitive analysis for many routine microbiology applications

The Attune® Acoustic Focusing Cytometer offers many advantages over traditional hydrodynamic focusing cytometers, including precise alignment of particles at increased collection rates (up to 1,000 µL/minute). As shown in Figure 1, consistent fluorescence emission is detected in samples of fluorescently labeled *Staphylococcus aureus (S. aureus)* analyzed at all collection rates using the Attune® cytometer. In addition, the Attune® cytometer is a valuable tool for cell vitality assessment (Figures 2 and 3), membrane potential measurement (Figure 4), and cell viability assays (Figure 5). To see a protocol for each assay used in this application note, go to invitrogen.com and search by catalog number.

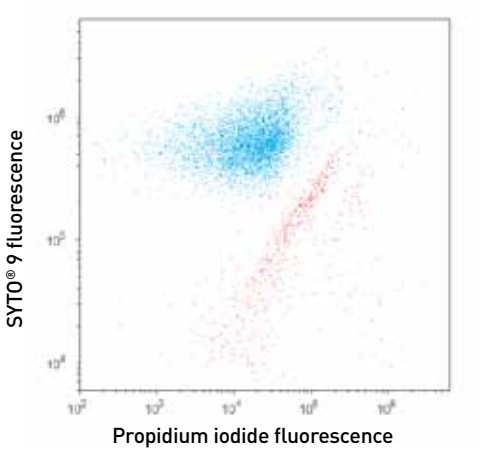

Figure 2. Analysis of relative cell viability within a bacterial culture using flow cytometry. *Escherichia coli (E. coli)* cells were stained with the LIVE/DEAD® *Bac*Light™ Viability Kit (Cat. No. L7012) before analysis using the Attune® Acoustic Focusing Cytometer equipped with 488 nm laser for SYTO® 9 and propidium iodide excitation. Samples were run at a collection rate of Standard 25 µL/min, and fluorescence emission was detected using a 530/30 bandpass filter for SYTO® 9 fluorescence and 640 longpass filter for propidium iodide fluorescence. Both live (L) and dead (D) cells fluoresce green (SYTO® 9) but only dead cells fluoresce red.

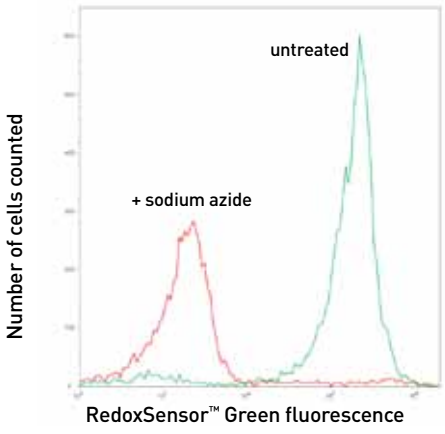

Figure 3. Analysis of relative cell vitality within a bacterial culture using flow cytometry. Untreated *E. coli* cells and cells treated with an electron transport chain uncoupler (sodium azide) were stained with the *Bac*Light™ RedoxSensor™ Green Vitality Kit (Cat. No. B34954) before analysis using the Attune® Acoustic Focusing Cytometer equipped with 488 nm laser. Samples were run at a collection rate of Standard 25 µL/min, and fluorescence emission was detected using a 530/30 bandpass filter for *Bac*Light™ RedoxSensor™ Green fluorescence. The histogram overlay indicates untreated cells have a brighter green fluorescence and greater redox potential than those treated with sodium azide.

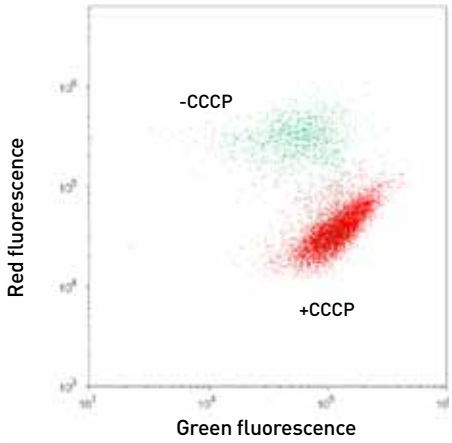

Figure 4. Analysis of relative membrane potential in an *S. aureus* culture before and after disruption with a proton ionophore. *S. aureus* cells were diluted to ~1 x 10^6 CFU/mL in PBS prior to staining with the *Bac*Light™ Bacterial Membrane Potential Kit (Cat. No. B34950) and 20 µM SYTOX® Blue (Cat. No. S34862). Samples stained with 30 µM 3,3'-diethyloxacarbocyanine iodide ($DiOC_2$) alone, and samples stained with $DiOC_2$ and treated with 5 µM carbonylcyanide 3-chlorophenylhydrazone (CCCP, for disruption of membrane potential), were analyzed on the Attune® Acoustic Focusing Cytometer equipped with 488 nm laser for $DiOC_2$ fluorescence excitation. At increased membrane potential, $DiOC_2$ molecules self-associate in the cytosol and shift $DiOC_2$ fluorescence emission from green (detected in the BL1 channel using a 530/30 bandpass filter) to red (detected in the BL3 channel using a 640 longpass filter). In this example, dead cells have been removed from analysis by excluding SYTOX® Blue–positive cells from analysis. The dot plot overlay indicates increased red-shifted $DiOC_2$ fluorescence in the untreated sample (-CCCP, green) as compared to the CCCP-treated sample (+CCCP, red).

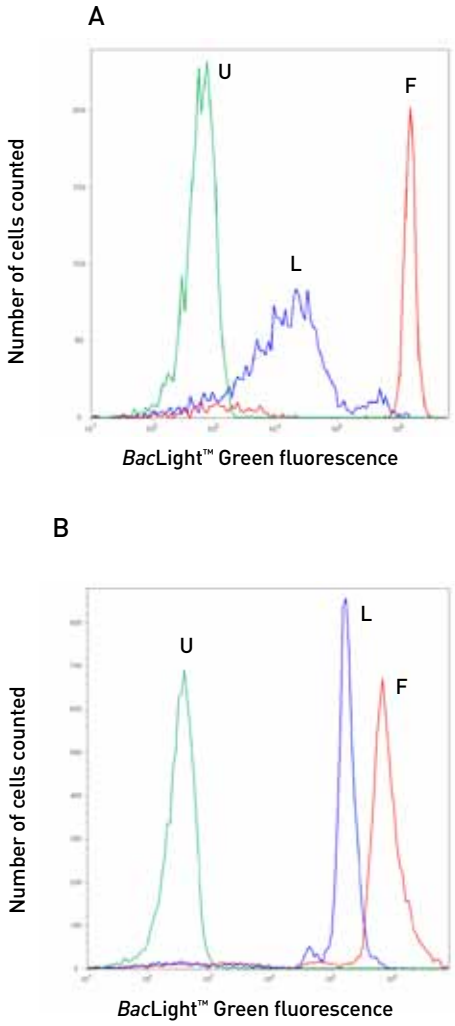

Figure 5. Staining of bacteria using *Bac*Light™ Green. Untreated and alcohol-fixed *E. coli* **(A)** and *S. aureus* **(B)** cells were stained with *Bac*Light™ Green (Cat. No. B35000) before analysis using the Attune® Acoustic Focusing Cytometer equipped with 488 nm laser. Samples were run at a collection rate of Standard 25 µL/min, and fluorescence emission was detected using a 530/30 bandpass filter for *Bac*Light™ Green fluorescence. The histogram overlays indicate that both untreated (L) and alcohol-fixed (F) gram-negative *(E. coli)* or gram-positive *(S. aureus)* cells have increased fluorescence over unstained (U) cells when stained with *Bac*Light™ Green. Fluorescence staining of fixed cells is greater than staining in both unfixed and unstained cells.

Ordering information

Application	Product	Quantity	Cat. No.
Bacterial viability	LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit	1 kit	L7007 L7012 L13152
Bacterial viability and cell counting	LIVE/DEAD® *Bac*Light™ Bacterial Viability and Counting Kit	1 kit	L34856
Bacterial cell counting	Bacteria Counting Kit, for flow cytometry	1 kit	B7277
Bacterial cell staining	*Bac*Light™ Green	20 x 50 µg	B35000
	*Bac*Light™ Red	20 x 50 µg	B35001
	SYBR® Green I Nucleic Acid Stain	500 µL	S7563
	SYTO® BC Green Fluorescent Nucleic Acid Stain (5 mM solution in DMSO)	100 µL	S34855
	SYTO® 9 Green Fluorescent Nucleic Acid Stain (5 mM solution in DMSO)	100 µL	S34854
Determination of bacterial Gram character	LIVE *Bac*Light™ Bacterial Gram Stain Kit, for microscopy and quantitative assays	1,000 assays	L7005
Microbial membrane potential/ microbial vitality	*Bac*Light™ Bacterial Membrane Potential Kit, for flow cytometry	100 assays	B34950
Microbial metabolism	*Bac*Light™ RedoxSensor™ Green Vitality Kit, for flow cytometry	1 kit	B34954
	*Bac*Light™ RedoxSensor™ CTC Vitality Kit, for flow cytometry and microscopy	1 kit	B34956

References

1. Sachidanandham R, Gin KY, Poh CL (2005) Monitoring of active but non-culturable bacterial cells by flow cytometry. *Biotechnol Bioeng* 89:24–31.
2. Hara-Kaonga B, Pistole TG (2007) A dual fluorescence flow cytometric analysis of bacterial adherence to mammalian host cells. *J Microbiol Methods* 69:37– 43.
3. Marie D, Partensky F, Jacquet S et al. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl Environ Microbiol* 63:186–193.
4. Sachidanandham R, Gin KY (2009) Flow cytometric analysis of prolonged stress-dependent heterogeneity in bacterial cells. *FEMS Microbiol Lett* 290:143–148.

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