



FLOW CYTOMETRY

CyAn™ ADP | Bacterial Viability

Assessment of bacterial viability is crucial to many applications ranging from the production of food and medicine to environmental monitoring. Traditionally, measurements have been obtained by diluting and plating of the bacterial cultures, which can take several days. Flow cytometric analysis offers accurate rapid assessment at rates up to 50,000 cells per second. Presented below is an approach using Molecular Probes LIVE/DEAD® BacLight™ Bacterial Viability Kits performed on a CyAn ADP LX 9 Color flow cytometer.

Materials and Methods

Escherichia coli was cultured at 37 °C in Tryptic Soy Broth. 1 mL was taken directly from the culture medium and centrifuged at 10,000 x g for 3 minutes, the pellet was then re-suspended in HBSS (Hank's Buffered Salt Solution) to a concentration of 1-5 x 10⁶ cells/mL.

The BacLight Kit is comprised of two DNA stains. Cell permeable SYTO 9 stains all cells and has a peak emission of 498 nm when excited by 488 nm. PI, a cell membrane impermeable stain with a peak emission of 630 nm when excited by 488 nm enters only non-viable cells with compromised membranes. 1.5 µL SYTO 9 and PI each was added to 1 mL of bacterial suspension which was then incubated in the dark for 10 minutes at room temperature before analysis on the CyAn ADP LX 9 Color. Control samples may also be prepared using known live cultures for viable and alcohol fixed cells (70% ethanol for 1 hour) for non-viable.

Results

Data was collected on a CyAn ADP LX 9 Color with standard filters using the 488 nm laser excitation. The bacteria population was isolated on a FS Log versus SS Log plot (Figure 1) using a FL1 trigger, which was then gated onto subsequent plots. The BacLight stained live/dead populations can be identified using FL1 Log (SYTO 9) versus FL3 Log (PI). Figure 2 shows the live/dead populations of an *E.coli* control sample. Figure 3 demonstrates the viability of a 7-day-old *E.coli* culture. This procedure was also tested on the malolactic bacteria *Oenococcus oeni*, its staining pattern is demonstrated in Figure 4.

Discussion

As shown here, this cell preparation and analysis is a simple and quick way to determine accurate bacterial viability. The near real time data provided expedites research and can be crucial in maintaining high volume production in industrial applications.

Figure 1

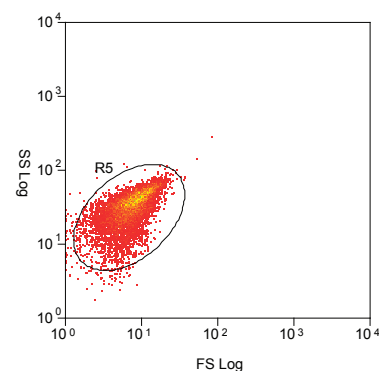


Figure 2

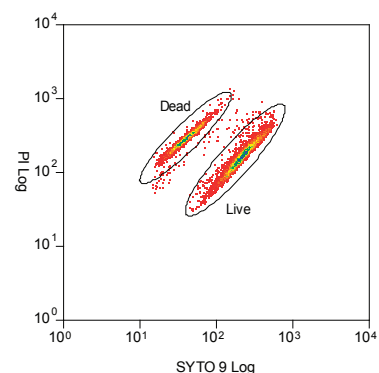


Figure 3

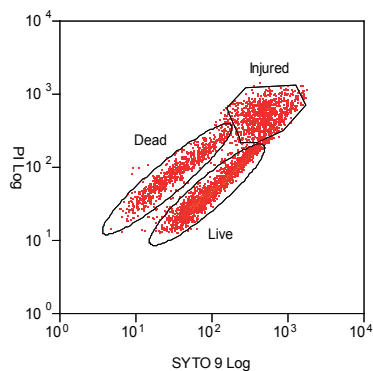


Figure 4

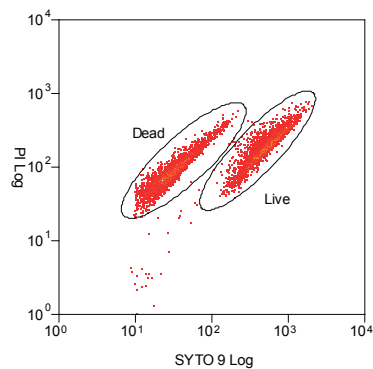
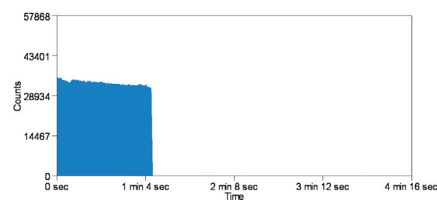


Figure 5



The acquisition rate exceeded 32,000 cells per second. This application can easily be run at high acquisition speeds. 66 seconds = 2,118,565 total events (32,099 events per second).

TECHNICAL TIPS

- > Running calibrated small particles that are similar to the size of your bacteria assists with instrument set-up. Typical cell arrangement (e.g. pairs, clusters or chains) should also be a consideration when selecting a bead size. Adjust the side scatter voltage and/or change the forward scatter ND (neutral density) filter to bring particles onto scale.
- > Analyze just the buffer solution to identify any noise it may generate, this can be reduced by gating or increasing threshold. Triggering off side scatter or a fluorescence parameter where appropriate will also reduce noise. If separation of the live/dead populations is not optimal when analyzed, try adjusting the dye ratio. Fluorescence microscopy will assist in determining that proper staining occurred and if the fluorescence of one dye is more prominent than another.
- > The flow cytometer sample injection system should be thoroughly cleaned after running SYTO 9 as it may contaminate subsequent samples. We recommend running 4 mL 70% ethanol in the acquire mode (boosting will speed up this process). To verify that proper cleaning has occurred we recommend running a log calibration particle such as 8 Peak FluoroSpheres before the experiment and after cleaning. Place bar regions around the first and last peak and verify they are the same after the clean cycle. If further ethanol cleaning is required, prepare fresh calibration particles for verification.

References

Stocks S.M. 2004. Mechanism and use of the Commercially Available Viability Stain, BacLight. *Cytometry Part A* 61A: 189-195.

PRODUCT

CyAn ADP LX 9 Color	CY201
8 Peak FluoroSpheres	K0112
Hank's Buffered Salt Solution	S1754

CODE

For research use only – not to be used in diagnostic procedures. Other vendor products used in this application: Molecular Probes and Sigma-Aldrich.

The protocols in this application note might deviate from the normal recommended protocol/specification guidelines which are included with the Dako product or any other non-Dako product.