CyAn™ ADP Yeast Viability

Introduction

Microorganisms such as yeast are utilized in many biotechnology applications, including genetic engineering, vaccine development and the production of food. Viability analyses of cultures are critical to many of these applications. Traditionally, a manual method using a hemocytometer and a microscope has been used to check viability of stock cultures.[1] This manual procedure method is cumbersome and time consuming. Results are subjective and dependent on the technician's level of training and experience. Flow cytometric analysis offers an easy to use, accurate and rapid assessment at rates of up to 50,000 cells per second. Using a flow cytometric approach, rapid and accurate analysis can provide real time data for high volume production applications such as the brewing industry and other commercial fermentation processes. Presented are two yeast viability methods using the yeast Saccharomyces cervisiae performed on a Beckman Coulter CyAn ADP 9 Color flow cytometer.

Materials and Methods

In the first method, yeast viability was assessed with LIVE/DEAD BacLight Bacterial Kits.* In the second method, viability was assessed with FUN1* cell stain. In both methods, the yeast Saccharomyces cervisiae was cultured in YPD (yeast extract, peptone, dextrose) broth at 25°C for 24–48 hours. Non-viable control cells were prepared by exposure to 70°C heat.

The BacLight Bacterial Kit is comprised of two nucleic acid stains, SYTO 9 and Propidium Iodide (PI). SYTO 9 is a cell permeable stain and has a peak emission wavelength of 498 nm when excited by the 488 nm laser. Non-viable cells with compromised membranes are stained by Propidium Iodide (PI), a live cell impermeable stain with a peak emission wavelength of 630 nm when excited by 488 nm. In the proper ratio, a mixture of these two dyes will fluoresce undamaged, intact cells as bright green (498 nm) and cells with damaged membranes (dead or dying) as red (630 nm) under a microscope.

The yeast culture was centrifuged at 10,000 x g for 5 minutes; the pellet was then re-suspended in PBS to a concentration of 1 x 10^6 cells/mL. A solution containing 100 µmol/L SYTO 9 and 600 µmol/L PI in PBS was prepared from the BacLight Kit, according to the manufacturer's recommendation, and was mixed with an equal volume of the yeast solution. After incubation in the dark at room temperature for 10 minutes, the sample was ready for flow cytometric analysis.

FUN1 is a membrane permeant, nucleic acid binding dye that initially stains both live and dead cells bright green (530 nm) when excited by 488 nm. However, after appropriate incubation, the dye is converted by metabolically active cells into an orange-red fluorescent product. This metabolic conversion occurs intracellularly in Cylindrical Intra Vacuolar Structures (CIVS). CIVS are easily viewed by fluorescence microscopy but difficult to detect by flow cytometry. Production of CIVS by viable cells removes the bright 530 nm fluorescence that is retained by non-viable cells so the two populations can be clearly distinguished. The cell suspension was centrifuged at 10,000 x g for 5 minutes. The pellet was then resuspended in a Glucose HEPES solution (0.2 µm-filtered water containing 2% D-(+) - glucose and 10 µmol/L Na-HEPES; pH 7.2) to a concentration of 1 x 10^7 cells/mL. 5 µmol/L of the stain was added to the yeast suspension which was then incubated at 30°C for 30 minutes prior to analysis.

Results

Data was collected on a CyAn ADP 9 Color with standard filters, using the 488 nm excitation for both methods. The yeast population was isolated on a forward scatter (FS) log verses side scatter (SS) log dot plot and then gated onto subsequent dot plots (Figure 2a).

*LIVE/DEAD BacLight Kits and FUN1 are registered trademarks of Molecular Probes, Inc.

The BacLight Kit stained live/dead populations can be identified using either SYTO 9 (log FL1) versus PI (log FL3) (Figure 1a) or SYTO 9 log versus side scatter log (Figure 1b) dotplots.

The difference in live and dead yeast cells stained by FUN1 is demonstrated by the dotplot FUN1 (log FL1) versus side scatter log is shown in Figure 2b

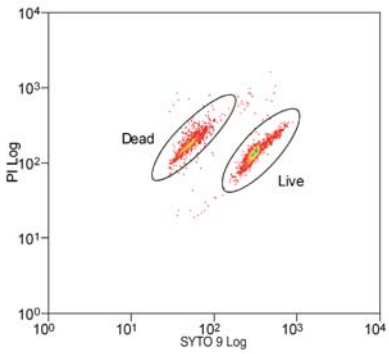

Figure 1a

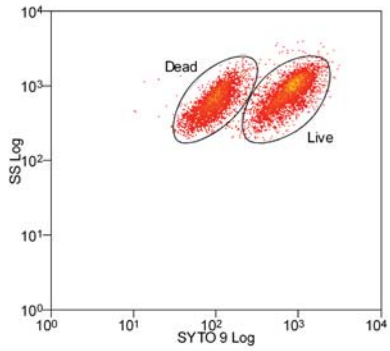

Figure 1b

Discussion

Flow cytometric assessment of yeast cultures stained with the BacLight Kit as described in this application note provided viability results that deviated by 1.4% when compared to microscopic quantification of a traditional methelyne blue stain method.[2]

When comparing BacLight and FUN1, the resulting percentages of the live/dead populations were within 1% of each other when analyzed on a CyAn ADP.

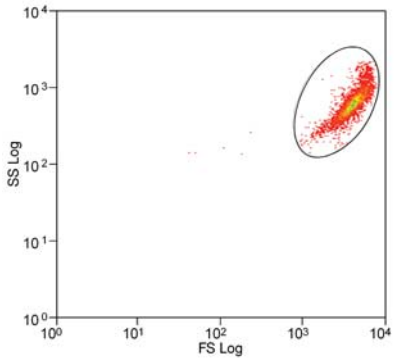

Figure 2a

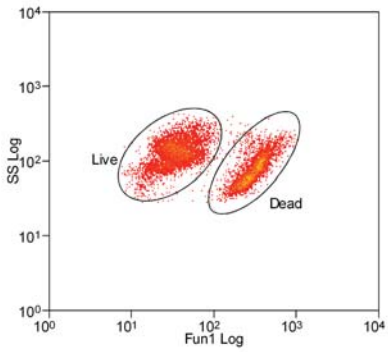

Figure 2b

The BacLight Kit method provides faster results than FUN1. The FUN1 method is easy to prepare and analyze, as it is a single stain.

Technical Tips

- Scatter parameters should be set to log to isolate small particles such as yeast. Running size calibrated particles may assist with voltage adjustment. Run just the buffer solution to identify any "noise" it may generate. "Noise" from the buffer can be reduced by gating or increasing threshold. A side scatter trigger can also be used to reduce noise.
- If separation (live and dead) is not observed as expected in the dot plot, check the sample under the microscope to ensure proper staining occurred. For example, improper incubation or dye loading of FUN1 into the cells can restrict CIVS formation and may leave viable cells with some green fluorescence.
- 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 of 70% ethanol in acquire mode (boosting will speed up this process). To verify 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 to verify they are the same before and after the clean cycle. If further ethanol cleaning is required, be sure to prepare fresh calibration particles for verification.

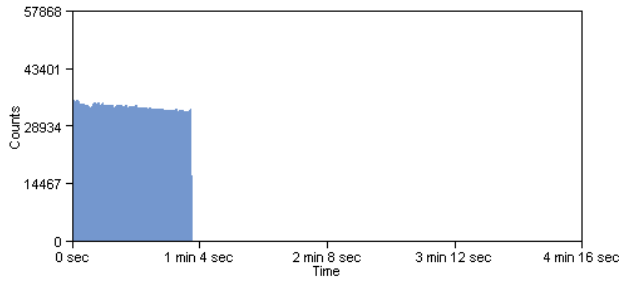

Figure 5

The acquisition rate exceeded 33,000 cells per second. This application can easily be run at high acquisition speeds. 59 seconds = 1,947,429 total events (33,000 events per second).

References

1. Smart K, Chambers, KM, Lambert I, Jenkins C. 1999. Use of Methylene Violet Staining Procedures to Determine Yeast Viability and Vitality. Am. Soc. Brew. Chem. 57(1):18-23.
2. Zhang T & Fang HHP. 2004. Quantification of Saccharomyces cerevisiae viability using BacLight. Biotechnology Letters 26: 989-992.

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