

Bacteria Counting Kit

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYTO® BC bacteria stain (Component A)	100 µL	1000X in DMSO	<ul style="list-style-type: none"> • 2–6°C • DO NOT FREEZE • Protect from light 	When stored as directed the kit is stable for at least 6 months.
Microsphere standard, 6 µm (Component B)	1 mL	1 × 10 ⁸ beads/mL in distilled water containing 2 mM sodium azide		

Number of assays: Sufficient material is supplied for approximately 100 flow cytometric assays.

Approximate fluorescence excitation and emission maxima: 480/500 nm for SYTO® BC stain bound to DNA.

Introduction

Accurate enumeration of bacteria in a sample is an important aspect of many experimental procedures in biotechnology. Molecular Probes' researchers have now devised a kit to perform such tests with the aid of a flow cytometer. The Bacteria Counting Kit provides our SYTO® BC bacteria stain, as well as a suspension of polystyrene microspheres that serve as a reference standard to indicate sample volume. The SYTO® BC stain is a high-affinity nucleic acid stain that easily penetrates both gram-positive and gram-negative bacteria and results in an exceptionally bright green fluorescent signal. The calibrated suspension of beads in the microsphere standard has size characteristics and relatively low fluorescence appropriate for use in combination with any type of bacteria that may be present in the sample. The Bacteria Counting Kit is particularly valuable for monitoring antibiotic sensitivity, as it provides a convenient and accurate means for assessing changes in a bacterial population over time. A sample of the population is simply diluted, stained briefly with the SYTO® BC dye, mixed with a fixed number of microspheres and applied to a flow cytometer for analysis. Bacteria and microsphere particles are easily distinguished in a plot of forward scatter versus fluorescence (Figure 1); the density of bacteria in the sample can be determined from the ratio of bacterial signals to microsphere signals in the cytogram.

Before You Begin

Caution No data are available addressing the mutagenicity or toxicity of the SYTO® BC stain. Because the reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Please exercise appropriate care and judgment when using this reagent, and dispose of the stain in compliance with all pertaining local regulations.

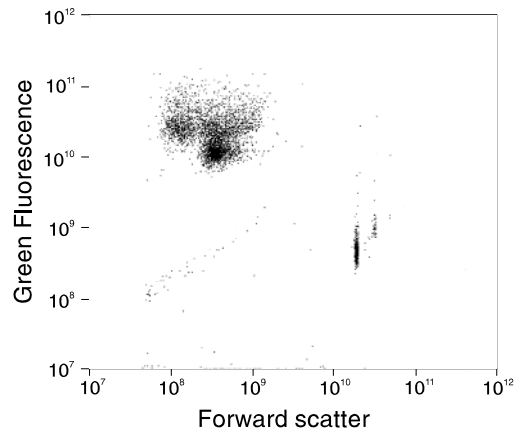


Figure 1. Flow cytometric enumeration of *Bacillus cereus* using the Bacteria Counting Kit. In this plot of forward scatter versus fluorescence, signals in the upper lefthand frame represent bacteria stained with SYTO® BC bacteria stain; signals in the lower righthand frame represent microsphere particles, which serve as a standard used to indicate sample volume.

Experimental Protocol

The following protocol can be adapted for many types of bacteria. Growth medium, cell density, the presence of other cell types and other factors may influence staining. In general, best results are obtained using diluted low-phosphate medium or buffers that do not contain phosphate. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present. All glassware should be washed in a mild detergent and rinsed thoroughly with hot tap water followed by several rinses with deionized, distilled water.

Growth Medium and Diluent

For a bacterial growth medium, we recommend tryptic soy broth (TSB, DIFCO Laboratories). Other low-phosphate media may also be suitable. Filter the medium through a sterile 0.2 µm-pore size filter to remove particulates.

For diluting the bacterial cultures, prepare 5% TSB in 0.15 M NaCl (saline). For example, mix 5 mL of TSB and 95 mL of 0.15 M NaCl and filter through a sterile 0.2 µm-pore size filter.

Sample Preparation

Note: Prior to opening, warm the kit vials to room temperature and centrifuge briefly.

- 1.1 Pregrow bacteria in filter-sterilized TSB medium.
- 1.2 Dilute the bacterial culture with filter-sterilized 5% TSB, 0.15 M NaCl, serially if necessary, to a final density of about 10^6 cells/mL. A 1.0 mL volume will be used for each assay.
- 1.3 Add 1 µL of SYTO® BC bacteria stain (Component A) to 1.0 mL of the diluted cells. Incubate at room temperature, or up to 37°C, for at least 5 minutes.
- 1.4 Thoroughly resuspend the microsphere standard suspension (Component B) by sonication in a waterbath for about 5 minutes. Add 10 µL of the microsphere suspension to the stained cell preparation, mix well and analyze by flow cytometry.

Analysis by Flow Cytometry

- 2.1 Stained bacteria can be assayed in a flow cytometer equipped with a laser emitting at 488 nm. Typical laser settings are 100 mW in the light-regulation mode. Fluorescence is collected

in the fluorescein channel; filters used for detecting fluorescein (e.g., 530 ± 10 nm bandpass filter) are generally suitable. The forward scatter and fluorescence should be collected with logarithmic signal amplification.

- 2.2 Instrument adjustments are especially critical for detecting relatively small particles such as bacteria. To avoid contamination of the data by electronic noise, we recommend the following procedure for instrument setup. Stain a sample of bacteria with the SYTO® BC dye, but do not add the microspheres. Acquire signals with the forward light scatter amplifier set to logarithmic amplification and the side light scatter (90°) on a suitable level of linear amplification. Set the amplification of the signals from forward and side scatter such that the bacteria are in the middle of the data space. We recommend using side scatter as the parameter for setting the acquisition trigger. Adjust the trigger level (also named “threshold level” on some instruments) to minimize electronic noise appearing on the monitor. To check for exclusion of electronic noise, briefly interrupt the sample flow; if the instrument is correctly adjusted, the signal rate should drop to nearly zero. Set the amplification of the fluorescein fluorescence channel such that the signals from the bacteria appear in the top range of the signal axis.
- 2.3 After adjusting the flow cytometer as described above, apply an experimental sample (stained bacteria plus microspheres) and process the data by framing two regions in the forward scatter versus fluorescence cytogram (see Figure 1). Counting the number of signals in the microsphere frame (lower right) provides an accurate estimate of the volume analyzed in the data file. The microsphere standard (Component B) contains 1.0×10^8 beads/mL; thus, after ~100-fold dilution into the stained cell preparation, the microsphere density is 1.0×10^6 beads/mL, and one bead represents 10^{-6} mL. The number of signals in the bacteria frame (upper left) divided by the number of signals in the microsphere frame provides the number of bacteria per 10^{-6} mL of the sample.
- 2.4 In the flow cytometer, bacteria are identified solely on the basis of their size and stainability with the SYTO® BC stain. *We recommend that you inspect each sample by fluorescence microscopy to confirm that the particles detected are indeed bacteria.*
- 2.5 Note that with the long data-acquisition times required for very dilute bacteria samples, the number of noise signals acquired in the bacteria frame may become significant. It may be useful to run a control sample—exactly like the experimental sample but lacking bacteria—for the same time duration. The results attained with the control sample provide an estimate of the noise contribution in the experimental data files.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
B7277	Bacteria Counting Kit *for flow cytometry*	1 kit

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