Introducing BD FACS™ EGFP Calibration Beads—the ultimate in calibration standards for flow cytometry. BD FACS EGFP Calibration Beads consist of five preparations of microbeads manufactured by Bangs Laboratories, Inc., exclusively for BD Biosciences Clontech. One preparation is an unlabeled blank; the remaining four are labeled with increasing densities of BD Living Colors™ Enhanced Green Fluorescent Protein (EGFP). Using these standards, you can actually quantify your EGFP flow cytometry data—enabling you to compare results between instruments, samples, or experiments.

Microbead technology enhances the utility of BD Living Colors™ EGFP

Bangs Laboratories meticulously selects its microbeads for uniform size and shape so that all microbeads within a standard set produce the same amount of light scatter. The four EGFP-labeled bead populations span a range of molecules of equivalent soluble fluorochrome (MESF), which correspond to increasing EGFP densities. By converting fluorescence intensities of cell population readings to MESF, you can compare data from different instruments and experiments (1, 2). You can also determine the linearity and stability of your flow cytometer’s readouts.

The blank population (unlabeled microbeads) emits a low level of autofluorescence (Figure 1), a signature similar to that of unstained cells. This blank allows you to measure the fluorescence detection threshold and the background noise level of your flow cytometer.

• Quantify the fluorescence intensity of biological samples
• Compare results between instruments, samples, or over time
• Determine the fluorescent threshold of your flow cytometer

Figure 1. Log fluorescence histogram of BD FACS™ EGFP Calibration Beads. When the standards are run through the flow cytometer, a log histogram of the fluorescent peaks can be created by gating around the singlet populations of beads. Each peak has a mean fluorescence value that should be consistent for those flow cytometer settings.

Figure 2. MESF standard curve. Mean fluorescence for each BD Living Colors™ EGFP standard is determined from the peaks of the log fluorescence histogram (Figure 1). By plotting these values versus the MESF for EGFP, you can generate a standard curve for determining MESF values of unknown samples. Representative MESF values are shown; actual values for each lot of beads are reported on the bottle labels.

Using BD FACS™ EGFP Calibration Beads

To use BD FACS EGFP Calibration Beads in your experiments, run the blank and four standards on your flow cytometer, producing five separate peaks of fluorescence as shown in Figure 1. Then, within the forward vs. side scatter plot, gate the singlet population of beads and use the average fluorescence intensity of these peaks to generate a standard curve (Figure 2). You can use this standard curve to calculate the MESF values for your unknown samples and quantify the actual number of fluorochromes in each cell.

References

Notice to purchaser for BD Living Colors™ Products

Use of BD Biosciences Clontech’s BD Living Colors™ products containing DNA sequences coding for mutant Aequorea victoria green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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