## Flow Cytometer Calibration Beads Protocol-At-A-Glance

This protocol describes how to calibrate flow cytometers with 488 nm laser lines that excite the green fluorescent proteins AcGFP1 (*Aequorea coerulescens* GFP) and EGFP, or flow cytometers with 561 nm laser lines that excite the red fluorescent protein, mCherry. Calibrate 488 nm laser lines using the **AcGFP Flow Cytometer Calibration Beads** (Cat. No. 632594). Calibrate 561 nm laser lines using the **mCherry Flow Cytometer Calibration Beads** (Cat. No. 632595).

## **Protocol: Preparing Calibration Beads for Flow Cytometer Analysis**

- 1. Add 1 ml of 1X Flow Cytometer Calibration Beads Dilution Buffer to a standard flow cytometer sample tube (BD Falcon 5 mL Polystyrene Round-Bottom Tubes, Disposable, VWR Cat. No. 60819-310).
- 2. Invert the stock tube of Calibration Beads 5 to 10 times to resuspend the beads.
- 3. After the beads are fully resuspended, transfer 20  $\mu$ l of the bead suspension to the flow cytometer sample tube containing the dilution buffer.
- 4. Cap the sample tube and invert it 5 times to mix the diluted bead suspension.
- 5. Insert the sample tube into the sample port of the flow cytometer and run an analysis on the instrument.

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