# Quantum MESF Kits

| Catalog # | Description |
|---|---|
| FCSC555PA | Quantum FITC-5 MESF (premixed), 20 tests, 2 x 1 mL bottles |
| FCSC555PB | Quantum FITC-5 MESF (premixed), 100 tests, 2 x 5 mL bottles |
| FCSC555A | Quantum FITC-5 MESF, 20 tests, 6 x 1 mL bottles |
| FCSC555B | Quantum FITC-5 MESF, 100 tests, 6 x 5 mL bottles |
| FCSC827A | Quantum R-PE MESF Medium Level, 20 tests, 5 x 1 mL bottles |
| FCSC827B | Quantum R-PE MESF Medium Level, 100 tests, 5 x 5 mL bottles |

**For Research Use Only. Not for use in diagnostic procedures.**

## Description

Quantum Molecules of Equivalent Soluble Fluorochrome (MESF) kits enable the standardization of fluorescein isothiocyanate (FITC) or phycoerythrin (PE) fluorescence intensity values for quantitative fluorescence cytometry. With this approach, bead controls are acquired to generate a calibration curve relating median fluorescence intensity (MFI) values to standardized fluorescence intensity units, i.e., MESF. This allows MESF values to be determined from cell samples stained with antibodies conjugated to the same fluorophore.

In addition to quantitative fluorescence cytometry, Quantum MESF kits may be used to assess the detection threshold, resolution, and linearity of the relevant detector. A free QuickCal template is provided with each kit to aid in determining cellular expression levels and for evaluating instrument linearity and detection threshold.

## Ordering Information

Quantum MESF kits can be ordered online at **bio-rad-antibodies/mesf-beads**

## Storage and Stability

Store all kit components at 2–8°C in the original opaque bottle and protected from light. Stability is guaranteed until the expiration date indicated on the product label.

## Kit Contents

The kit bottles contain blank microbeads or prestained microbead populations surface-labeled with increasing amounts of FITC or PE, with each population calibrated in MESF units. Bead concentrations are approximately 2 x $10^6$ beads/mL.

Quantum FITC-5 MESF and R-PE MESF Medium Level kits include individual bottles of each stained bead population. The Quantum FITC-5 MESF (premixed) kits include two bottles: one containing the blank bead population and another containing five fluorescent bead populations combined.

An access code for downloading an appropriate QuickCal analysis template is also provided.

## Reagents and Equipment

- Cell staining buffer, e.g., phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA) or equivalent

## Protocol

### General Considerations

- Remove the bottles from refrigerator storage and return them as quickly as possible (<5 min) to minimize temperature cycling effects
- Manually shake the bottles to ensure uniform microsphere suspensions. Do not vortex or sonicate the bottles
- Prepare all suspensions immediately prior to use
- Use the cell staining buffer to dilute the beads and resuspended cells. As the fluorescence intensity of fluorophores can be highly dependent upon the pH, ionic strength, etc., of the buffer, use the same buffer for samples
- Protect the beads from light to guard against photobleaching
- Acquire the beads and stained cell samples on the same day, on the same instrument, and using the same fluorescence (photomultiplier tube, PMT) settings. The forward and side scatter (FSC, SSC) settings may be adjusted to optimally visualize bead and cell populations
- Bead populations may be run individually (standard kits) or as mixed populations (premixed kits). If the bead population resolution is suboptimal for the standard kits, running the populations individually will ensure accurate gating

## Experimental Steps

1. Add one drop of reference blank beads "B" to 400 µL cell staining buffer.
2. Acquire the beads on the flow cytometer. Adjust the flow rate or suspension concentration to achieve a count rate of 100 beads/sec.
3. Gate around the bead population using an FSC vs. SSC dot plot (Figure 1).
4. Create a fluorescence histogram for the appropriate detector of the events in the bead gate. The histogram should be positioned to start around the origin; adjust the voltage if necessary (Figure 1).

   **Note:** The excitation/emission maxima of FITC and PE are 490 nm/525 nm and 496 nm/578 nm, respectively.
5. Add prestained bead populations to 400 µL of cell staining buffer. For premixed beads, add 1 drop to the buffer. For non-premixed beads, add 1 drop of each separate stained bead sample to the buffer.
6. Acquire the prestained beads on the flow cytometer. If needed, adjust the voltage so that the samples are on scale.

   **Note:** The blank population will also need to be acquired again if the settings are altered. Separate fluorescence peaks should be observed (Figure 2).
7. Cells should be stained with the antibody of interest conjugated to the same fluorophore as the stained beads. Acquire the stained cell samples on the flow cytometer. Suitable controls (e.g., unstained cells) should be run to aid in interpreting the fluorescence intensity results.

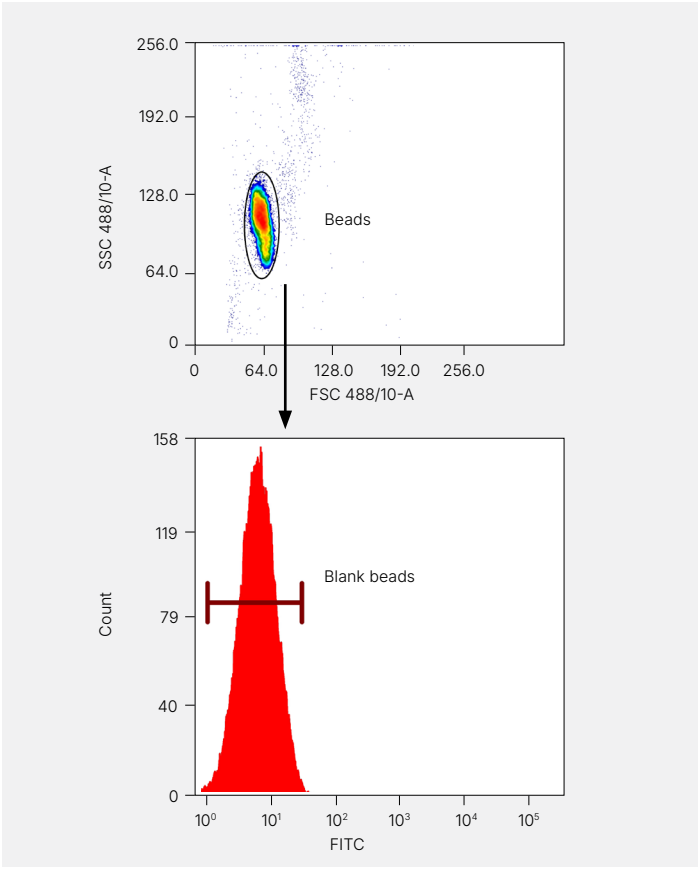


**Fig. 1. Blank bead population identification.** Data were generated using the blank bead population from the Quantum FITC-5 MESF (FCSC555A) kit. The arrow shows the gating strategy. FITC, fluorescein isothiocyanate; FSC, forward scatter; SSC, side scatter.

# Analysis

1. Download the QuickCal analysis template at **bangslabs.com/quickcal**. Use the QuickCal access number in your Quantum MESF kit. The software is used to:
   - Generate a calibration curve
   - Determine detection threshold
   - Determine linearity
   - Quantitate the fluorescence intensity of stained cell samples
2. Determine the MFI of the blank and each prestained bead population, as shown in Figure 2, in the fluorophore target filter (FITC or PE).
3. Enter the MFI values of the bead populations into the channel column of the QuickCal template. A calibration curve and regression coefficient ($r^2$) value and detection threshold value will be calculated (Figure 3). For accurate MESF assignments, instrument linearity must be assured. A regression coefficient ≥0.99 is required for accurate results.
4. Determine the MFI of the positive population of stained cells in the fluorophore target filter (FITC or PE).
5. Enter the MFI into the channel column in the sample section of the QuickCal template. The MESF value of each sample will be determined automatically.

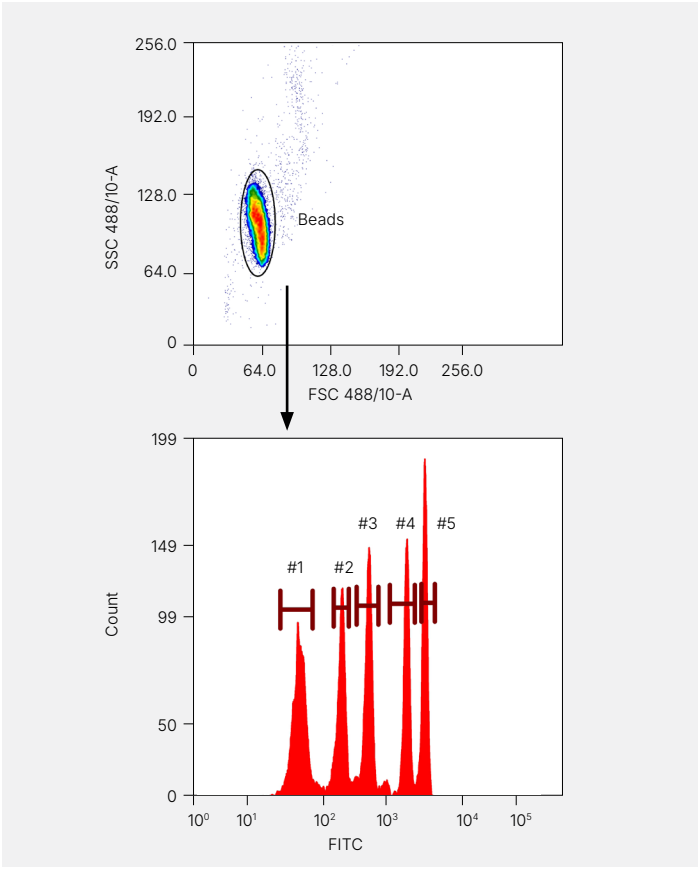


**Fig. 2. Stained bead population identification.** Data were generated using the five stained bead populations from the Quantum FITC-5 MESF (FCSC555A) kit. The arrow shows the gating strategy. FITC, fluorescein isothiocyanate; FSC, forward scatter; SSC, side scatter.

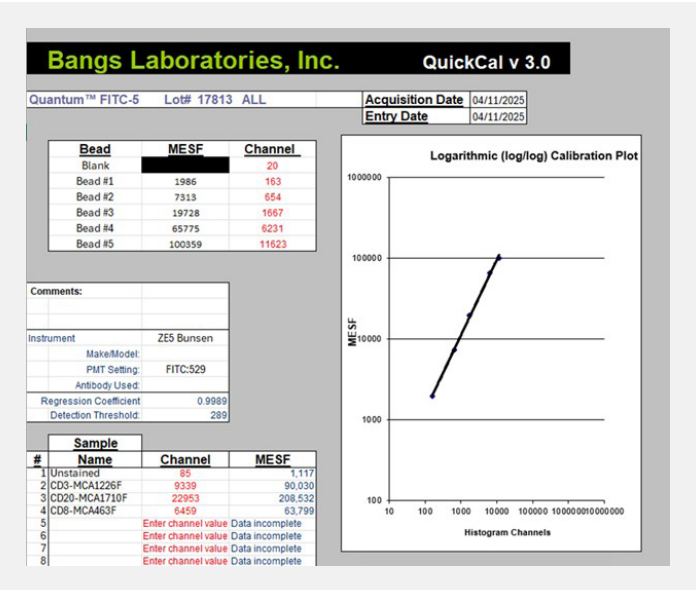


**Fig. 3. Example QuickCal analysis.** MFI values determined from the bead populations and unknown cell samples are shown in red. MESF values from the cell samples are shown in blue. FITC, fluorescein isothiocyanate; MESF, molecules of equivalent soluble fluorochrome; MFI, median fluorescence intensity; PMT, photomultiplier tube.

## Using MESF Beads for Monitoring Flow Cytometer Performance

Quantum MESF kits may also be utilized to monitor instrument performance. The data can be graphed in a Levey-Jennings chart to identify changes in performance over time. Follow these steps:

- The blank and prestained beads are prepared as described above
- Acquire beads at different PMT settings (low, medium, and high), ensuring that all bead populations remain on scale
- Record the channel values of the bead populations for each different PMT setting and complete a QuickCal template
- Linear regression values should be comparable for each setting. Poor or disparate $r^2$ values may identify an issue with the cytometer. MESF values at each PMT setting can also be monitored over time to identify cytometer changes

Contact the technical services team at **bio-rad.com/Contact-Us** for further assistance.



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