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**B E A D S ● A B O V E T H E R E S T™**

Description

Quantum FITC-5 MESF kits are used in the quantitation of FITC fluorescence intensity in Molecules of Equivalent Soluble Fluorochrome (MESF) units. When used in conjunction with Simply Cellular® microspheres, Quantum FITC-5 MESF kits also allow quantitation of Antibody Binding Capacity (ABC).

Quantum FITC-5 MESF kits are comprised of 6 populations of microspheres: 5 populations having different levels of FITC fluorescence intensity and one blank population. The Quantum FITC-5 MESF kits have excitation and emission spectra matching those of cell samples labeled with FITC. The microsphere standards are suspended in sterile filtered, pH buffered PBS solution with a preservative.

The kit allows for the direct quantitation of the fluorescence intensity of a sample in terms of MESF units. Once flow cytometry results have been converted to MESF units, samples from different instruments may be accurately compared. The fluorescence intensity of each of the labeled populations in the Quantum FITC-5 MESF kit has been calibrated against solutions of laser grade fluorescent dye in units of MESF FITC per microsphere. The blank population is used to measure the fluorescence detection threshold of the instrument at test-specific instrument settings. Correct use of the Quantum FITC-5 MESF kit allows for: 1) quantitation of fluorescence intensity of samples in terms of MESF, 2) determination of instrument fluorescence detection threshold, 3) determination of instrument linearity, and 4) data comparison over time and between multiple instruments.

Characteristics

Mean Diameter: 7-9µm
Particle Concentration: 2 x 10⁶ microspheres/mL

Material

Material Supplied

- Quantum FITC-5 MESF microsphere kit: 6 bottles included (5 labeled, 1 blank)
- QuickCal® Template: Download from www.bangslabs.com using the access code provided at the time of kit purchase.

Material Required

- Cell samples
- Cell suspension solution
- Sample test tubes
- Vortex mixer
- Flow cytometer

Procedure

Researchers are advised to optimize the use of particles in any application. Prepare all suspensions immediately prior to use. The standards should be analyzed on the same day and at the same fluorescence (PMT and compensation) settings used to analyze the samples you wish to quantitate.

1. Briefly vortex the bottles to ensure uniform suspensions of microspheres. Do not sonicate.
2. Add 1 drop of the blank to 0.5mL of the same type of buffer or medium in which the cell samples will be suspended.
3. Analyze the microspheres on the flow cytometer. Adjust the flow rate or suspension concentration such that the count rate is

optimal for your instrument. A count rate of 100 beads per second is recommended.

4. Using the forward scatter versus side scatter dot plot, construct a live gate around the singlet population of the blank. (Figure 1)
5. Create an FL1 (FITC) histogram, including only the events falling in the singlet gate of the forward scatter versus side scatter dot plot.
6. Verify that the blank appears near the origin of the histogram. (Figure 2)
7. Combine 1 drop of each of the 5 fluorescence intensity populations to 0.5mL of the same type of buffer for analysis. Analyzing each intensity separately is also an option. We recommend running the reference blank separate from the labeled populations.
8. Analyze the microspheres on the flow cytometer. When establishing a calibration plot, make no further adjustments to the fluorescence settings (e.g., amplifier gains, PMT voltages, compensation, etc.). Record the peak (e.g. median or geo mean) channel value for each population.
9. Log into www.bangslabs.com/products/quickcal to use Bangs Laboratories' quantitative software, QuickCal, to establish a calibration curve, determine the instrument detection threshold, and quantitate the fluorescence of samples. To access this free service, you will need the QuickCal access number affixed to your Quantum FITC-5 MESF kit. See Product Data Sheet 819 for detailed QuickCal instructions.
10. If internet access is not available, the following steps may be used for manual generation.
 - a. *Establishing a Calibration Curve:*
 1. Establish a standard calibration curve by plotting the MESF (y-axis) versus the peak channel (x-axis) for each of the 5 fluorescence intensity populations. *Note:* If linear fluorescence is selected, a log-log plot of the data should give a 45° line. If log fluorescence is selected, the data should be plotted on a semi-log paper, and may not fall on a 45° line. The actual slope obtained will be characteristic of the particular log amplifier and PMT.
 - b. *Determining the Instrument Detection Threshold:*
 1. After completing the fluorescence intensity calibration and establishing a calibration curve procedure, determine and record the peak (median or geo mean) channel of the blank.
 2. Use the calibration plot to determine the MESF value associated with the fluorescence of the blank. This is the fluorescence detection threshold of the instrument at these instrument settings.
 - c. *Quantitating the Fluorescence of Samples:*
 1. After completing the fluorescence intensity calibration and establishing a calibration curve, analyze the unknown samples on your flow cytometer. *Note:* To correctly quantitate the fluorescence of samples, instrument settings used for MESF calibration must remain exactly the same for sample analysis.
 2. Record each samples' FITC fluorescence intensity peak channel (median or geo mean).
 3. Use the calibration plot to determine the MESF value that corresponds to each sample's peak channel.

Recommendation

For consistency of data across instruments and time, it is recommended that a unified analysis range (Unified Window of Analysis) be used. The Unified Window of Analysis may be achieved by setting the PMT's of the detectors with Bangs' Right Reference Standard™ or QC Windows® when performing your daily set-up.

Expected Values

Figures 2 and 3 depict the FITC fluorescence histogram of the Quantum FITC-5 MESF microspheres and the calibration curve from which the MESF values can be obtained, respectively.

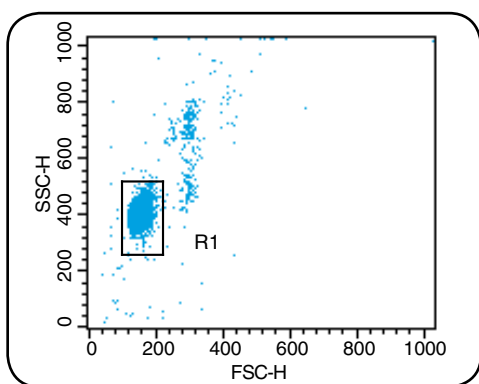


Figure 1

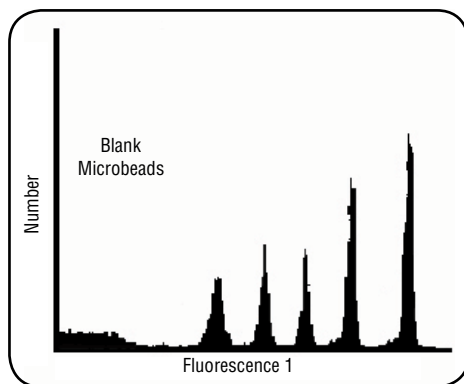


Figure 2

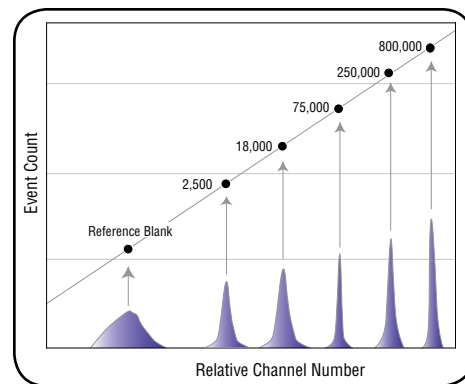


Figure 3

Notes

1. Proper storage and handling are essential. Exposure to direct light, even for limited periods, may result in photobleaching of the fluorochromes, substantially affecting performance. Therefore, the reagent should be kept in its original opaque bottle. See also the Storage and Stability section below.
2. Fluorescence intensity of the fluorochromes (e.g., FITC) is extremely sensitive to changes in pH. It is therefore important to resuspend the microspheres in the same cell suspension solution used with cell samples in order to maintain comparable spectral properties.
3. Fluorescence intensity of some fluorochrome molecules (e.g., FITC) is sensitive to temperature.
4. If there is a problem with the run, follow these steps:
 - Prepare and run a fresh sample.
 - Drain and fill the flow cell several times to eliminate air bubbles and debris.
 - Wash fluidics system by running a fresh solution of 10% household bleach. Follow manufacturer's instructions.
 - Check system for pressure leaks.
 - Check the properties of diluent and sheath fluid (such as pH).
 - Check alignment of the instrument.
 - Consult your service engineer.

References

1. **Vogt, R.F., G.D. Cross, L.O. Henderson, D.L. Phillips.** 1989. Model system evaluating fluorescein-labeled microbeads as internal standards to calibrate fluorescence intensity of flow cytometers. *Cytometry*, 10: 294-302.
2. **Sisken, J.E.** 1989. Fluorescent standards. In: Taylor, D.L., Wang, Y., Eds. *Methods in cell biology*. San Diego, CA: Academic Press Inc., pp. 30.
3. **Schwartz, A.** 1989. Instrument compensation and calibration methods: reducing theory to practice. Progress in Cytometry. Belgium: Reports from the Third European Cytometry Users' Meeting; June 5-7, 1989.
4. **Longobardi Givan, A.** 1992. Flow cytometry first principles. New York: Wiley Liss, pp. 88-90.
5. **Horan, P.K., K.A. Muirhead, S.E. Slezak.** 1990. Standards and controls in flow cytometry. In: Melamed, M.R., T. Lindmo, M.L. Mendelsohn, Eds. *Flow cytometry and sorting, 2nd ed.* New York, NY: Alan R. Liss.
6. **National Committee for Clinical Laboratory Standards.** 1993. Clinical applications of flow cytometry: immunophenotyping of leukemic cells. Proposed Guideline, NCCLS document H43-P (ISBN 1-56238-219-5). Villanova, PA: NCCLS.

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2. Cy™, including Cy5, is a trademark of GE Healthcare Limited. These products are manufactured under license from Carnegie Mellon University under U.S. Patent Number 5,268,486 and related patents.
3. Alexa Fluor® is a registered trademark of Life Technologies Corporation.

Storage and Stability

Store at 2-8°C. Do not freeze and do not sonicate. Prepared samples may be vortexed briefly, if necessary, to increase the % singlets. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

Ordering Information

Catalog Code	Description	Sizes
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL

Related Products

Catalog Code	Description	Sizes
555p	Quantum™ FITC-5 MESF Premix	1mL, 5mL, or 14mL

Related Products, cont.

Catalog Code	Description	Sizes
488	Quantum™ Alexa Fluor® 488 MESF	1mL, 5mL, or 14mL
647	Quantum™ Alexa Fluor® 647 MESF	1mL, 5mL, or 14mL
823	Quantum™ APC MESF	1mL, 5mL, or 14mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL, 5mL, or 14mL
815	Quantum™ Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
816	Quantum™ Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
817	Quantum™ Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL
810	Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
812	Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
813	Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL
845	QC Windows® (FITC/PE)	1mL, 5mL, or 14mL
846	QC Windows® (FITC/PE/PE-TR)	1mL, 5mL, or 14mL
847	QC Windows® (FITC/PE/PE-Cy™5)	1mL, 5mL, or 14mL
848	QC Windows® (FITC/PE/PE-Cy™5, APC)	1mL, 5mL, or 14mL

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