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

Description

Quantum FITC MESF Premix 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 MESF Premix kits also allow quantitation of Antibody Binding Capacity (ABC).

Quantum FITC MESF Premix kits are comprised of 5 populations of calibrated fluorescent standards: 4 populations of microspheres having different levels of FITC fluorescence intensity and 1 blank population. The Quantum FITC MESF Premix kits have excitation and emission spectra matching those of cell samples labeled with FITC. The microsphere standards are suspended in a sterile filtered, pH buffered PBS solution containing surfactant and preservatives.

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 5 populations in the Quantum FITC MESF Premix kits has been calibrated against solutions of laser grade fluorescent dye in units of MESF FITC per microsphere. The reference blank in each kit is used to measure the fluorescence detection threshold of the instrument. Correct use of the Quantum FITC MESF Premix kit allows for: 1) quantitation of the fluorescence intensity of the samples in terms of MESF, 2) determination of instrument fluorescence detection thresholds, 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 MESF Premix kit: 2 bottles included (1 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 fluorescent 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 Certified Blank to 0.5mL of the same type of buffer or medium in which 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 ~100-400 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 which fall 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. Add 1 drop of the FITC beads to 0.5mL of the same type of buffer of medium in which cell samples will be suspended. Record the peak (e.g. median or geo mean) channel value for the blank. We recommend running the reference blank separate from the dyed populations when using the premix kits.
8. Analyze the microspheres on the flow cytometer. When establishing a calibration plot, make no further adjustments to the instrument once you have begun collecting data. Record peak channels for each of the 4 calibrated FITC intensities. Also, record the instrument settings (e.g., amplifier gains, PMT voltages, etc.).
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 MESF Premix kit. 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 4 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) channel of the Certified Blank.
 2. Use the calibration plot to determine the MESF value associated with the fluorescence of the Certified 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 procedure, 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' FL1 fluorescence intensity peak channel.
 3. Use the calibration plot to determine the MESF value that corresponds to each samples' 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 FL1 fluorescence histogram of the Quantum FITC MESF microspheres and the calibration curve from which the MESF values can be obtained, respectively.

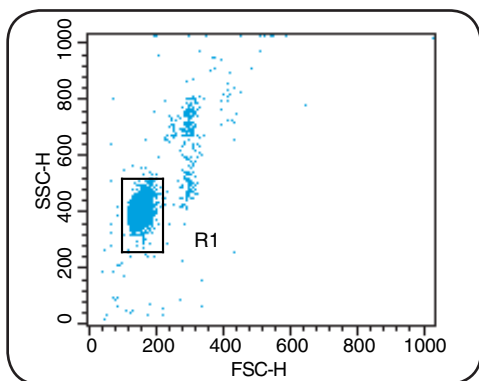


Figure 1

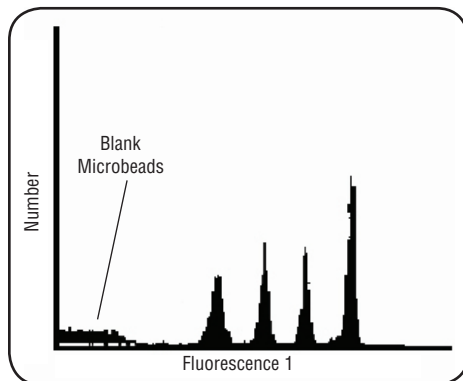


Figure 2

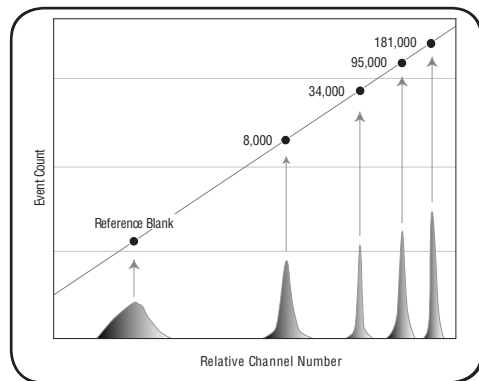


Figure 3

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 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 cells are not properly compensated, follow these steps:
 - 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).
 - Prepare fresh dilution of standards.
 - 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.

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 % 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
824p	Quantum™ FITC MESF - Low Level	1mL, 5mL, or 14mL
826p	Quantum™ FITC MESF - Medium Level	1mL, 5mL, or 14mL
825p	Quantum™ FITC MESF - High Level	1mL, 5mL, or 14mL



Related Products

Catalog Code	Description	Sizes
510	Right Reference Standard™ Fluorescein, Low Intensity	5mL
511	Right Reference Standard™ Fluorescein, Medium Intensity	5mL
512	Right Reference Standard™ Fluorescein, High Intensity	5mL
513	Right Reference Standard™ Phycoerythrin, Low Intensity	5mL
514	Right Reference Standard™ Phycoerythrin, Medium Intensity	5mL
515	Right Reference Standard™ Phycoerythrin, High Intensity	5mL
516	Right Reference Standard™ PE-Cy™5, Low Intensity	5mL
517	Right Reference Standard™ PE-Cy™5, Medium Intensity	5mL
518	Right Reference Standard™ PE-Cy™5, High Intensity	5mL
519	Right Reference Standard™ APC, Low Intensity	5mL
520	Right Reference Standard™ APC, Medium Intensity	5mL
521	Right Reference Standard™ APC, High Intensity	5mL
810	Simply Cellular® anti-Mouse 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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