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B E A D S ● A B O V E T H E R E S T™

Description

Flow cytometers are highly configurable, and results can vary dramatically with different instrument settings. Establishing a common “Window of Analysis” for each detector, with upper fluorescence limits defined, allows reference populations to be positioned in approximately the same place on the scale. This type of standardized instrument set-up ensures consistency of results from specific instruments and enables meaningful data comparison between instruments. Standardized instrument set-up using our QC3 products can ameliorate differences in range, relative scale, and reporting units, as well as daily fluctuation due to electronic noise and ambient temperature and humidity.

QC3 Reference Standard kits for instrument set-up include one or more bead population(s) surface-labeled with fluorochromes. The microspheres exhibit excitation and emission spectra matching those of cell samples labeled with the same fluorochromes. The QC3 microspheres approximate the size of human lymphocytes (7-9µm) and are suspended in a sterile-filtered, isotonic, buffered solution (pH 7.4).

Characteristics

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

Material

Material Supplied

- QC3 microspheres

Material Required

- Cell suspension solution
- Sample test tubes
- Flow cytometer

Procedure

Researchers are advised to optimize the use of particles in any application.

Establishing Target Conditions

1. On the cytometer, acquire a sample that is representative (e.g., a positive control) of the samples you wish to analyze.
2. Adjust the forward and side scatter signals for optimal visualization.
3. Construct a live gate around the cell population of interest.
4. Adjust the PMT and compensation levels of the fluorescence signals such that the cell populations are located where you would expect them to be.

Target Channel Determination

1. Place 1-2 drops of the QC3 Reference Standards in a labeled test tube. Add ~0.5mL suspension solution and pulse vortex.
Note: For QC3 (FITC/PE/PE-Cy5, APC) [Catalog Code 844], prepare a separate tube for each of the two labeled populations (FITC/PE/PE-Cy5 and APC).
2. Run the microspheres on the cytometer, acquiring a minimum of 5000 events.

3. Gate on the singlet population of microspheres in a bivariate histogram (dot plot) showing side scatter (SSC) versus forward scatter (FSC). (Figure 1)
4. Record the peak channels for the forward scatter and fluorescence channels.
5. The recorded peak channels are the new instrument-specific Target Channels for these specific target conditions.

Daily Instrument Setup and Quality Control

1. Set the instrument PMT and compensation settings to the same settings used in the previous section.
2. Place 1-2 drops of the QC Reference Standards in a labeled test tube. Add 0.5mL suspension solution and pulse vortex. *Note:* For QC3 (FITC/PE/PE-Cy5, APC) [Catalog Code 844], prepare a separate tube for each of the two labeled populations (FITC/PE/PE-Cy5 and APC).
3. Run the microspheres on the cytometer, acquiring a minimum of 5000 events.
4. Observe the peak channels for the forward scatter and fluorescence channels. They should fall in the respective Target Channels, as determined in the previous section. If they do, then the instrument is operating the same as when the Target Channels were determined. If the peak channels are substantially different than the Target Channels, small PMT adjustments may be used to correct the microspheres' peak channels.

QC3 as an Internal Standard

QC3 microspheres may be added directly to fluorescently-labeled cell samples.

Expected Results

Except for the APC population in Catalog Code 844, the QC3 Reference Standards are labeled with multiple fluorochromes. The microspheres will be visible as an intense fluorescent peak in each of the applicable fluorescent channels. (Figure 3) The microspheres will appear in the dual-labeled quadrants of bivariate fluorescence dot plots. (Figure 2)

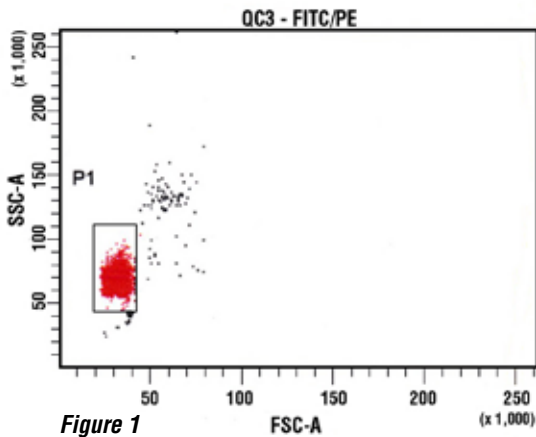


Figure 1

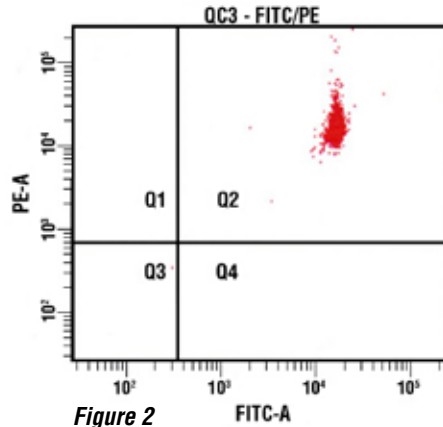


Figure 2

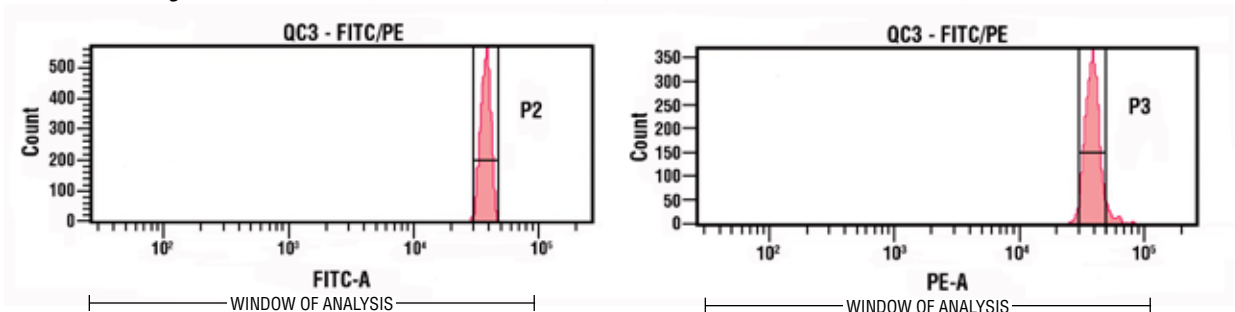


Figure 3

Notes

Prior to acquiring the QC3 microspheres, the operator should ensure that the flow cell is free of debris. This can be accomplished by running a 10% solution of household bleach for 5 minutes followed by distilled water for another 5 minutes. (Follow manufacturer's instructions.) Should this fail, 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.

Reference

1. **Purvis, N., G. Stelzer.** 1998. Multi-platform, multi-site instrumentation and reagent standardization. *Cytometry*, 33: 156-165.
2. **Schwartz, A., E. Fernandez-Repollet.** 1993. Development of clinical standards for flow cytometry. *Clinical Flow Cytometry*, Ann NY Acad Sci, 677: 28-39.

Trademarks

1. QC Windows®, QC3™, and Quantum™ are registered trademarks and trademarks of Bangs Laboratories, Inc.
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. Freezing may result in irreversible aggregation. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations. The reagent should be kept in its opaque bottle.

Safety

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

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
841	QC3™ (FITC/PE) - 1 bottle	1mL, 5mL, or 14mL
842	QC3™ (FITC/PE/PE-TR) - 1 bottle	1mL, 5mL, or 14mL
843	QC3™ (FITC/PE/PE-Cy™5) - 1 bottle	1mL, 5mL, or 14mL
844	QC3™ (FITC/PE/PE-Cy™5, APC) - 2 bottles	1mL, 5mL, or 14mL

Related Products

Catalog Code	Description	Sizes
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
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
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL
555p	Quantum™ FITC-5 MESF (Premix)	1mL, 5mL, or 14mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL, 5mL, or 14mL

Order online anytime at www.bangslabs.com.