

DESCRIPTION

Right Reference Standard™ microspheres are suspensions of highly uniform microspheres labeled with a single fluorochrome at a high intensity level. These microspheres exhibit excitation and emission spectra matching those of cell samples labeled with the same fluorochromes and approximate the size of human lymphocytes (7-9µm). Right Reference Standard™ microspheres are suspended in a sterile-filtered, isotonic, buffered solution (pH 7.4). Quantum™ QC or Quantum™ MESF microspheres may be used for applications requiring multiple intensities.

CHARACTERISTICS

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

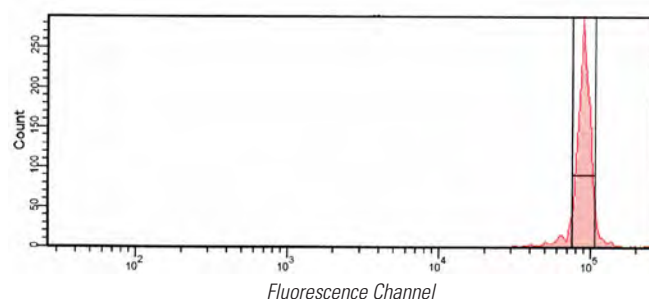
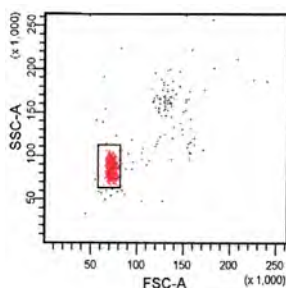
MATERIAL

Material Supplied

Right Reference Standard™ microspheres

Material Required

Cell suspension solution
Appropriate sized test tubes
Flow cytometer



PROCEDURE

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

Determining Instrument Specific Target Channels

1. On the cytometer, acquire a stained sample that is representative (e.g., a positive control) of the samples you wish to analyze. Adjust settings as needed to have the sample fall into the desired range.
2. Prepare Right Reference Standard microspheres by lightly shaking bottle (do not sonicate or vortex).
3. Add a drop (~ 50 ul) to 400 ul of buffer for analysis. Using the same settings as for the positive sample in step 1, gate on the singlet population of microspheres in a bivariate histogram (dot plot) showing side scatter (SSC) vs. forward scatter (FSC) (as shown above) and apply to a fluorescence histogram.
4. Repeat runs 3 times with independent prepared samples for determination of inherent run variability.
5. Use the mean value of these runs as your instrument specific target channel. Outliers should be discarded and the run repeated.

Note: Initial settings may need to be adjusted to fit both the positive control and reference standard within the same window of analysis. If adjustments are made, record the new instrument specific target channel.

Subsequent Daily Set-up and Quality Control

1. Set the PMT and compensations to values used in the previous section.
2. Run the Right Reference Standard to ensure that they fall near the instrument specific target channels. Tolerance on absolute differences between the set target channel value and the measured channel value will need to be determined by the user, we encourage the use of Levey-Jennings charts to assist with this, as well as the use of daily QC/setup beads.

Expected Results

The Right Reference Standard™ microspheres are labeled with a single fluorochrome. The microspheres will be visible as an intense fluorescent peak in the applicable fluorescent channel.

NOTES

Prior to acquiring the Right Reference Standard™ microspheres, the flow cell should be free of debris. This can be accomplished by running a 10% solution of household bleach (follow instrument manufacturer's recommendations) for 5 minutes, followed by distilled water for another 5 minutes at the highest flow rates allowable (will assist in clearance). Should this fail, follow the following steps:

- Run the cytometer's debubble/purge cycle.
- Verify that the instrument is properly compensated.
- Perform Manufacturer's recommended monthly cleaning procedure.
- Check the properties of diluent and sheath fluid (e.g., especially changes in pH).
- Check alignment of the instrument.
- Prepare a new sample and run once again.

REFERENCES

1. Schwartz, A., E. Fernandez-Repollet. 1993. *Development of clinical standards for flow cytometry*. Clinical Flow Cytometry, Ann NY Acad Sci. 677: 28-39.
2. Shapiro, H.M. 1995. *Practical flow cytometry*, 3rd ed. New York: Wiley Liss, Inc.

TRADEMARKS AND REGISTERED TRADEMARKS

Right Reference Standard™ is a trademark of Bangs Laboratories, Inc.

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. Freezing may result in irreversible aggregation. Stable for 6 or 12 months from date of purchase, as noted on the product label, provided the product is handled in accordance with the manufacturer's recommendations. The reagent should be kept in its opaque bottle.

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

ORDERING INFORMATION

Cat. Code	Description	Sizes
512	Right Reference Standard™ Fluorescein, High Intensity	5mL
515	Right Reference Standard™ Phycoerythrin, High Intensity	5mL
518	Right Reference Standard™ PE-Cy™5, High Intensity	5mL
521	Right Reference Standard™ APC, High Intensity	5mL

RELATED PRODUCTS

Cat. Code	Description	Sizes
725	Quantum™ QC Multi-Color, Multi-Intensity Fluorescence Reference Standard	5mL
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
822	Quantum™ Cy™5 MESF	1mL, 5mL, or 14mL
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL
555p	Quantum™ FITC-5 MESF (Premix)	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL

Order online anytime at www.bangslabs.com.