Product Data Sheet 510

Right Reference Standard™

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B E A D S ● A B O V E T H E R E S T ™

Description

The Right Reference Standards are designed to establish unified fluorescence intensity ranges that will standardize data for a variety of flow cytometry applications. Flow cytometric assays can cover a range of six decades of fluorescence intensity; however, the majority of commercial instruments have an analysis range of four decades. Therefore, to cover the six decades, three unified fluorescence intensity ranges have been established for each specific fluorochrome with three Right Reference Standards of specific intensity. They have been designated as low for RBC or platelet assays, medium for leukocyte phenotyping or high for cell cultures or abnormal lymphoma cells, respectively. The uniformity of the ranges across instruments is accomplished due to matching of the excitation and emission spectra of the standards to the assay samples. Establishing unified fluorescence intensity ranges helps ensure comparability and reproducibility of assay data, as well as simplify comparison of instrument performance across instruments and time.

A specific unified fluorescence intensity range (low, medium or high) is achieved by adjusting the PMT setting such that the median channel of the Right Reference Standard falls in the middle of the reporting scale. If only moderate precision is required, the Right Reference Standard can be placed in the middle channel of the reporting scale.

The Right Reference Standards are suspensions of highly uniform microspheres labeled with multiple fluorochromes. The Right Reference Standards exhibit excitation and emission spectra matching those of cell samples labeled with the same fluorochromes. The Right Reference Standards 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 10^6 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.
2. Gate on the singlet population of microspheres in a bivariant histogram (dot plot) showing side scatter (SSC) vs. forward scatter (FSC). (Figure 1)
3. Adjust the PMT voltages such that the peak of the cells falls in the middle of the sample space. Record the PMT settings.

4. Run the Right Reference Standard microspheres and record their peak channel levels. You have now determined the Instrument Specific Target Channels.

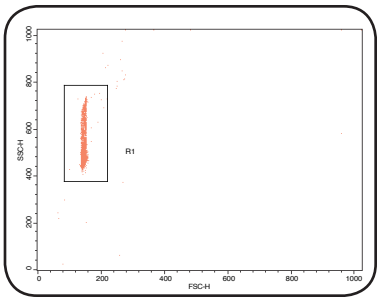


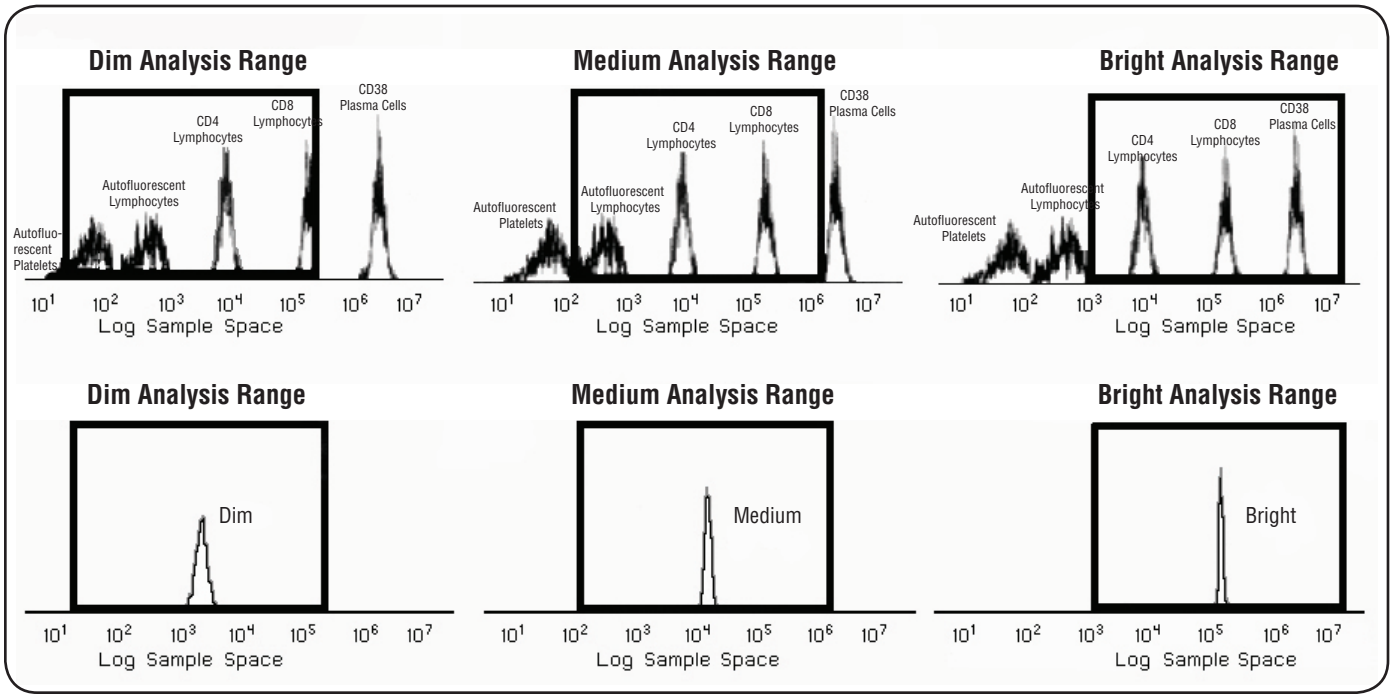
Figure 1

Subsequent Daily Set-up and Quality Control

1. Set the PMT and compensations to those determined in the previous section.
2. Run the Right Reference Standard microspheres to insure that they fall in the Instrument Specific Target Channels. The position of the Right Reference Standard microspheres should be within ~1.5% of the Instrument Specific Target Channels based on Histogram channels, i.e., +/- 3 channels on a 256 histogram scale and +/- 12 channels on a 1024 histogram scale.

Expected Results

The Right Reference Standards 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 Standards, 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. Should this fail, follow the following steps:

- Drain and fill the flow cell several times to eliminate air bubbles and debris.
- 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

1. Right Reference Standard™ is a trademark 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.

Storage and Stability

Store at 2-8°C. Freezing may result in irreversible aggregation and loss of binding activity. 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 is 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

Catalog Code	Description	Size
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

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