Product Data Sheet 880

Flow Cytometry Absolute Count Standard™

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

The Flow Cytometry Absolute Count Standard™ is intended for use as an internal counting standard. It is designed for use in the proper set-up of flow cytometers and cell counters, and for the accurate enumeration of cells or particles.

The Flow Cytometry Absolute Count Standard™ consists of 1 bottle containing a 10mL microsphere suspension of a known concentration, which are internally labeled with multiple fluorochromes. The combination of dyes allows the beads to be excited by a common argon laser (488nm) and emit in the three standard channels of a flow cytometer (FL1, FL2, FL3). The Flow Cytometry Absolute Count Standard™ 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:	1 x 10^6 microspheres/mL (see the Certificate of Analysis for the lot-specific concentration)

MATERIAL

Material Supplied

- Flow Cytometry Absolute Count Standard™ microspheres

Material Required

- Cell suspension solution
- Test tubes
- Sodium hypochlorite solution (household bleach)
- Flow cytometer

PROCEDURE

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

Instrument Preparation

1. Run a 10% sodium hypochlorite solution (household bleach) for 5 minutes to eliminate any debris in the flow cytometer's fluidics.
2. Run distilled water for 5 minutes.
3. Adjust instrument settings to those used to acquire your cell samples.

Percent Singlet Determination

1. Shake the Flow Cytometry Absolute Count Standard™ bottle to re-suspend particles. Pipette 100µL of the standard into a labeled test tube. Add 0.5mL suspension solution.
2. Acquire 1000 events.
3. Create a bivariate histogram (dot plot) showing side scatter (SSC) versus forward scatter (FSC). Gate on the singlet population. (Figure 1)
4. Using the singlet gate, determine the percent singlets.

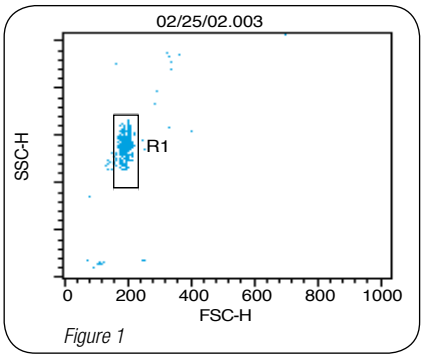

Figure 1

5. Create a second dot plot showing SSC versus fluorescence in any channel (FL1, FL2, or FL3). Gate on the singlet population. (Figure 2)

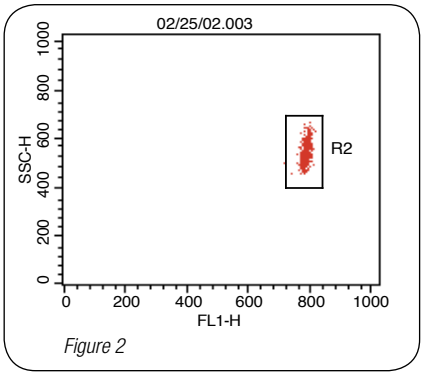

Figure 2

Sample Collection and Analysis

1. Shake the Flow Cytometry Absolute Count Standard™ bottle to re-suspend particles, and pipette 500µL into a labeled test tube. Add 100µL of the cell sample to be analyzed and mix thoroughly.
2. Collect at least 100,000 events of the combined sample and count standard.
3. In the SSC versus fluorescence histogram, place a gate around the single population of microspheres, and a gate on the cells you wish to count. Determine the number of events collected for each of the 2 populations. (Figure 3)

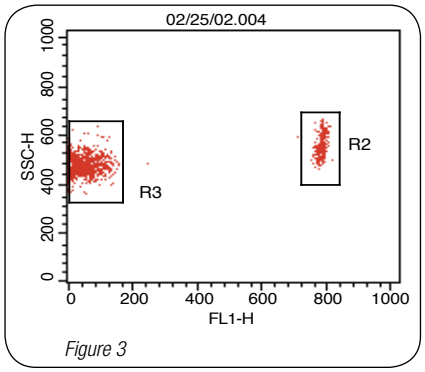

Figure 3

Absolute Count Calculations
Calculate the absolute count of the cells per the following equation:

$$\frac{[\text{beads}]}{\#\text{ of beads counted}} = \frac{X}{[(\#\text{ cells counted}) \times 5]}$$

where:
X = cells/mL
beads = (% singlets) (concentration reported on CoA)

NOTES

1. Prior to acquiring data for counts, the flow cell should be free of debris. This can be accomplished by running at 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 these steps:
    - Drain and fill the flow cell several times to eliminate air bubbles and debris.
    - Verify 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.
2. The Flow Cytometry Absolute Count Standard™ should not be vortexed as beads may come into contact with and cling to the cap of the bottle.

REFERENCES

1. **Jennings, C.D., K.A. Foon.** 1997. Recent advances in flow cytometry: application to diagnosis of hematologic malignancy. *Blood*, 90:2863-2892.
2. **Shapiro, H.M.** 1988. *Practical flow cytometry.* New York: Wiley-Liss.

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. Store in reagent's 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	Size
580	Flow Cytometry Absolute Count Standard™	10mL

Order online anytime at **www.bangslabs.com.**