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B E A D S ● A B O V E T H E R E S T™

Description

The Size Calibration Standards Kit contains 5 populations of unstained microsphere standards of different sizes. The standards are suspended in a sterile, filtered, isotonic buffered solution containing a surfactant and a preservative. Each bottle contains microspheres at a concentration of 2×10^6 particles/mL. The Size Calibration Standards Kit is to be used to construct calibration plots for electronic volume and light scatter instrumentation.

Characteristics

Mean Diameter: 4-11 μ m
Particle Concentration: 2×10^6 particles/mL

Material

Material Supplied

- Size Calibration Standards Kit: 5 populations

Material Required

- Isotonic phosphate saline (pH 7.2)
- Appropriate sized tubes
- Flow cytometer

Procedure

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

The Size Calibration Standards Kit is to be used to construct calibration plots for electronic volume and light scatter instrumentation. Factors which compensate for the difference in refractive indices between the microsphere standards and biological cells need to be used. These factors can be derived by measuring a cell of known size (by scanning electron microscopy), e.g. a lymphocyte, on the calibration plot and determining the difference between the known size and its measured size, and using this number as a correction factor in subsequent size determinations. This correction factor can be employed by horizontally shifting the calibration plot to where the cell should have fallen on the plot. Note that fixation may significantly change the refractive index of cellular components and thus affect the light scatter properties of the cell as a whole.

On the other hand, when using an electronic volume sensing instrument, hydrodynamic shape factors must be considered. That is, only cells which are spherical, such as lymphocytes, may have their size directly determined against the calibration plot derived from spherical microsphere standards. In the case of measuring the size of erythrocytes, which have a concave disk shape, the measured value will appear lower than expected and the hydrodynamic factor of 1.5 must be used to multiply the reading from the volume calibration plot for an accurate size determination of these cells. A more complete discussion of such hydrodynamic factors is presented by V. Kachel.¹

A size calibration plot for a flow cytometer may be generated by plotting the relative channel number (linear or log) of the peak maximum against the appropriate size value of the microsphere standards. A linear relationship may be obtained from a log-log plot if a linear amplifier is used. Plotting data from linear amplifiers on log-log paper has the advantage of not only conveniently extending the range over which measurements can be made, but it also reduces all the size parameters to linear relationships. When using a log

amplifier, a linear relationship is obtainable if the plot is made on semi-log paper. Linear relationships for all these size parameters are obtained when plotted as suggested, because the above equations are transformed into log expressions with the power of the diameter converted to relative slope of the relationship, as follows:

$$\begin{aligned} \text{Diameter} &= \log (D) \\ \text{Cross-sectional Area} &= 2 \log (D) + \log (\pi/4) \\ \text{Surface Area} &= 2 \log (D) + \log (\pi) \\ \text{Volume} &= 3 \log (D) + \log (\pi/6) \end{aligned}$$

This method of data representation allows multiple-size calibration (i.e., diameter, cross-sectional area, surface area, and volume) to be plotted as linear functions on the same graph, or linear and log amplifier, respectively. With these simultaneous calibration plots, the operator is able to directly determine all size parameters just by knowing the peak maximum channel number of the sample.²

Caution: Size Interpretation

Having a graduated series of uniform microspheres to calibrate the size channel of a flow cytometer can be useful when considering cellular sample sizes; however, additional factors must be considered. When sizing samples by light scatter, the refractive index is an important factor in the measurement. Although forward scatter is not as sensitive to differences in refractive index as 90° scatter, differences in refractive index between the microsphere standards and cellular samples can affect size measurements.³

Although the FSC detector of flow cytometers will provide some indication of bead or cell size, it is important to note that cytometers are not sizers, per se, and do not afford the same accuracy and resolution as dedicated sizing instruments. FSC amplitude is not a monotonic function of particle size, and FSC response may also vary by instrument make and model, i.e. with different optical systems.

Considerations in Size Determination of Samples

The term “size” in this document refers not only to the diameter of the microscope or cell, but also to other size parameters, including the cross-sectional area, the surface area, and the volume. Accurate determination of any of these size parameters by means of a flow cytometer is not as straight-forward as it may seem.⁴

For example, it is important to remember which sizing parameters are being measured by the instrument. With flow cytometers that have volume sensors, one cannot simply plot the diameter of the microsphere standards against the relative peak channel on a linear scale and expect to obtain a straight line. Instead, the volume of each microsphere would have to be calculated from a given diameter first, and then that volume would be plotted against the relative channel number to obtain a straight line. Similarly, with instruments that have light scatter detectors, the cross-sectional area is the parameter that is being measured, and therefore the one that should be plotted. As a convenience, the size formulae as a function of diameter are presented below:

$$\begin{aligned} \text{Diameter} &= D \\ \text{Cross-sectional Area} &= \pi D^2/4 \\ \text{Surface Area} &= \pi D^2 \\ \text{Volume} &= \pi D^3/6 \end{aligned}$$

Size Calibration

1. Bath sonicate for 30 seconds or vigorously shake the bottle to ensure uniform suspension of standard.
2. Add 1 drop of each of the 5 sized beads into isotonic phosphate saline (pH 7.2).
3. Run the beads. *Note:* When establishing a calibration plot, make no further adjustments to the instrument once you have begun collecting data.
4. Determine the FSC Peak Channel Number for each population.
5. Plot the diameter, surface area, or volume (y-axis) vs. the Peak Channel (x-axis) for the 5 sized beads

Expected Value

A generally linear relationship should be obtained when the five populations are plotted. FSC response may vary by instrument make and model, i.e., with different optical systems.



References

1. **Kachel, V.** 1979. Electronic resistance pulse sizing (Coulter sizing). In: M.R. Melamed, ed., *Flow cytometry and sorting*. New York: Wiley & Sons, Ltd., pp. 61-104.
2. **Schwartz, A., H. Sugg, T.W. Ritter, E. Fernandez-Repollet.** 1983. Direct determination of cell diameter, surface area, and volume with an electronic volume sensing flow cytometer. *J Cytomet*, 3(6):456-458.
3. **Salzman, G.C.** 1982. Light scattering of single cells. In: N. Catsimpoilas, ed. *Cell analysis, Vol. 1*. New York: Plenum Publishing Corp., pp. 111-143.
4. **Shapiro, H.M.** 1988. *Practical flow cytometry*. New York: Wiley-Liss.

Storage and Stability

Store at 2-8°C. Freezing of particles 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. Specifically, 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
829	Size Calibration Standards Kit	5mL or 14mL

Order online anytime at www.bangslabs.com.