

Nanobead Calibration Kit

DESCRIPTION

Interest in exosome and microvesicle analysis has grown rapidly in recent years as another way to explore disease states, cancer progression, and for drug discovery and delivery.^{1,2,3,4} Due to their small size (30 – 100 nm) however, these particles are difficult to detect using traditional methods, and require extra processing to segregate/characterize from debris commonly found in biological samples.^{5, 6, 7} Flow cytometry has emerged as a popular method of analysis and sorting for small particle characterization.^{8, 9, 10} The Nanobead Calibration Kit comprises highly defined microspheres with an internalized fluorescent dye to allow users to determine the capabilities of their cytometer and appropriate instruments settings, as size references for small particle characterization, and assessing small particle handling processes.

CHARACTERISTICS

Nominal Diameter: 50 nm & 100 nm
Particle Concentration: ~ 1x10⁸ beads/mL

MATERIALS

Nanobead Calibration Standards: 2 bottles: 50nm x 3mL, 100nm x 3mL

Materials Required:

Filtered flow buffer/diluent (see procedures)
Appropriate sized tubes
Vortex Mixer/sonicator
Flow cytometer

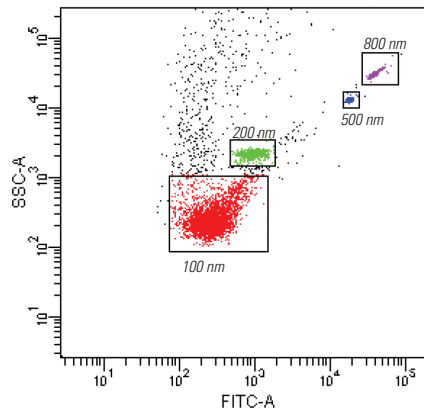
PROCEDURE

Small particle analysis is highly demanding and requires that the flow cytometer is in peak operating condition, and that the user have a thorough understanding of instrument operation and associated software. In some cases, all beads are not able to be identified as discrete populations even under optimal conditions, as these particle sizes are at the lower bounds of many cytometers' detection limits. Optimal settings used will be highly dependent on the instrument and will need to be determined for the specific analysis. It is recommended to run each bead independently initially to establish detection capabilities, appropriate settings, and expected scatter profile. Once the user has become more comfortable with using each bead, they can mix as desired.

1. Shake the spheres or vortex for 30 seconds to ensure a uniform suspension of microspheres.
2. Add 1 drop of the beads into 1 mL of filtered buffer
3. Run the beads and adjust settings as needed until the scatter population is resolved as desired.

EXPECTED RESULTS

Exact results and resolution will differ instrument to instrument, and is highly dependent on settings used.



Settings:
SSC log 500 - Threshold 200
FITC log 650 - Threshold 300
Windows Ext. 2.0 Events: 5000

Figure 1: Scatter plot of FITC vs SSC of a mixture of 100 nm, 200 nm, 500 nm and 800 nm beads (Nanobead Kit + Submicron Kit) on a BD FACSCanto II

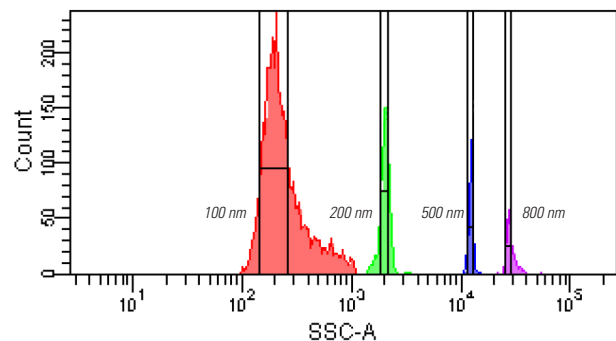


Figure 2: SSC Histogram for a mixture of 100 nm, 200 nm, 500 nm, and 800 nm beads (Nanobead Kit + Submicron Kit) on a BD FACSCanto II

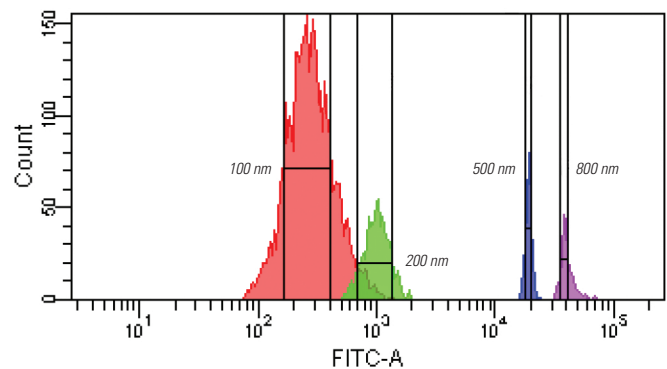


Figure 3: FITC Histogram for a mixture of 100 nm, 200 nm, 500 nm, and 800 nm beads (Nanobead Kit + Submicron Kit) on a BD FACSCanto II

TIPS AND SUGGESTIONS

The following are steps that can be taken to potentially improve the resolution of the nanobeads. Care should be taken before modifying your cytometer, as some changes could prove damaging if done improperly and possibly void service contracts, product maintenance (PM's), or warranties. We highly encourage users to consult the appropriate flow cytometry professional or responsible party before undertaking any physical modifications to the cytometer.

1. Filter any sheath fluid or diluent used with an initial 100 nm filter, and then a final 50 nm filter. These filters can be sourced from common manufacturers such as Pall or Whatman.
2. Many flow cytometers employ a 200 nm inline filter. Users can add in an additional 100 nm inline filter (or smaller), although this may impact the flow rate, reducing it to unacceptably low levels or causing pressure concerns.
3. Flush the system for a minimum of 1 hour with filtered sheath. Running a debubble cycle can also help reduce background events or noise.
4. Run the filtered sheath using the same settings as for nanobead acquisition to establish the noise threshold, scatter profile of background, and baseline event rate of sheath. This will greatly assist in discerning the small particles from noise.
5. Many studies have indicated side scattered light (SSC) as a better signal to trigger/threshold than forward scatter light (FSC). This can be coupled with a fluorescence trigger to increase the ability to discern the particles above background.^{11, 12}
6. Monitor the abort rate, high values could indicate an unacceptable amount of contribution from background (either due to poor filtration, or inappropriate settings), or too high of a sample concentration (the nanobeads are provided at approximately 1x10⁸, which typically isn't concentrated enough to cause high levels of coincident events). Performing serial dilutions of beads and monitoring scatter and fluorescence histograms is another useful method to control for coincident/swarm events.^{9, 13, 14}
7. Establish the largest bead/sample of interest in the top right quadrant of the scatter plot, this maximizes the available room for smaller particles which often will have more heterogeneous scatter profiles.
8. Reduced flow rates have been shown to improve scatter cv, however users should investigate several flow rates to determine the impact on their resolution (not all cytometers have this feature).¹⁴
9. Starting with larger particles and working down to the smallest is usually easier. Users may also want to include a larger reference bead in the samples to aid in establishing the initial window of analysis and settings, as well as detecting aggregation
10. Adjustment of the windows extension (WE) could improve signal resolution by reducing background contribution. Consult the user manual for more info, some instruments may not have this option.¹⁵
11. If possible, establish a buffer between the background and smallest particle on the scatter plot.
12. Due to the small size of nanobeads, PMT's may need to be increased past typical values. It is not always the case however that maximum settings are the best settings.
13. The use of a 405 nm laser has been shown to decrease signal cv, although this may reduce signal intensity.¹⁴
14. Adequately flush lines with filtered sheath between runs to reduce contribution from prior particles.

15. Consult the cytometer manufacturer, as they may have suggestions on potential modifications or settings for small particle characterization

STORAGE

Store at 2-8° C. Freezing of suspensions is expected to 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 suspensions should be kept in their supplied opaque bottles.

SAFETY

These particle suspensions contains sodium azide, which 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 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

Cat. Code	Description	Size
BLI834	Nanobead Calibration Kit (50 nm & 100 nm)	3mL

RELATED PRODUCTS

Cat Code	Description	Size
BLI832	Submicron Bead Calibration Kit (0.2 µm, 0.5 µm, 0.8 µm)	1 Kit
BLI833	Micron Bead Calibration Kit (1.0 µm, 3.0 µm, 6.0 µm)	1 Kit
BLI829	Size Calibrations Standards Kit (5 populations, ~ 4- 12 µm)	1 Kit

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FURTHER READING

Lannigan J., Nolan J., Zucker R (Eds.). (2016). Special Issue: *Measurement*

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