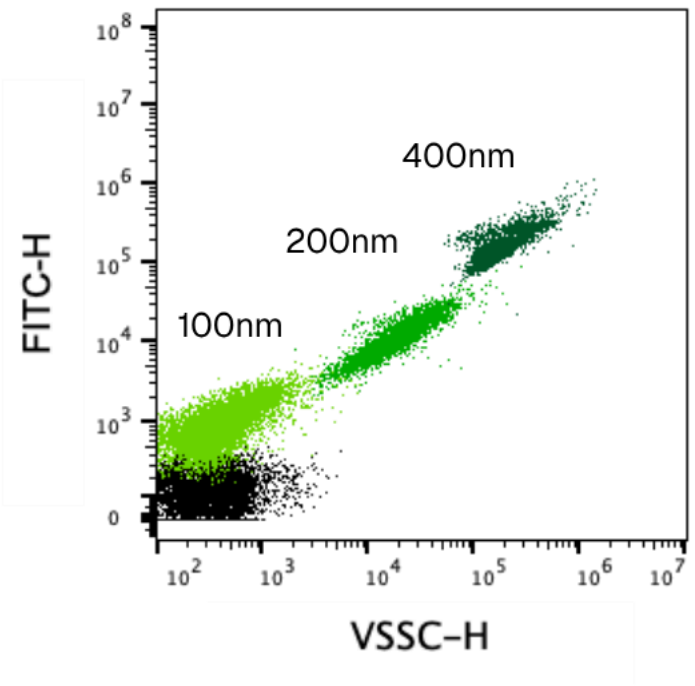
1. Technical Data Sheet

Summary	The NanoCytes™ Blue-525 Ladder Kit is a set of fluorescent synthetic particles with an excitation maximum at ~495nm and an emission maximum at ~525nm. The kit contains particles in three sizes, estimated at 100 nm, 200 nm, and 400 nm, each with a refractive index designed to mimic the optical properties of sub-micron biological particles such as extracellular vesicles. *For Research Use Only. Not for use in diagnostic or therapeutic procedures.*
Application	NanoCytes™ are stable, monodisperse reference controls with distinct scatter and fluorescence resolution when analyzed via techniques such as flow cytometry and nanoparticle tracking analysis. This material allows instrument performance to be qualified at high resolution. Refractive index-matched NanoCytes™ provide a stable, reproducible framework for estimating sizes of biological EVs or cross-site instrument benchmarking.
Materials	Fluorescent NanoCytes™ are synthetic particles in liquid suspension.
Safety	No special safety precautions are necessary. See the Safety Data Sheet (SDS) at www.slingshotbio.com.
Storage and Handling	Store liquid products at 2-8°C upon receipt. Keep protected from light.
Expiration	Refer to Certificate of Analysis (CoA) and product label.

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Instructions for Use	1. Spin down each NanoCytes tube before use to consolidate the liquid suspension. 2. Dilute the product by pipetting the liquid suspension into pre-filtered buffer or deionized water, and pipette to mix. 3. Diluent buffers should be prepared by filtering through a ≤0.02 µm pore filter. 4. Optimal dilution factors will vary by analytical method and should be determined by the user. For flow cytometry analysis, serial dilutions are recommended to avoid swarm detection. *For flow cytometry measurements, the following best practices are recommended:* 1. Clean the flow cytometer to prevent carry-over from previous experiments. 2. Set the scatter plot axis to log-scale. 3. Prior to sample measurement, acquire the diluent (pre-filtered buffer or deionized water) to determine background noise levels and event rates. 4. Sample dilution factors should be optimized to avoid swarm detection. 5. As an example dilution: pipette 10µL of the NanoCyte sample into 990µL of filtered diluent (100× dilution factor). 6. Acquire the sample using the instrument's best practices and recommended flow rate for sub-micron particles. 7. Use short-wavelength lasers (e.g. violet, VSSC) for side scatter characterization. 8. Apply a fluorescence threshold to help isolate and identify fluorescent NanoCytes. 9. Run the pre-filtered buffer or deionized water at a high flow rate between sample measurements to prevent carry-over.

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QC Data	 Figure 1. Violet Side Scatter (VSSC, X-axis) versus Fluorescence (FITC-H, Y-axis) plot for NanoCytes™ Blue-525 Ladder particles with 100nm, 200nm, and 400nm nominal sizes indicated, as reported on a CytoFLEX Nano cytometer. All particles can be clearly resolved from each other, and above the 1× PBS buffer background (in black).

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