

## Contents:

### 1. Description

- 1.1 Background
- 1.2 Uses
- 1.3 Reagent and instrument requirements

### 2. Protocol

- 2.1 Care Instructions
- 2.2 Before running a calibration

### 3. Example acquisition of NanoCollect Calibration Beads on the WOLF®

**Warning:** Product contains small amounts of Sodium Azide and Tween-20. Please handle and dispose of properly.

## 1. Description

### 1.1 Background information

Components: NanoCollect Biomedical Calibration Beads.

Capacity: 1.5mL for greater than or equal to 20 individual dilutions.

Product Format:

- 1.5mL dropper bottle.
- Aqueous solution containing 0.02% Sodium Azide and 0.01% Tween-20.

Storage: Protected from light at 4-10°C temperature.

DO NOT FREEZE! Beads can irreversibly aggregate, affecting product performance.

Discard beads after expiration date (indicated on the vial label).

### 1.2 Uses

This product is to be used only in accordance with the calibration step for the WOLF Cell Sorter.

### 1.3 Reagent and requirements

- NanoCollect WOLF Cell Sorter
- WOLFViewer Software
- Sterile, lint-free Polypropylene tubes, e.g., 5-mL round-bottom tubes or microcentrifuge tubes.

- Sterile, 0.22µm-filtered Phosphate Buffer Saline or sterile 0.22µm-filtered deionized water.

## 2.0 Protocol

### 2.1 Care Instructions:

For detailed instructions on how to use the NanoCollect WOLF Cell Sorter, please refer to the product manual.

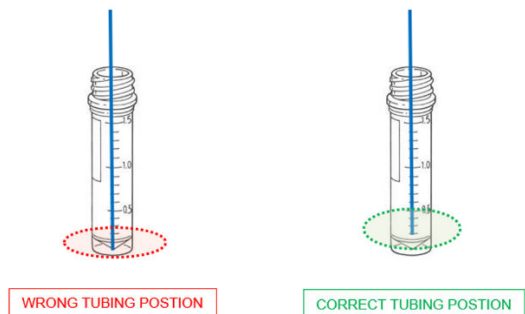
5. Open the beads under the hood to maintain sterility. If the beads are opened outside of a biosafety cabinet, NanoCollect cannot ensure sterility.
6. Keep the tip of the bottle clean and lint-free.
7. Do not spin the beads down as it may cause irreversible aggregation that can lead to cartridge clogging.

### 2.2 Before running a calibration:

*We recommend that you bring the dropper bottle to room temperature 10 minutes prior to preparing a dilution.*

1. Resuspend dropper stock beads by vortexing on maximum speed for 10 seconds, **pulsing the beads**.
2. Pipette **650µl of 0.22um sterile filtered PBS or DI water** into a tube.
3. Place the dropper bottle vertically over the tube with PBS. Squeeze 1 drop of stock solution into the tube.
4. Resuspend the diluted beads by vortexing on maximum speed for 10 seconds, pulsing the beads.
5. Be aware of the position of the cartridge sample, inlet tubing in the beads tube. The tubing should be freely able to float around the tube and not be up against the bottom (see figure below).
6. Leave the rest of the stock beads inside the fridge, never freeze.
7. Proceed to the calibration step in the setup process.

*Note: The system performs calibration automatically. Please follow steps within WOLFViewer when prompted.*



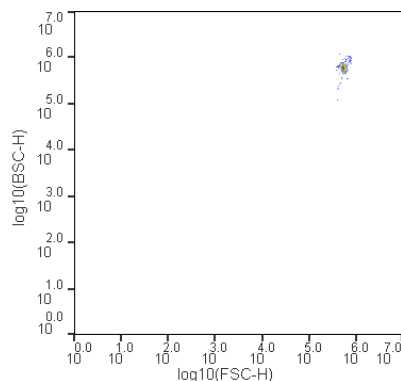
*Note: depending on how the user vortexes and dispenses the solution from the dropper, the concentration can vary slightly. This is normal, and calibration will still proceed as intended.*

8. This should yield an approximate concentration of 50-80 beads/ $\mu$ l.
9. When calibration is complete, WOLFViewer will give the following dialog: "Calibration complete. Detection Settings changed to Cells."
10. Proceed with analysis and sorting workflow.

### 3.0 Example Acquisition Information

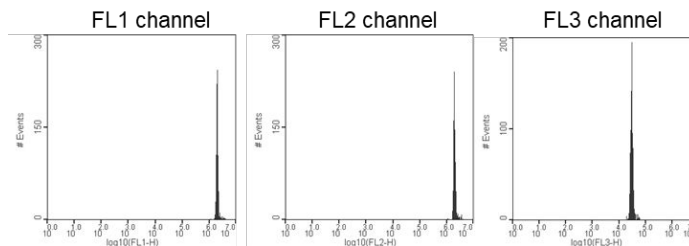
#### Scatter

Forward Scatter (FSC-H) versus Back Scatter (BSC-H)



#### Fluorescence Intensity

The following histograms show fluorescence intensity after excitation with the 488 nm laser on 3 channels (FL1, FL2, FL3).



#### **Warranty:**

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. NanoCollect Biomedical makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. NanoCollect Biomedical's liability is limited to either replacement of the products or refund of the purchase price. NanoCollect Biomedical is not liable for any property damage, personal injury or economic loss caused by the product.