vFC™ Instrument Setup and QC

Qualify and monitor instrument performance using vCal™ nanoRainbow Beads

Purpose

Assess vFRed™ fluorescence resolution, calibrate FITC and PE fluorescence channels, assess laser alignment, and calibrate instrument flow rate.

Materials

Kit Component	Size	Store
vCal™ nanoRainbow beads	1 mL	4°C

Materials to be provided by User

Gloves
Microwell plate (Sartstedt 82.1583.001)
Pipettes (5 uL – 300 uL)
Pipette tips

Procedure

1. Open vCal™ nanoRainbow Instrument Calibration experiment template
2. Place undiluted nanoRainbow Beads in a well and record for a fixed amount of time at the specified flow rate:

	CytoFlex	CellStream
Volume	100 uL	20 uL
Flow rate	60 ul/min (High)	3.66 uL/min (Low)
Time	30 sec	60 sec
Trigger (threshold_	VSSC (10,000)	Not applicable

3. Measure at same fluorescence channel gains as for EV analysis.
   Note: Scatter gains should be set to allow resolution of bead singlets from doublets and background

Analysis

1. Open the **vCal™ nRB Report Layout** and load the nanoRainbow Beads data file.
2. On the **Gating Tab**, inspect the time history and adjust the nanoRainbow gate on the population of single nanoRainbow Beads.

Assess vFRed™ Fluorescence Resolution and

3. On the **vFRed™, FITC, PE Tab**, inspect the univariate plots of marker fluorescence intensity in the appropriate channel to visually evaluate the separation of bead populations.
4. Note the CV of the bright nRB bead (peak 4), which should be <6% or less, and the vFRed™ staining index calculated using the two dimmest peaks (peaks 1 and 2), which should be >3.0.

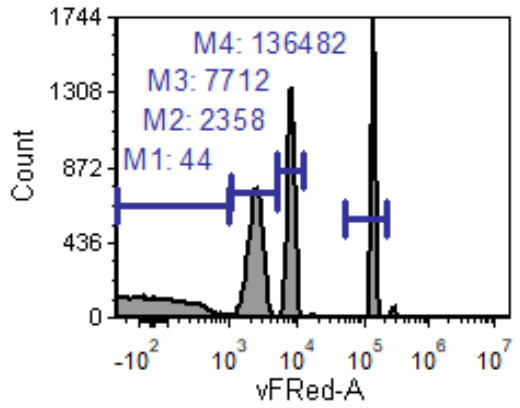

Figure 1. vFRed™ nanoRainbow beads

Table 1. vFRed™ SI Calculation

	A	B	C
1	Pos med	2358	
2	Neg med	44	
3	Neg sd	246	
4	**Sep Index**	**4.71**	
5			

Laser Alignment and Performance

5. On the **Lasers Tab**, Inspect the CV and peak shape of the brightest bead (Peak 4) in the representative histogram from each laser. CVs should be >8%. If CVs are higher, or there is an apparent leftward shoulder to the peak, alignment on that laser may be sub-optimal.

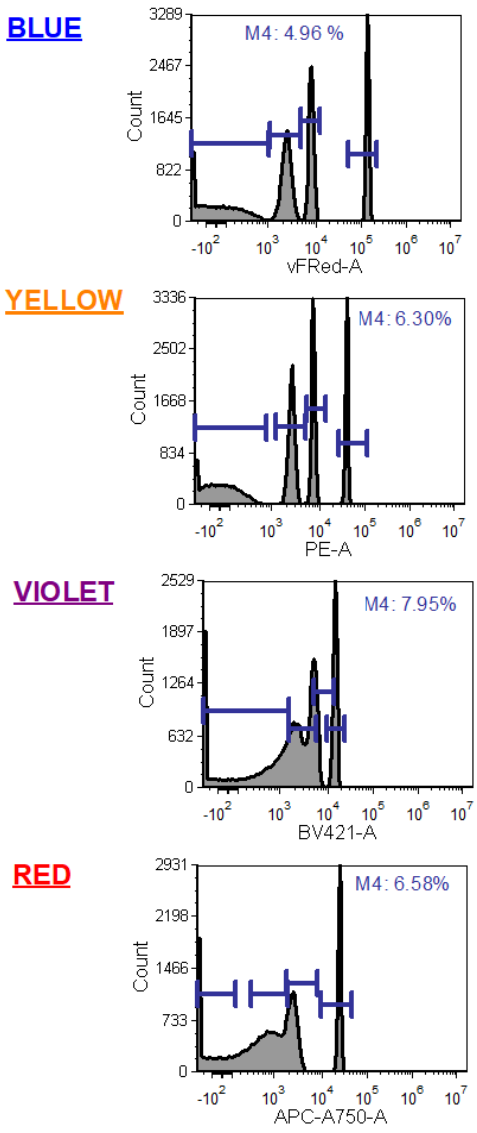

Figure 3. Assessing laser alignment from Peak 4 CVs.

Calibrate FITC and PE Channels

6. Use the FCS Express Channel Calibration Tool to create a channel calibration file (Tools>Channel Calibration) using the nanoRainbow lot-specific MESF values (provided on the product data sheet).
7. Apply the calibration to the selected plots to validate calibration. *Note that these MESF assignments are nominal and are likely to be similar across similarly configured instruments (lasers, filters, detectors, etc.), but may vary when configurations are dissimilar.*

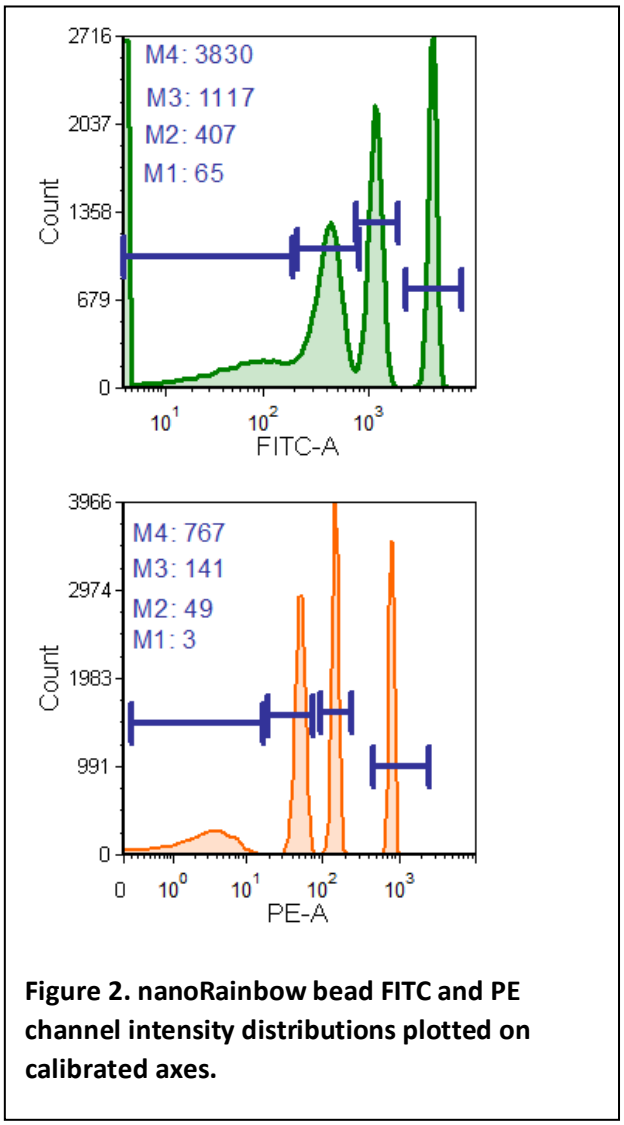

Figure 2. nanoRainbow bead FITC and PE channel intensity distributions plotted on calibrated axes.

Instrument Flow Rate Calibration

8. On the **Flow Rate Tab**, adjust the gates selecting the Peak 4 singlets, doublets and triplets. The Layout will calculate the sample flow rate using the known concentration of the nanoRainbow beads (1e7/mL). The Layout will also calculate the apparent Peak 4 bead concentration, assuming the instrument-reported volume estimate is correct.

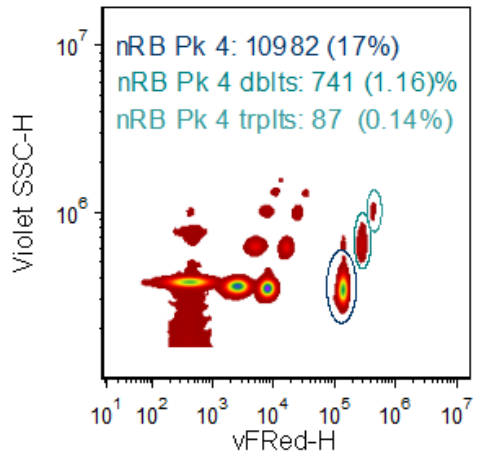

Figure 3. Assessing flow rate Peak 4 counts.

Table 2. Flow rate and concentration calculations

	A	B	C
1	Pk 4 conc	1.00E+04	/uL
2	Pk4 singlets	10982	
3	Pk4 doublets	741	
4	Pk4 triplets	87	
5	Gated	12725	
6	Flow rate	1.27	uL/sec
7			
8	Volume	5275	nL
9	Conc	2.41E+03	/uL

Notes

Developed, Manufactured, and Distributed By

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