

ProLine™ Rainbow Beads

Catalog #	Description
145-1085	ProLine Rainbow Beads , 1 x 5 ml

For research purposes only.

Description

ProLine Rainbow Beads are designed for routine calibration of the S3™ or S3e™ Cell Sorter. Each bottle contains a mixture of 3.0–3.4 µm beads with eight different fluorophore intensities that can be excited at any wavelength from 365 to 650 nm.

Contents

1 x 5 ml bottle.

Storage Buffer Composition

Deionized water with 0.02% sodium azide and 0.01% NP-40.

Storage

Store at 2–8°C.

Important: Do not freeze. Protect from light.

Directions for Use

Preparation of Beads

1. Shake or vortex the bottle vigorously.
2. Place three to five drops of solution into a 5 ml tube containing 1 ml of phosphate buffered saline.

Procedure

1. Place the 5 ml tube containing the diluted ProLine Rainbow Beads on the loading stage.
2. Move the loading stage to the run position.
3. Open a new protocol in ProSort™ Software.
4. Create one density plot and four histogram plots in the protocol. Set the axis of each plot as described in the table below.

Plot	X-Axis	Y-Axis
Density	SSC/area	FSC/area
Histogram 1	Count	FL1/area/log
Histogram 2	Count	FL2/area/log
Histogram 3	Count	FL3/area/log
Histogram 4	Count	FL4/area/log

FL, fluorescence; FSC, forward scatter; SSC, side scatter.

5. Click **Play** in the Instrument Control panel.
6. In the PMT Control panel, adjust the voltage for FSC and SSC (Figure 1) until a single dense population appears in the density plot.

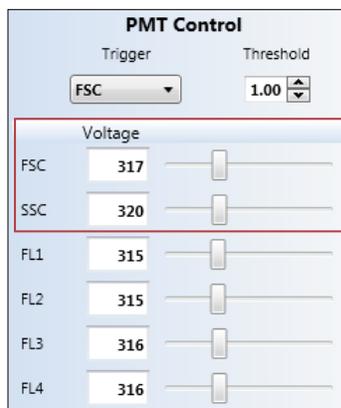


Fig. 1. PMT Control panel with FSC and SSC voltage controls highlighted. FL, fluorescence; FSC, forward scatter; SSC, side scatter.

7. Select a new rectangle region (Figure 2) and create a gate around the dense population (Figure 3).

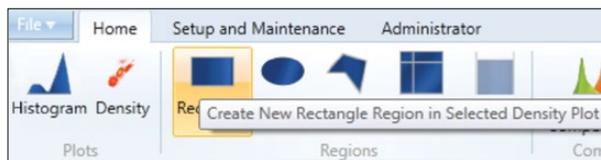


Fig. 2. Home tab with a new rectangle region highlighted.

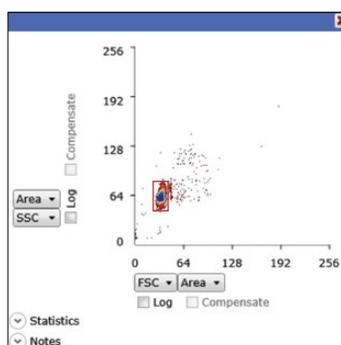


Fig. 3. Density plot with a gate on the dense population. FSC, forward scatter; SSC, side scatter.

8. Right click each histogram plot and select **Apply Gate > R1**.

- In the PMT Control panel, adjust the voltage for FL1, FL2, and FL4 until the corresponding histogram displays eight distinct peaks (Figure 4).

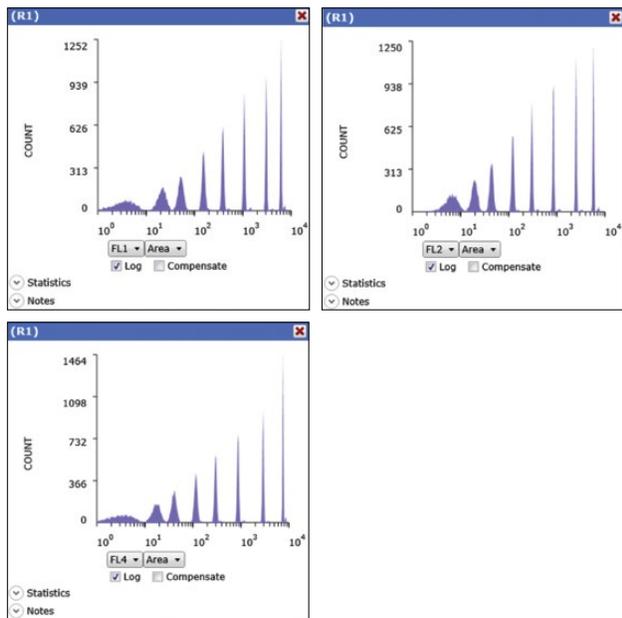


Fig. 4. FL1, FL2, and FL4 histograms displaying eight peaks after setting the appropriate voltages. FL, fluorescence.

- For FL3, two different sets of voltages are required to distinguish all eight peaks. Adjust the voltage for FL3 until the FL3 histogram displays peaks 1–7 (Figure 5). Save the flow cytometry standard (FCS) file for future reference.

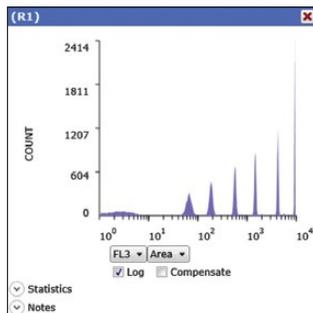


Fig. 5. FL3 histogram displaying peaks 1–7. FL, fluorescence.

- Adjust the voltage for FL3 until the FL3 histogram displays peaks 2–8 (Figure 6). Save the FCS file for future reference.

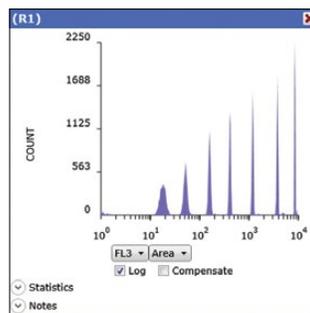


Fig. 6. FL3 histogram displaying peaks 2–8. FL, fluorescence.

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