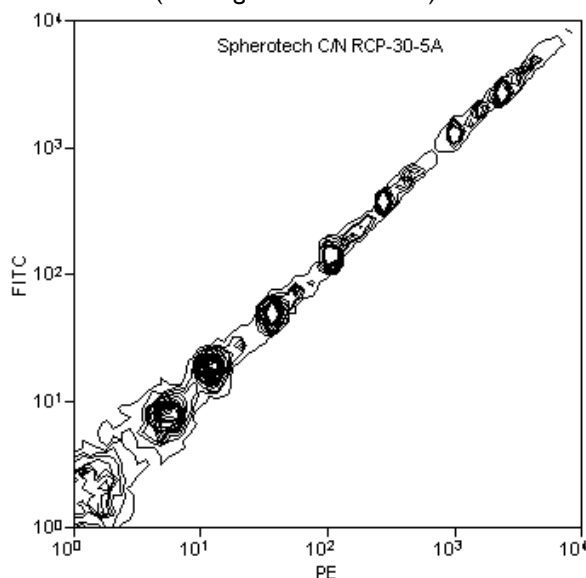# SPHERO™ Calibration Particles

SPHERO™ Calibration Particles are designed for routine calibration of flow cytometers. They are used extensively by many laboratories for QC and long term performance tracking of the flow cytometer. In addition, they are also used for routine alignment and calibration in fluorescence and confocal fluorescence microscopy.

SPHERO™ Rainbow Calibration Particles

- Contains multiple fluorophores incorporated in the same particle to be used in multiple channels of the flow cytometer

- Available with different fluorescent intensities on the same size particles

- Stable for several years when stored properly

- Withstand freeze-thaw cycles; diluted particles can be stored frozen for later use

- Can be sanitized by treating with 70% ethanol or other antibiotic agents.

Particle Type and Surface	Size, μm	Catalog No.	Unit
Rainbow Calibration, 4 peaks, 10^7/mL	1.8-2.2	RCP-20-5	5 mL
Rainbow Calibration, 6 peaks, 10^7/mL	3.0-3.4	RCP-30-5	5 mL
Rainbow Calibration, 8 peaks, 10^7/mL	3.0-3.4	RCP-30-5A	5 mL
Rainbow Calibration, 6 peaks, 10^7/mL	3.2 (+/-0.1)	RCP-32-5	5 mL
Rainbow Calibration, 4 peaks, 10^7/mL	3.5-4.0	RCP-35-5	5 mL
Rainbow Calibration, 6 peaks, 10^7/mL	6.0-6.4	RCP-60-5	5 mL

Figure 20   The contour plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A).

The **Rainbow Calibration Particles** (RCPs) contain a mixture of several similar size particles with different fluorescence intensities. Every particle contains a mixture of fluorophores that allows excitation at any wavelength from 365 to 650 nm. As a result, most channels in the flow cytometer can be calibrated using the same set of particles.

These particles are also used to determine the relative voltage range for each flow cytometry detector. This will determine the dynamic range of specific PMT detectors*.

*Perfetto, S. P., D. Ambrozak, et al. (2006). "Quality assurance for polychromatic flow cytometry." Nat. Protocols 1(3): 1522-1530.

The RCPs provide a reliable and reproducible particle mixture for calibrating flow cytometers. They are very stable since the fluorochromes are entrapped within the particles instead of being located on the surface. In addition, Spherotech uses fluorophores that are non-spectral matching to the commonly used fluorophores such as FITC, PE or PE-Cy5. As a result, the RCPs are stable in terms of fluorescence.

The RCPs are convenient and affordable to use for long term performance tracking or routine calibration. They are packaged in a dropper bottle to facilitate dispensing and storage. The diluted particles can be stored in the freezer for later use if desired to reduce costs. Dilution of a few drops of the particles from the dropper bottle to 1 mL of a diluent will provide adequate particle concentration for flow cytometer calibration. The diluted Rainbow Calibration Particles remain stable following repeated freezing and thawing.
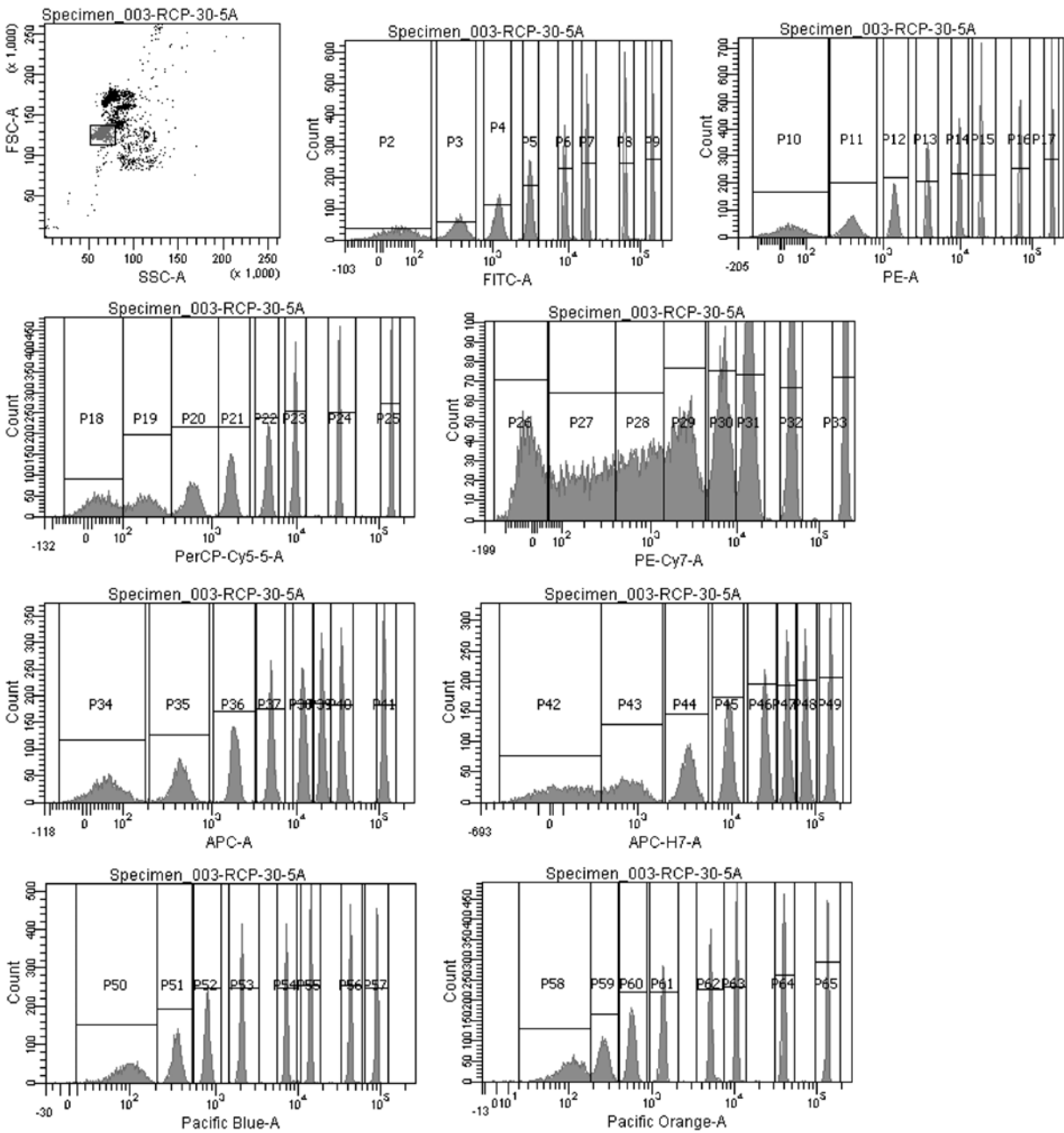
Figure 21   Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. RCP-30-5A, Lot No. AC02) are shown below.
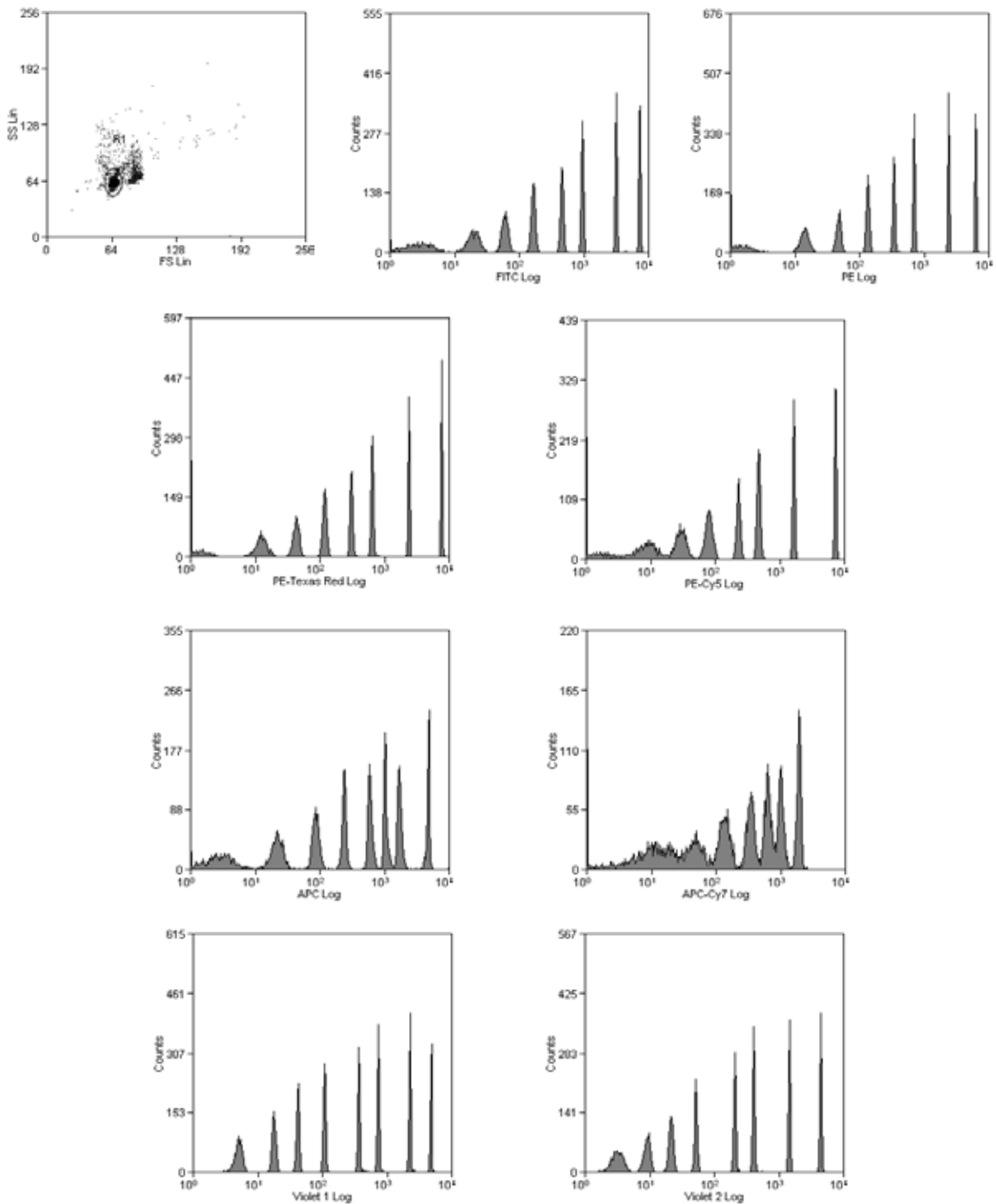
Figure 22   Histograms of the Rainbow Calibration Particles
(Cat. No. RCP-30-5A, Lot No. AA01) on a BD FacsCanto™ II.

* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 23 Histograms of the Rainbow Calibration Particles
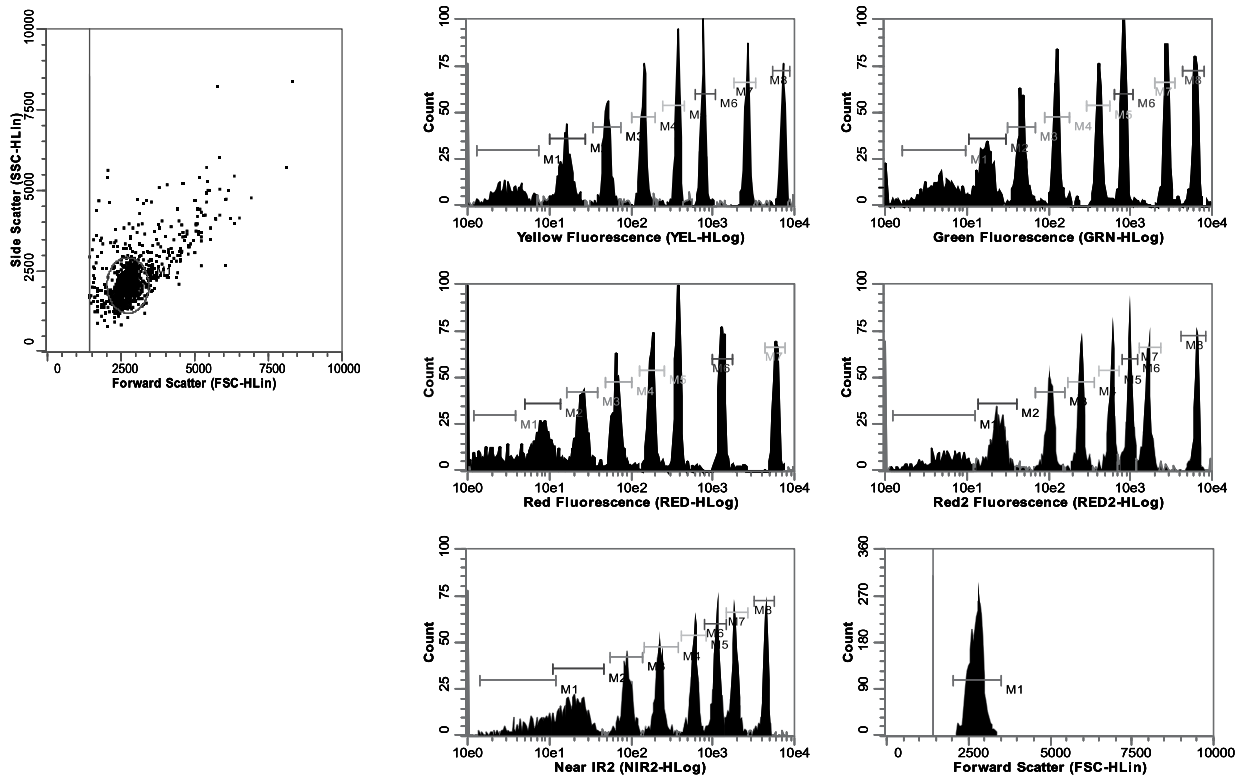(Cat. No. RCP-30-5A, Lot No. AC02) on a Millipore guava easyCyte™ 8.

Figure 24 Histograms of the Rainbow Calibration Particles
(Cat. No. RCP-60-5, Lot No. AC02) on a Millipore guava easyCyte™ 8.
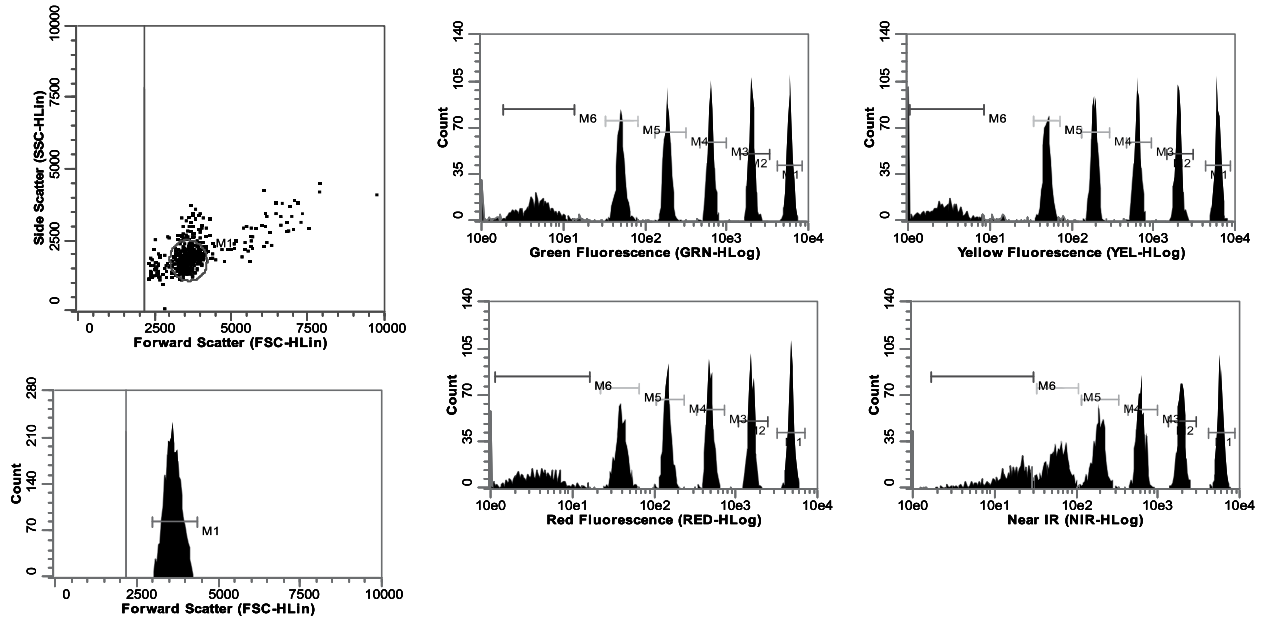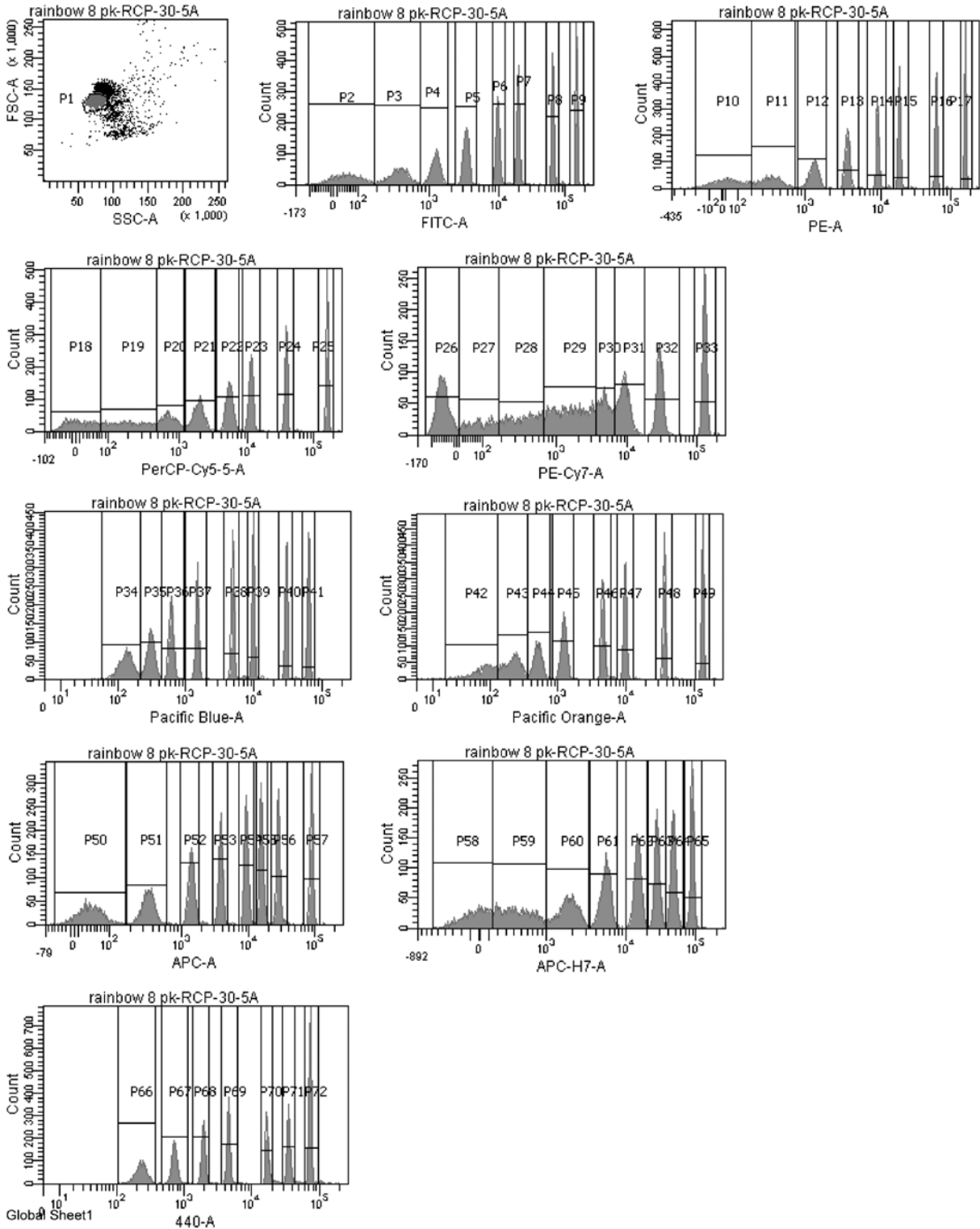
Figure 25   Histograms of the Rainbow Calibration Particles
(Cat. No. RCP-30-5A, Lot No. AA01) on a BD LSR™ II.

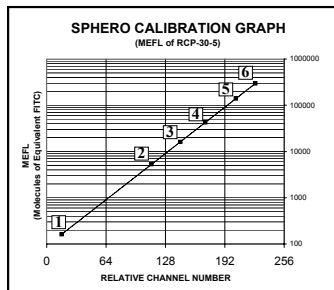* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

The relative number of fluorophores per particles has been determined for every peak of RCP-30-5 (Lot# AC01) in FL1 (FITC, MEFL), FL2 (RPE, MEPE), FL3 (RPE-Cy5, MEPCY) and FL4 (APC, MEAP) channels of flow cytometer to plot the calibration graph as shown below. The calibration graph is used to check the linearity of the PMT in each channel. In addition, the relative number of fluorophores can be cross calibrated with cells or particles stained with known number of spectral matching fluorophores such as FITC, PE, RPE-Cy5 to estimate the number of fluorophores on stained cells. The RCP-30-5A, which is identical to RCP-30-5 with the exception of two additional peaks between the blank and the dimmest peak of RCP-30-5 to give a total of 8 peaks is shown on Page 19. The RCP-30-5A is very useful in checking the sensitivity and resolution of the flow cytometer.

A Template for MS Excel files, as shown below, is available free of charge upon request. The template will allow the user to check and report the linearity of PMT in all channels easily by using RCP-30-5, RCP-30-5A, RCP-60-5, URCP-38-2K, URCP-50-2K, RQC-4K or ACP-30-5K.

PMT LINEARITY QC RECORD

PEAK #	CH #	MEFL	MEFL LOG	CALC.	RESIDUAL	CALC. MEFL
1	22.42					
2	77.13	692	2.840	2.840	0.04%	692
3	108.17	2192	3.341	3.341	0.03%	2192
4	135.42	6028	3.780	3.780	0.01%	6027
5	164.11	17493	4.243	4.243	0.01%	17491
6	183.31	35674	4.552	4.552	0.00%	35673
7	217.49	126907	5.103	5.104	0.00%	126914
8	239.84	290983	5.464	5.464	0.01%	291016
		Ave Residual			0.02%	

Slope: 0.0161
Intercept: 1.5964
Rsq: 1.0000

SPHERO CALIBRATION GRAPH
(FITC Channel)

$R^2 = 1.0000$

Rainbow Calibration Particles (RCP-30-5A)		Lot No.:		File #
Acceptable:				
Action taken if not linear:		By:		Date:

FITC Channel

PEAK	CH#	MEFL
1	16	-------
2	113	6078
3	144	18080
4	171	37488
5	204	127549
6	226	292408
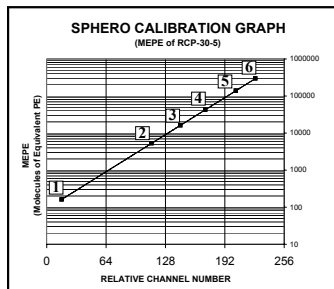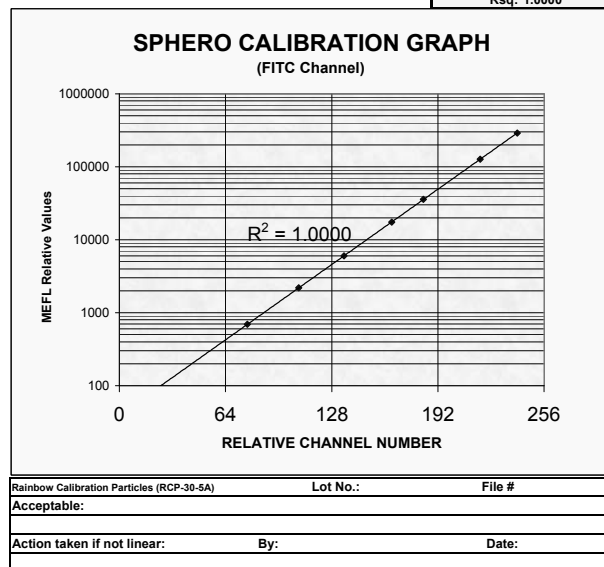
PE Channel

PEAK	CH#	MEPE
1	11	-------
2	109	5023
3	140	13251
4	167	27327
5	203	96553
6	228	259881

Figure 26 The Rainbow Calibration Particles are available in sizes of 2 to 6 μm to suit various applications. Additional histograms of Rainbow Calibration Particles are shown on Pages 24-26.
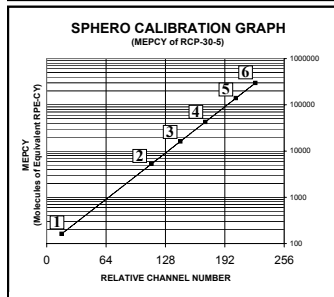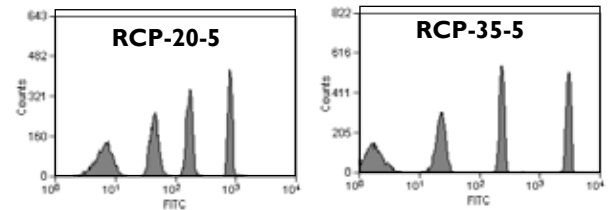
PE-CY5 Channel

PEAK	CH#	MECY
1	21	--------
2	91	12977
3	118	34975
4	148	71893
5	190	258366
6	221	1120607

RCP-20-5

RCP-35-5

APC Channel

PEAK	CH#	MEAP
1	50	--------
2	84	8892
3	99	20919
4	118	35336
5	154	62371
6	180	160356

Figure 27   Histograms of the Rainbow Calibration Particles
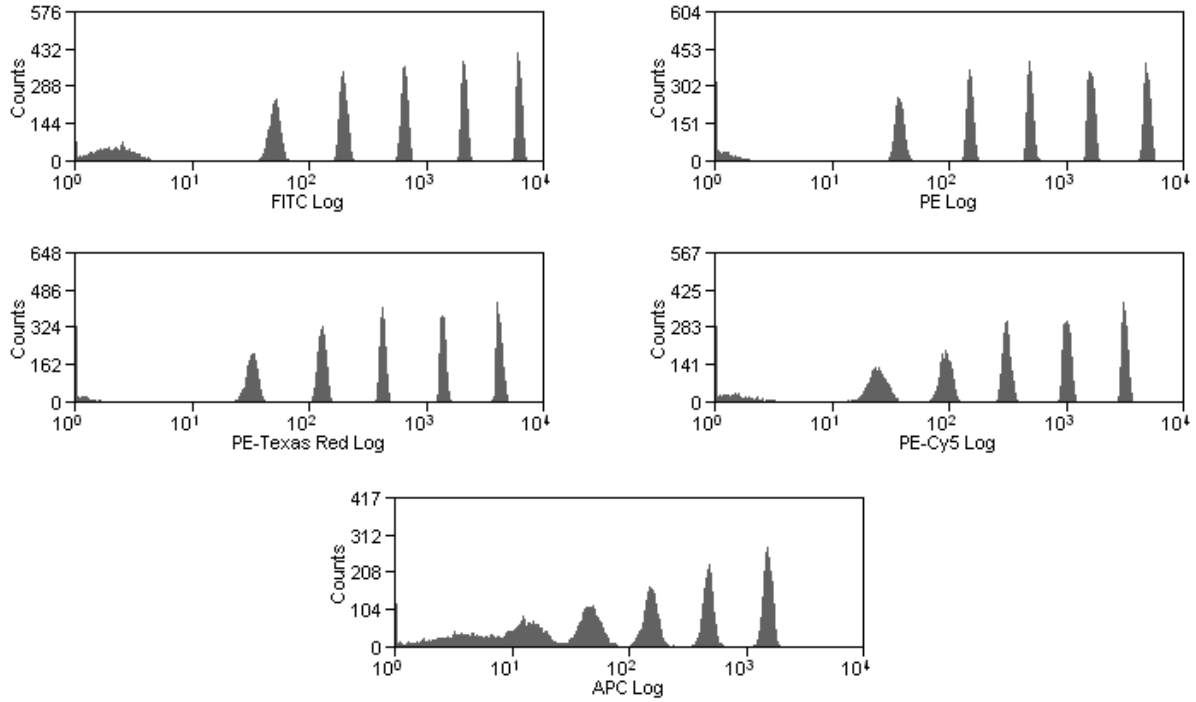(Cat. No. RCP-60-5, Lot No. AC02).

Figure 28   Histograms of the Rainbow Calibration Particles
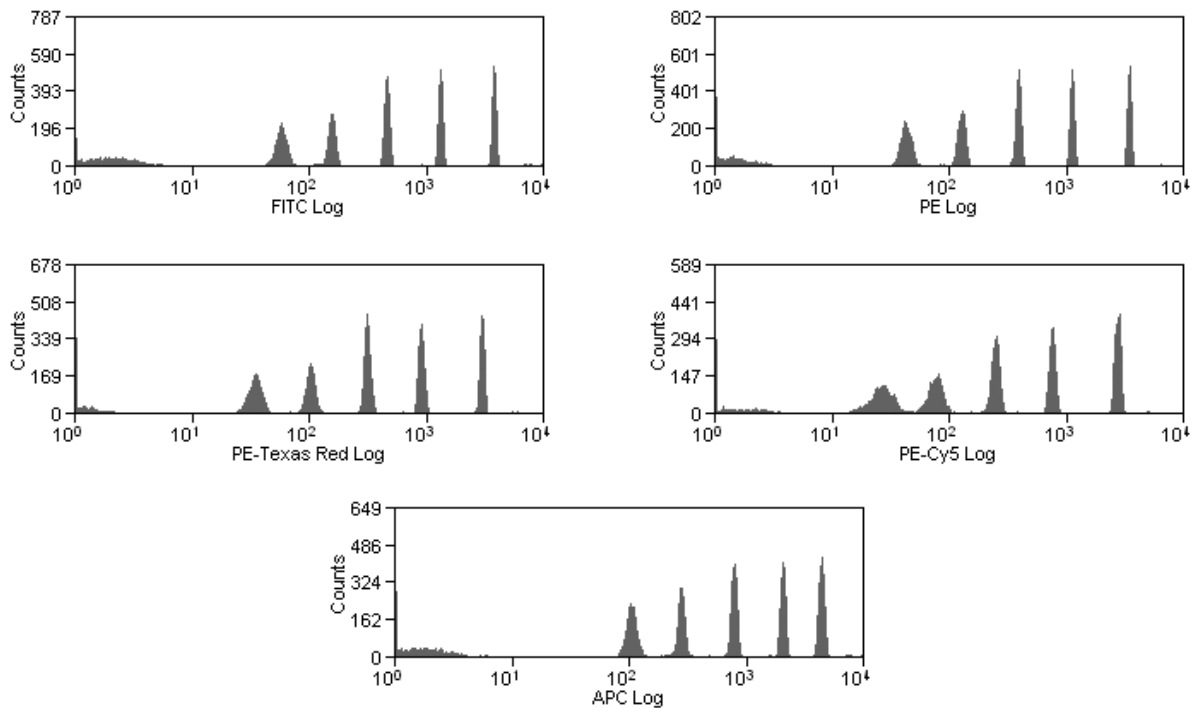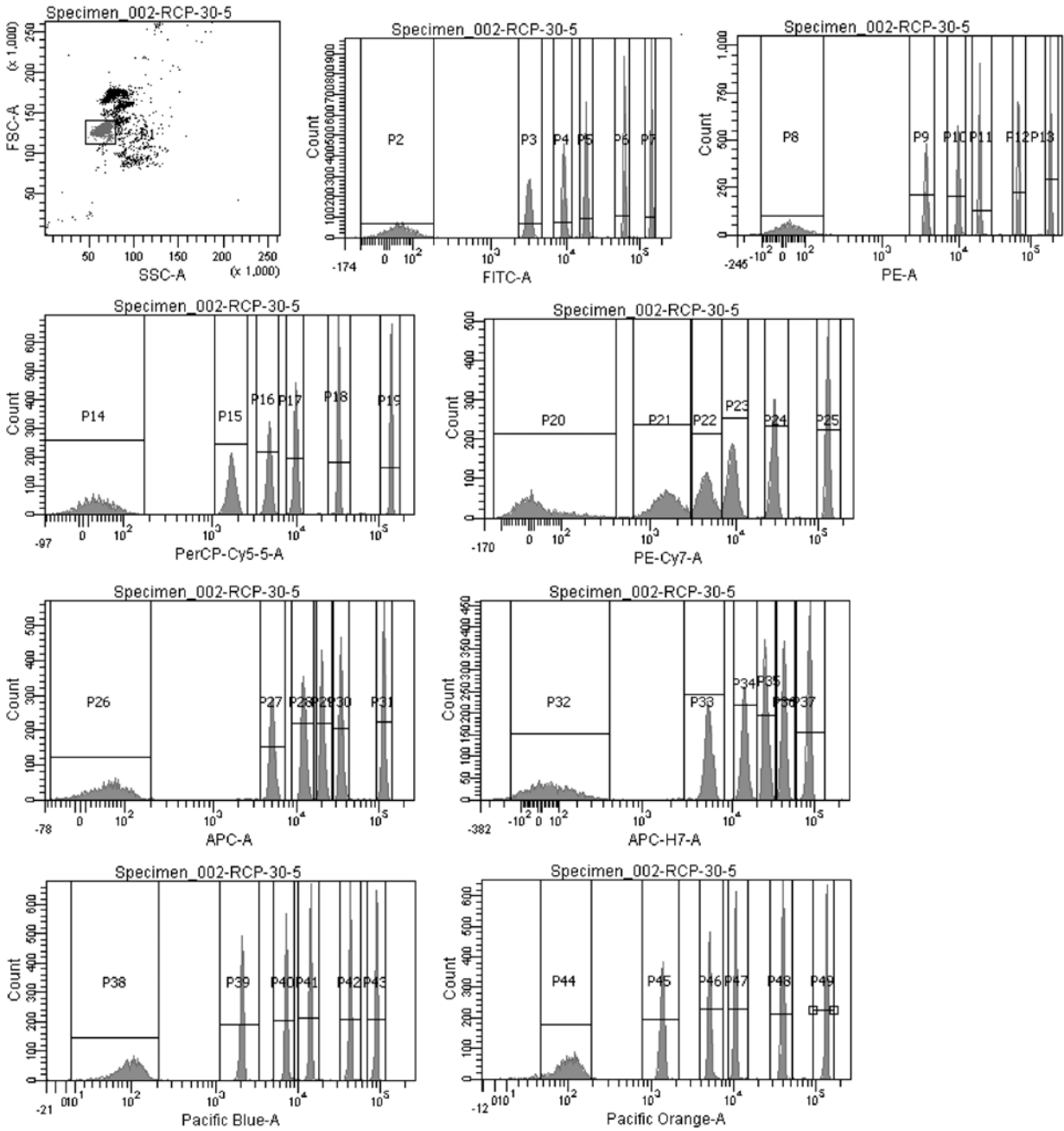(Cat. No. RCP-32-5, Lot No. AC01).

Figure 29   Histograms of the Rainbow Calibration Particles
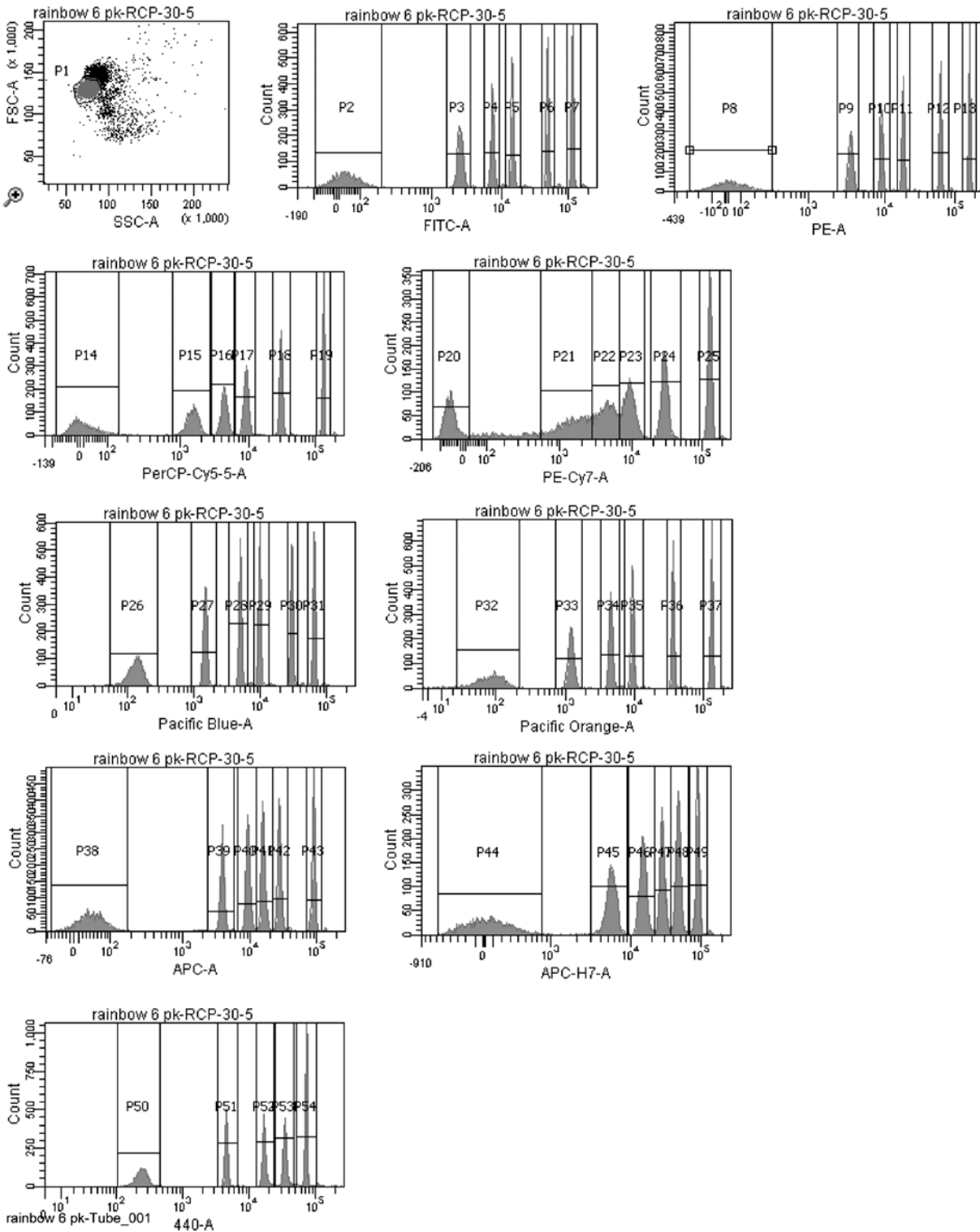(Cat. No. RCP-30-5, Lot No. AA01) on a BD FacsCanto™ II.

* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 30   Histograms of the Rainbow Calibration Particles
(Cat. No. RCP-30-5, Lot No. AA01) on a BD LSR™ II.

* Data provided by Laura Marszalek, Northwestern Memorial Hospital.