

## 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.

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\*.

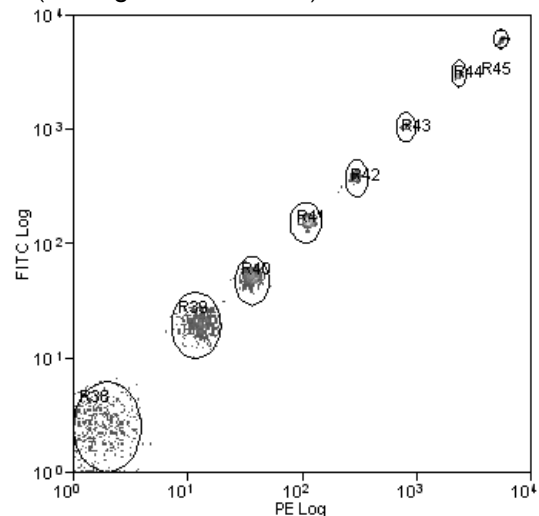
\*Perfetto, S.P., D.Ambrozak, et al. (2006). "Quality assurance for polychromatic flow cytometry." Nat. Protocols 1(3): 1522-1530.

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.

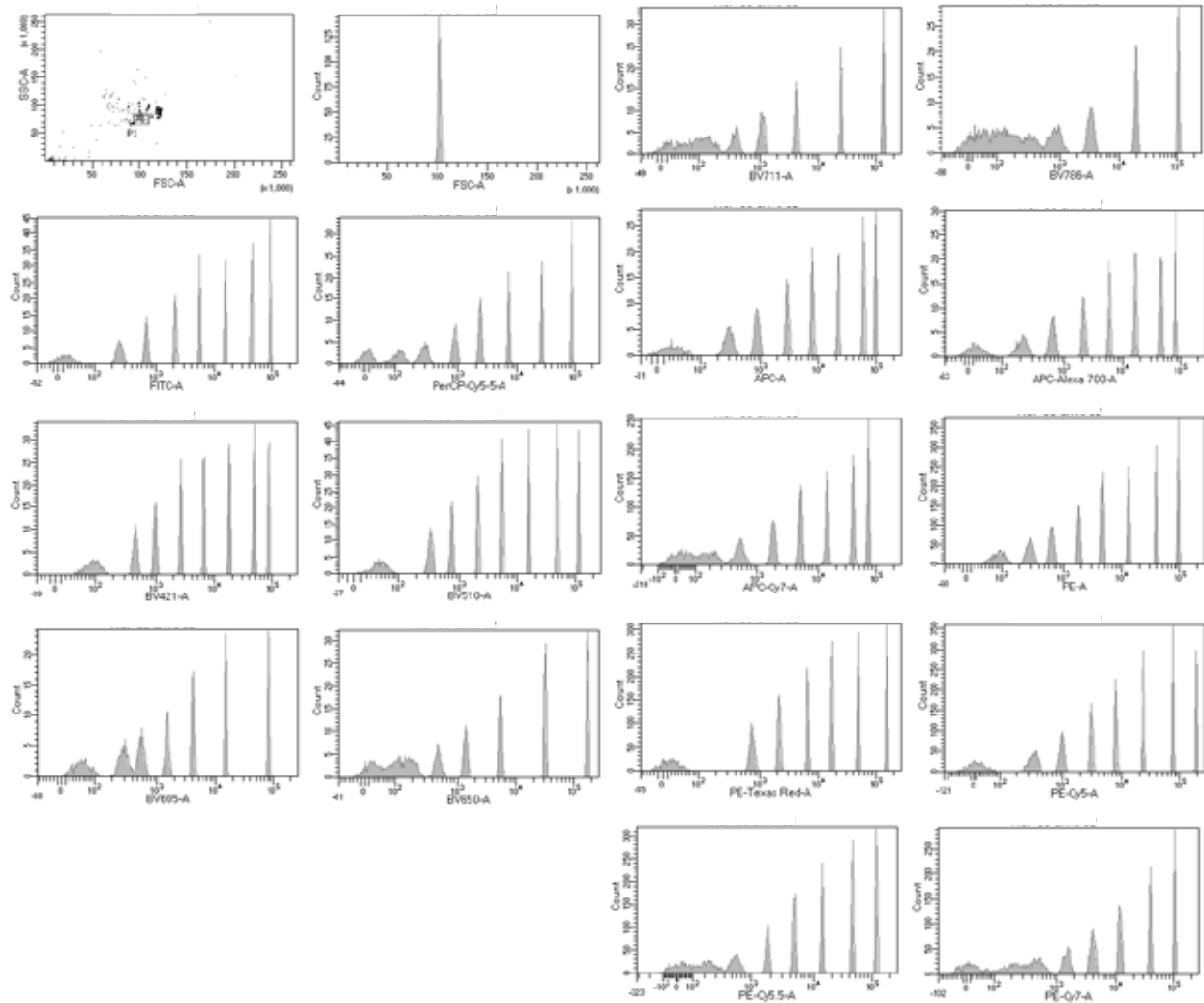
The RCPs are convenient and affordable to use for long term performance tracking or routine calibration. They are packaged in a dropper bottle to facilitate dispensing and storage. The diluted particles can be stored in the freezer for later use if desired to reduce costs. Dilution of a few drops of 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.

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

**Figure 28** The dot plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A).



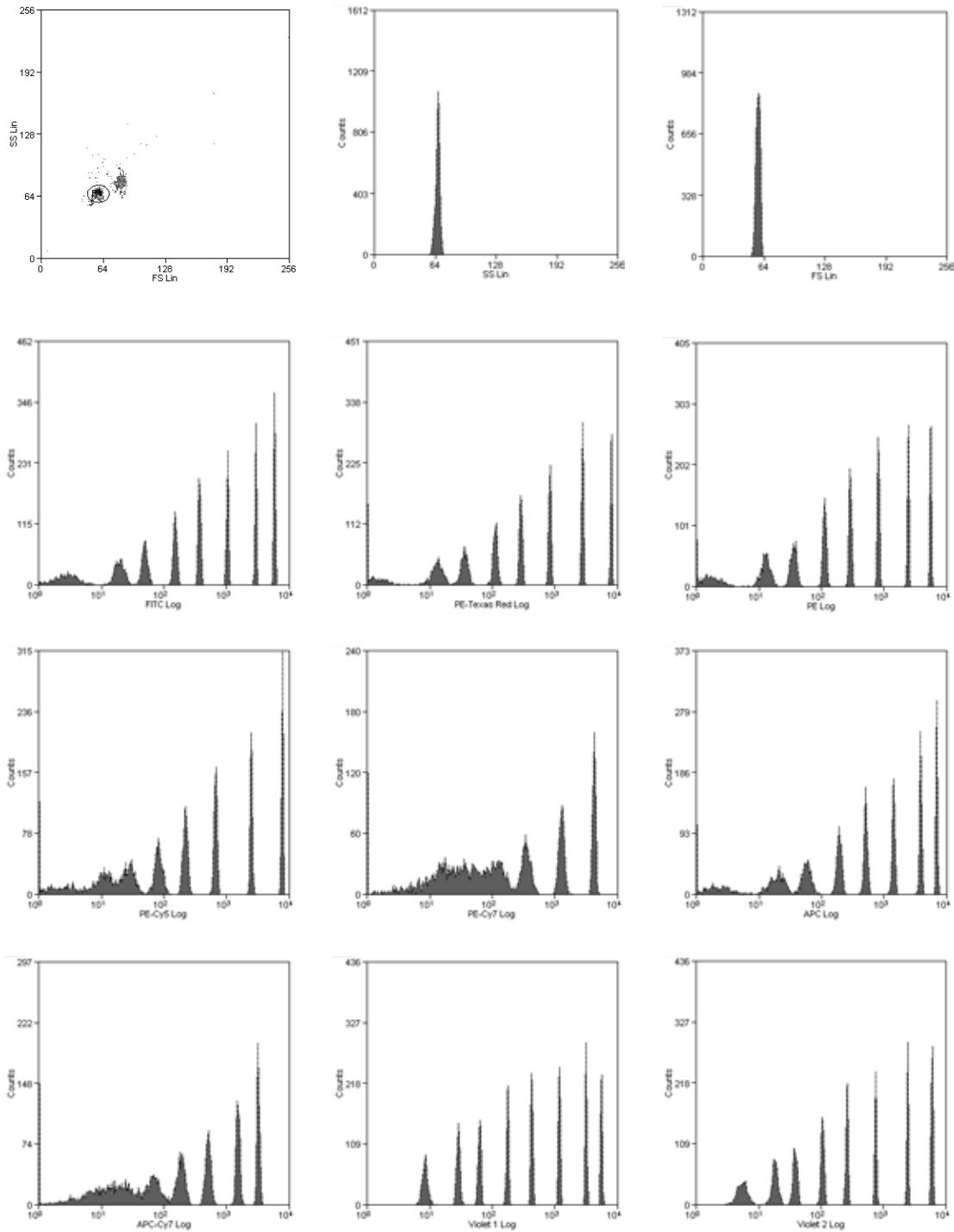
**Figure 29** Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. RCP-30-5A, Lot No. AF02) on a BD Bioscience LSRFortessa™ X-20 are shown



#### New Selected References: Flow Cytometry Calibration Particles

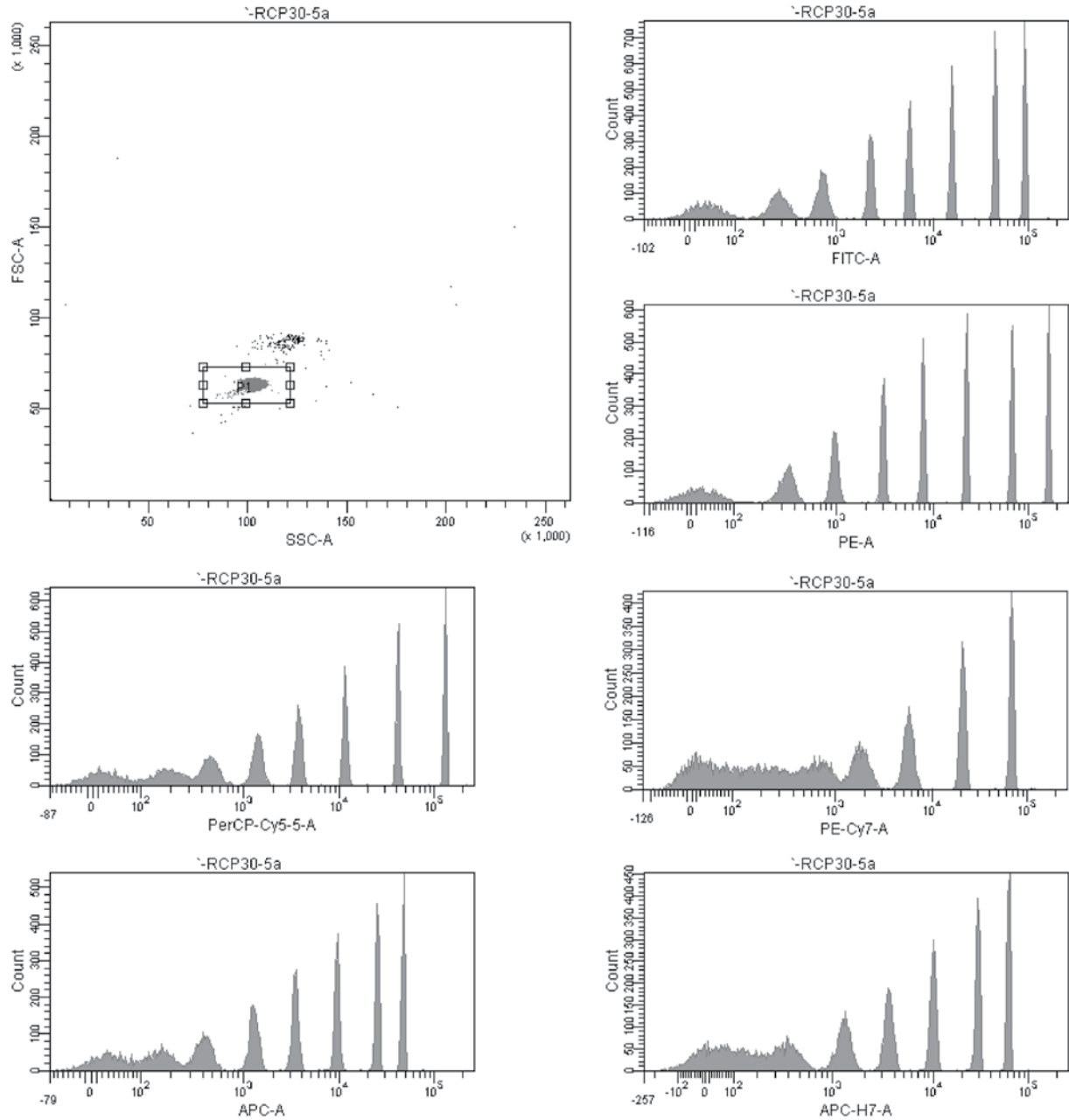
- Vera S. Donnenberg, Albert D. Donnenberg, Coping with artifact in the analysis of flow cytometric data, *Methods*, Volume 82, 1 July 2015, Pages 3-11, ISSN 1046-2023, <http://dx.doi.org/10.1016/j.ymeth.2015.03.012>. (<http://www.sciencedirect.com/science/article/pii/S1046202315001188>) - Using RCP-30-5A to calibrate to predetermined photomultiplier target channels prior to each use using 8-peak Rainbow Calibration Particles
- Frankowski, M., Simon, P., Bock, N., El-Hasni, A, Schnakenberg, U., Neukammer, J. (2015) " Simultaneous optical and impedance analysis of single cells: A comparison of two microfluidic sensors with sheath flow focusing ". *Eng. Life Sci.* 15(3): 286-296 - Using RCP-30-5A to determine the stability of hydrodynamic focusing by measuring the coefficients of variations of calibration beads with specified size and fluorescence intensities.

**Figure 30** Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. RCP-30-5A, Lot No. AF02) on a Beckman Coulter CyAn™ ADP are shown below.



Flow Cytometry Particles -  
Linearity and Sensitivity

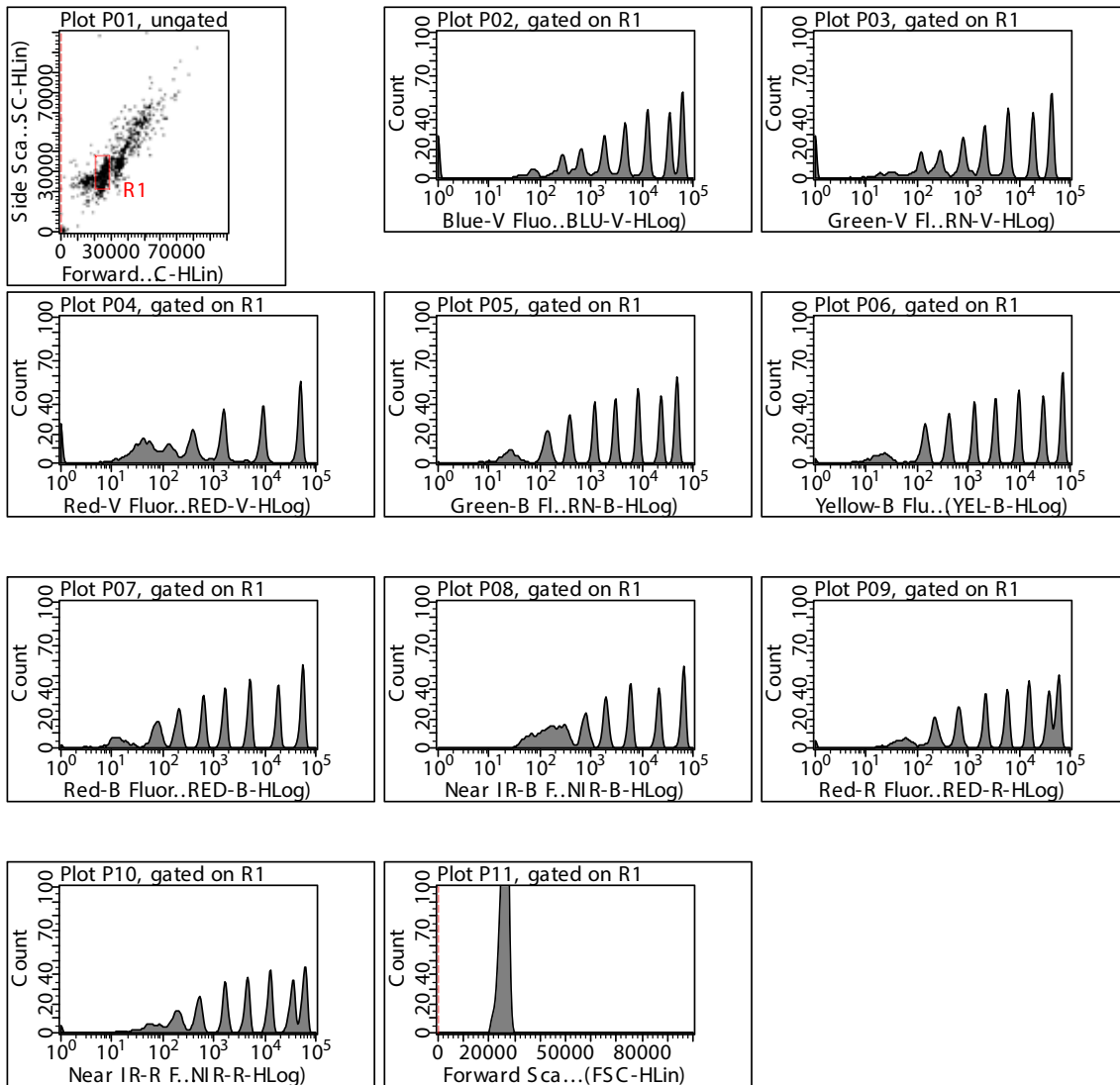
**Figure 3I** Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5A, Lot No. AE01) on a BD FACSanto™ II.



Flow Cytometry Particles -  
Linearity and Sensitivity

\* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

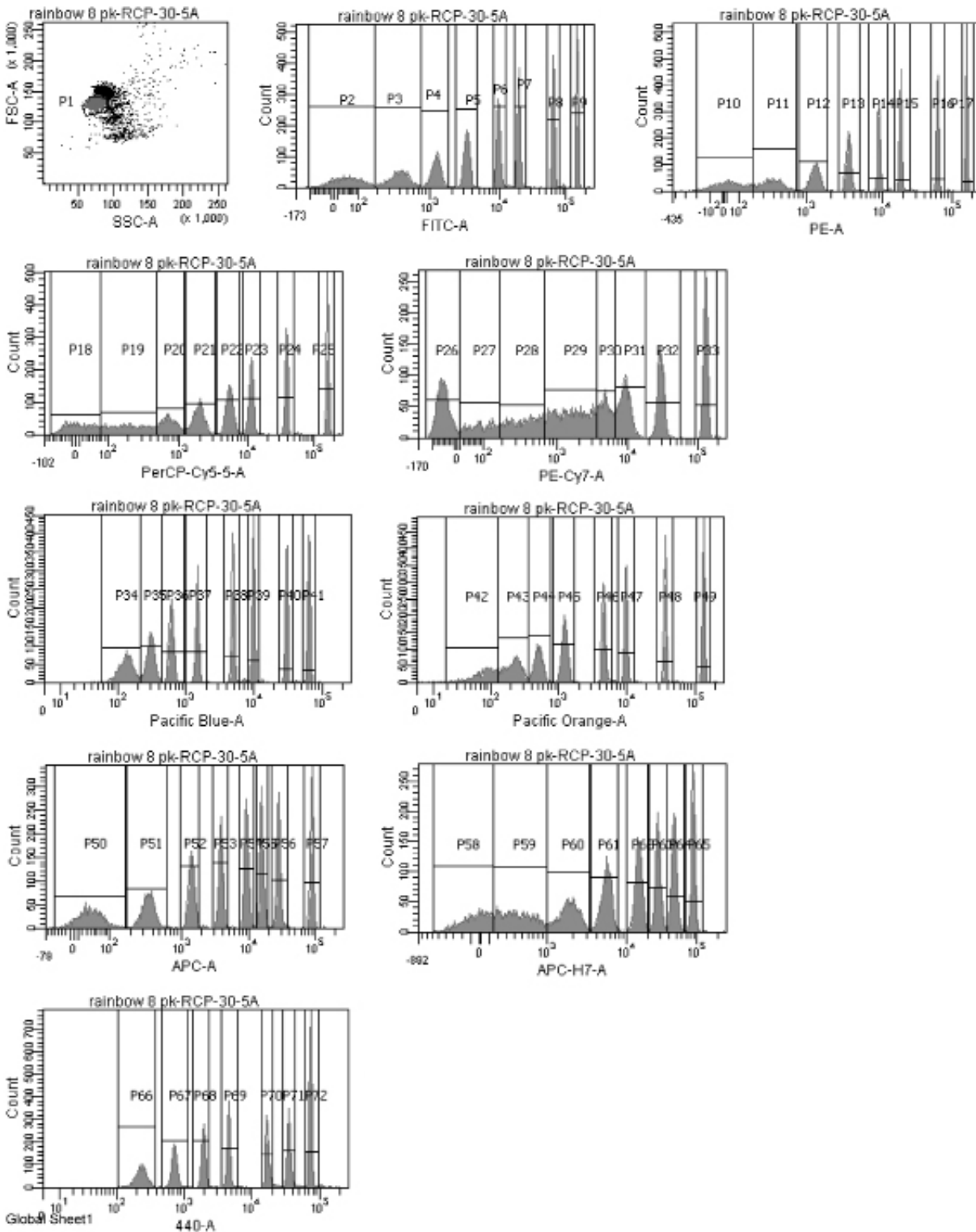
**Figure 32** Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5A, Lot No. AF01) on a Millipore guava easyCyte™ I2.



#### New Selected References: Flow Cytometry Calibration Particles

- Brown M, Stafford LJ, Onisk D, Joaquim T, Tobb A, et al. (2013) Snorkel: An Epitope Tagging System for Measuring the Surface Expression of Membrane Proteins. PLoS ONE 8(9): e73255. doi: 10.1371/journal.pone.0073255 - Flow cytometry was performed on a Guava EasyCyte Plus (Millipore) while calibration was performed using Rainbow Calibrator Particles Spherotech Cat. No. RCP 30-5A.

**Figure 33** 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.

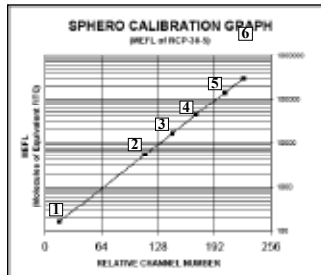
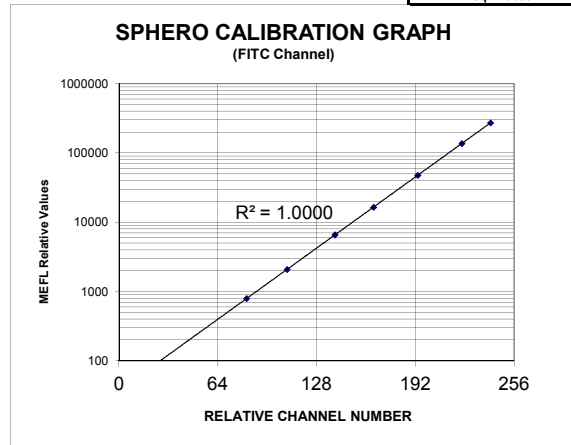
Flow Cytometry Particles -  
Linearity and Sensitivity

The relative number of fluorophores per particles has been determined for every peak of RCP-30-5 (Lot# AG01) 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 21. 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.

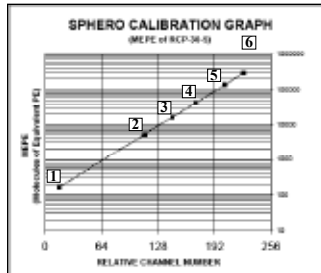
**PMT LINEARITY QC RECORD**

PEAK #	CH #	MEFL	MEFL LOG	CALC.	RESIDUAL	CALC. MEFL
1	23.98			1.958		91
2	82.63	792	2.899	2.898	0.15%	791
3	108.85	2079	3.318	3.319	0.25%	2083
4	139.92	6568	3.819	3.817	0.45%	6562
5	164.94	16471	4.217	4.218	0.37%	16531
6	193.56	47497	4.677	4.677	0.15%	47575
7	222.14	137049	5.137	5.136	0.23%	136680
8	240.75	271647	5.434	5.434	0.04%	271771
<b>Ave Residual</b>					<b>0.23%</b>	
					<b>Slope: 0.0160</b>	
					<b>Intercept: 1.5730</b>	
					<b>Rsq: 1.0000</b>	



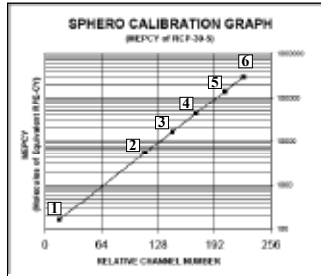
**FITC Channel**

PEAK	CH#	MEFL
1	26	-----
2	126	4447
3	158	14227
4	189	46322
5	218	133924
6	238	276897



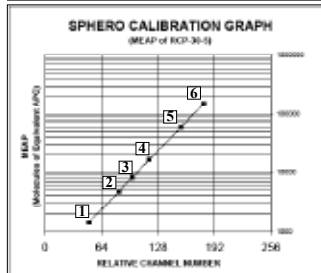
**PE Channel**

PEAK	CH#	MEPE
1	13	-----
2	119	3236
3	152	10754
4	184	34842
5	214	104483
6	237	245894



**PE-CY5 Channel**

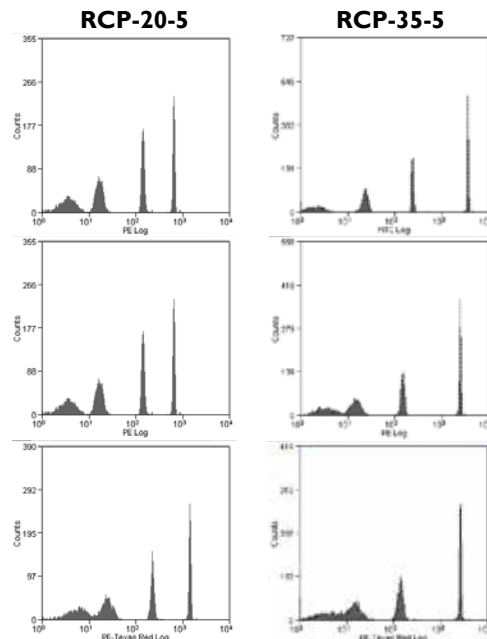
PEAK	CH#	MECY
1	17	-----
2	108	8737
3	141	28177
4	176	93996
5	211	334087
6	243	1023447



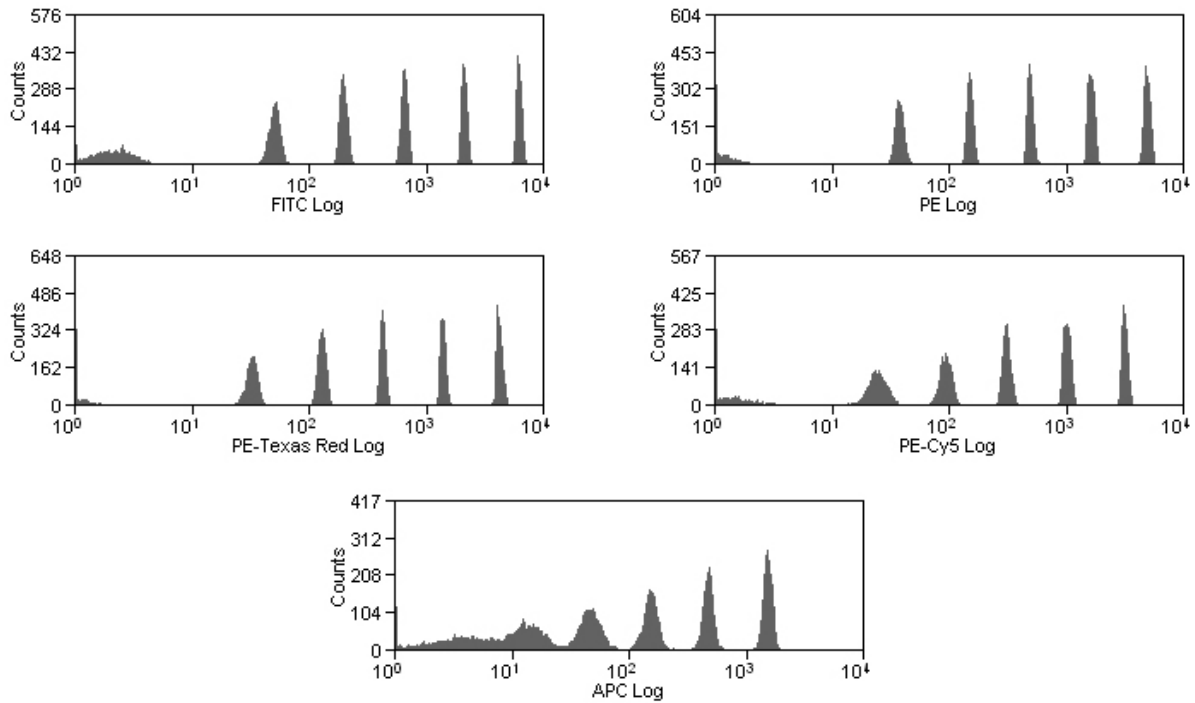
**APC Channel**

PEAK	CH#	MEAP
1	23	-----
2	128	2395
3	161	8273
4	194	27652
5	221	75669
6	239	145428

**Figure 34** 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 21-29.

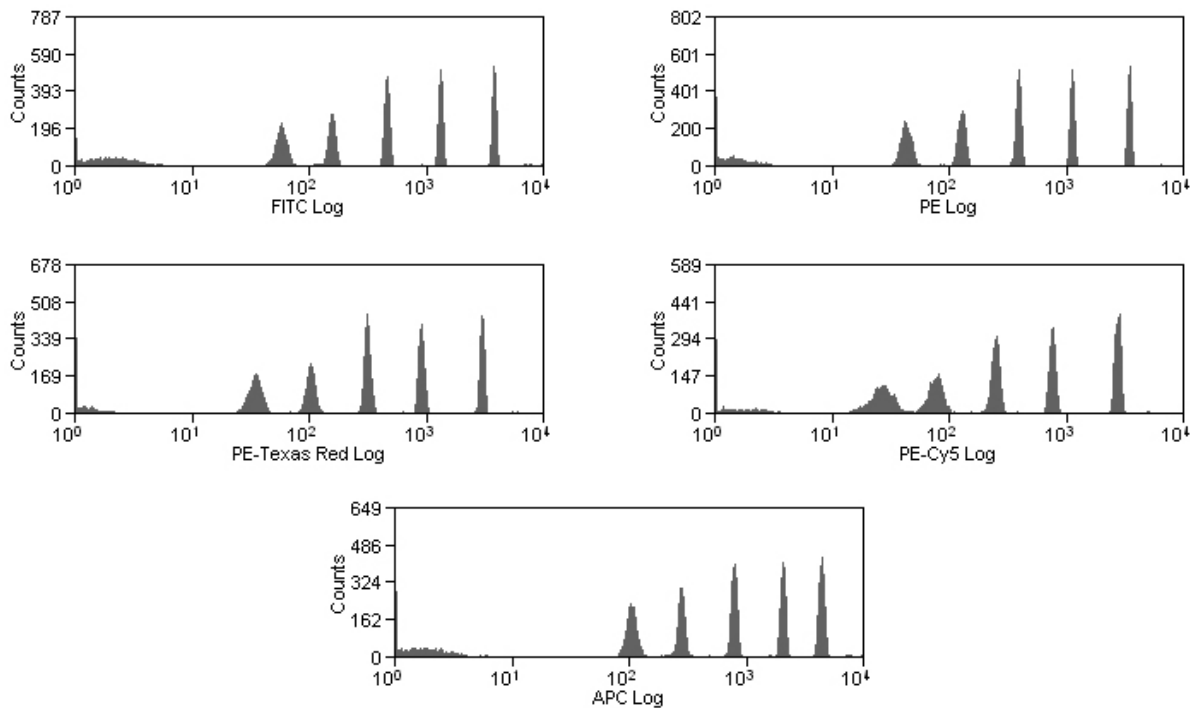


**Figure 35** Histograms of the Rainbow Calibration Particles (Cat. No. RCP-60-5, Lot No. AF01).



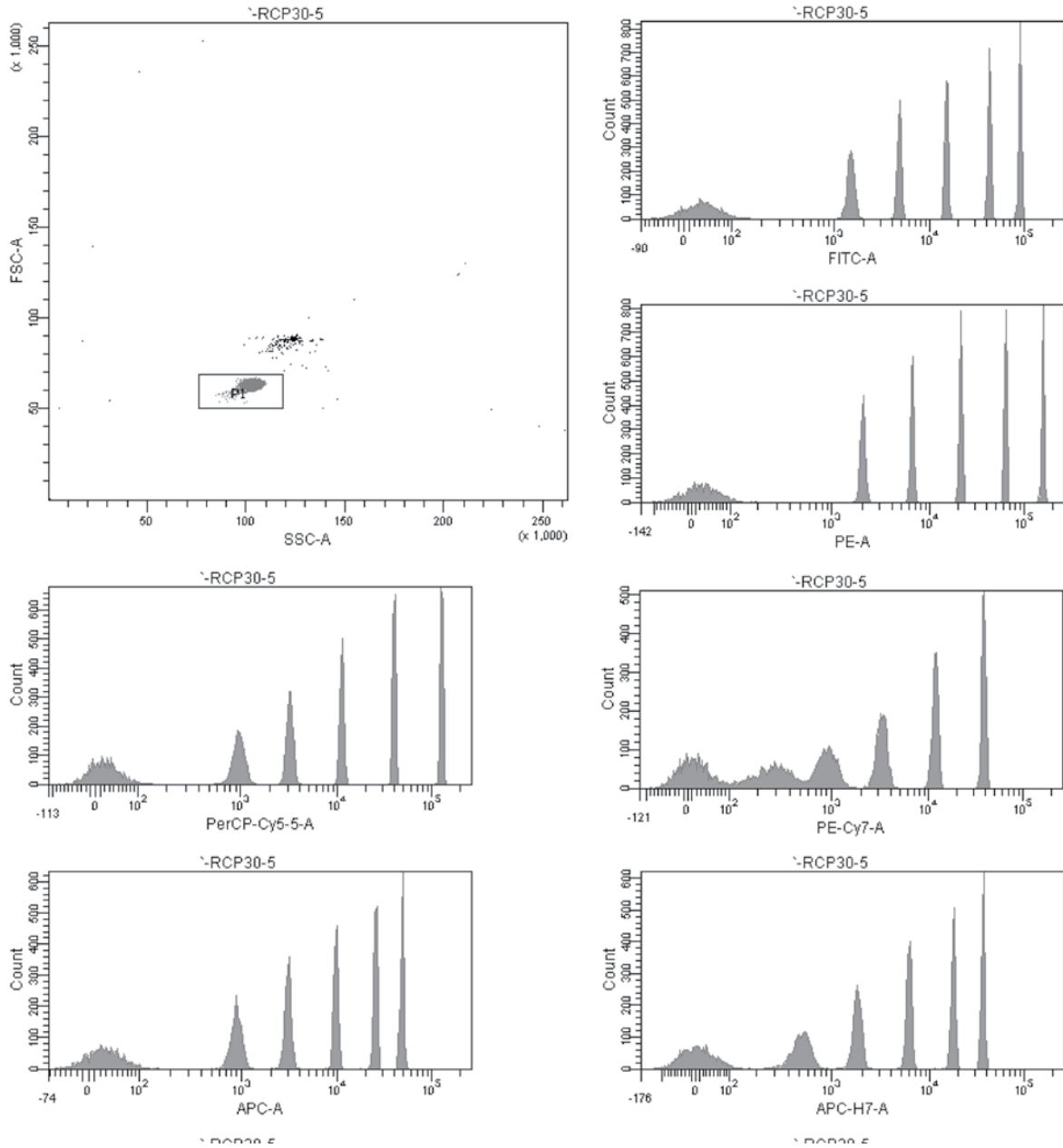
Flow Cytometry Particles -  
Linearity and Sensitivity

**Figure 36** Histograms of the Rainbow Calibration Particles (Cat. No. RCP-32-5, Lot No. AF02).





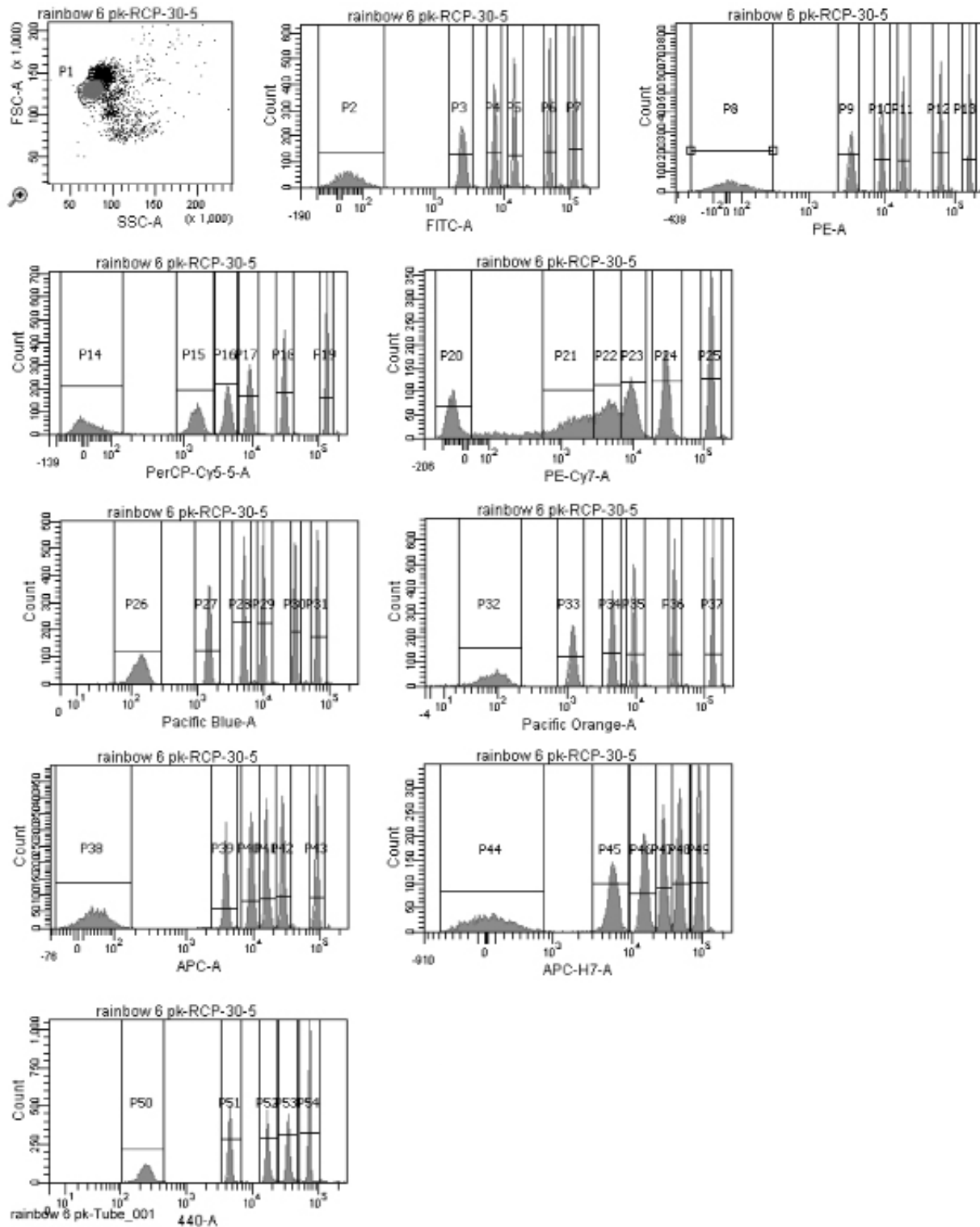
**Figure 37** Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5, Lot No. AD03) on a BD FACSanto™ II.



\* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Flow Cytometry Particles -  
Linearity and Sensitivity

**Figure 38** 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.

Flow Cytometry Particles -  
Linearity and Sensitivity