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. In addition, they are used for routine alignment and calibration in fluorescence and confocal fluorescence microscopy.

SPHERO™ Rainbow Calibration Particles

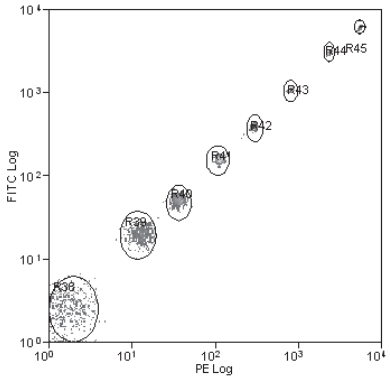
- Contains multiple fluorophores incorporated in the same particle to be used in multiple channels of the flow cytometer
- Available with different fluorescent intensities on the same size particles
- Stable for several years when stored properly
- Withstand freeze-thaw cycles; diluted particles can 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	0.4-0.6	RCP-05-5	5 mL
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, 8 peaks, 10^7/mL	3.0-3.4	RCP-30-20A	20 mL
Rainbow Calibration, Peak 2, 10^7/mL	3.0-3.4	RCP-30-5A-2	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-60-20	20 mL
Rainbow Calibration, 6 peaks, 10^7/mL	6.0-6.4	RCP-60-5	5 mL

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 have been used to determine the relative voltage range for each flow cytometry detector. This will determine the dynamic range of specific PMT detectors*.

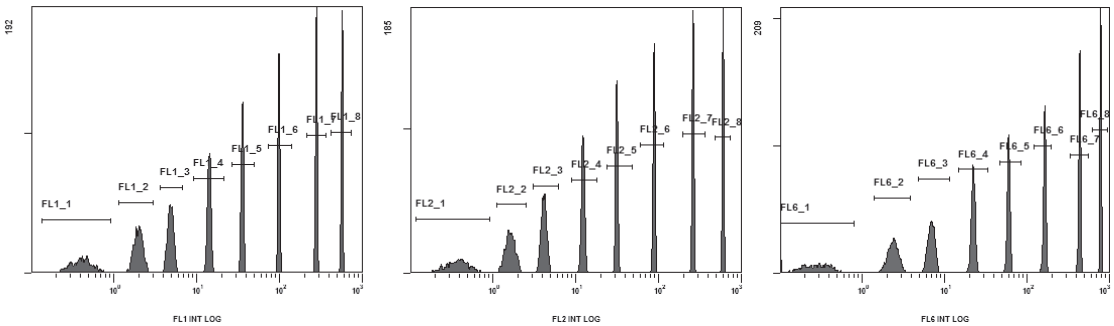
**Perfetto, S. P., D.Ambrozak, et al. (2006). "Quality assurance for polychromatic flow cytometry." Nat. Protocols 1(3): 1522-1530.*



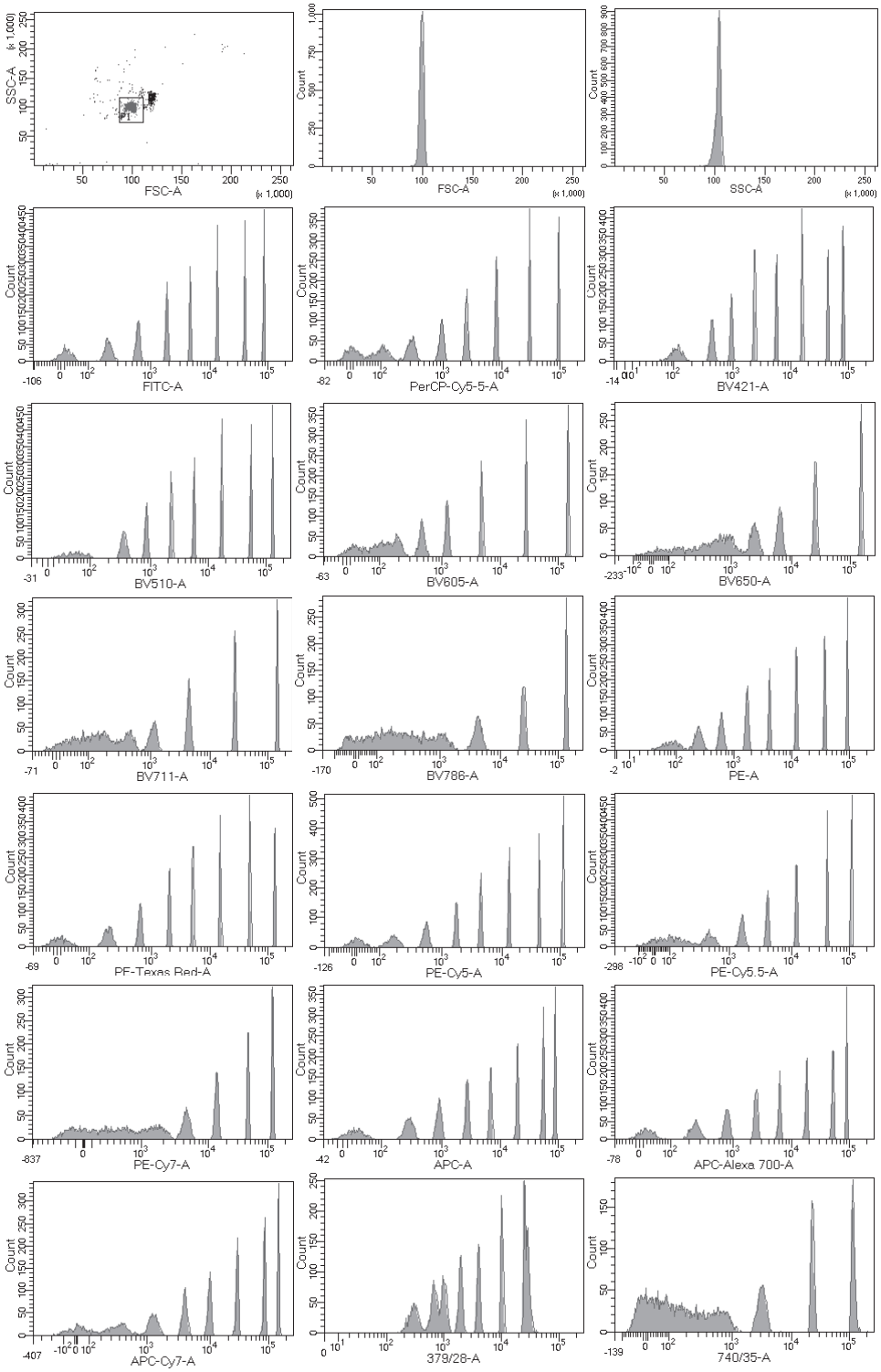
Dot plot of RCP-30-5A
from a BD Bioscience LSRFortessa™ X-20

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. 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.



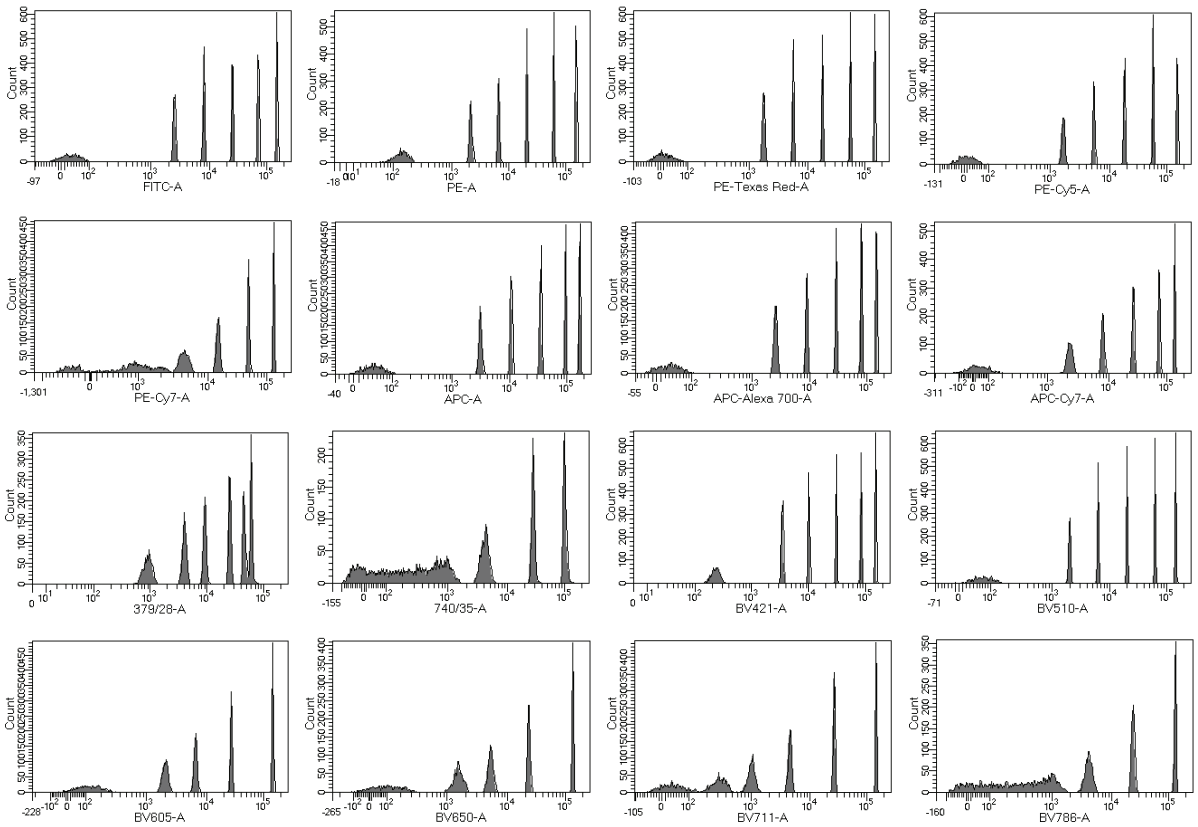
Histograms of RCP-30-5A from a Beckman Coulter Gallios in three channels



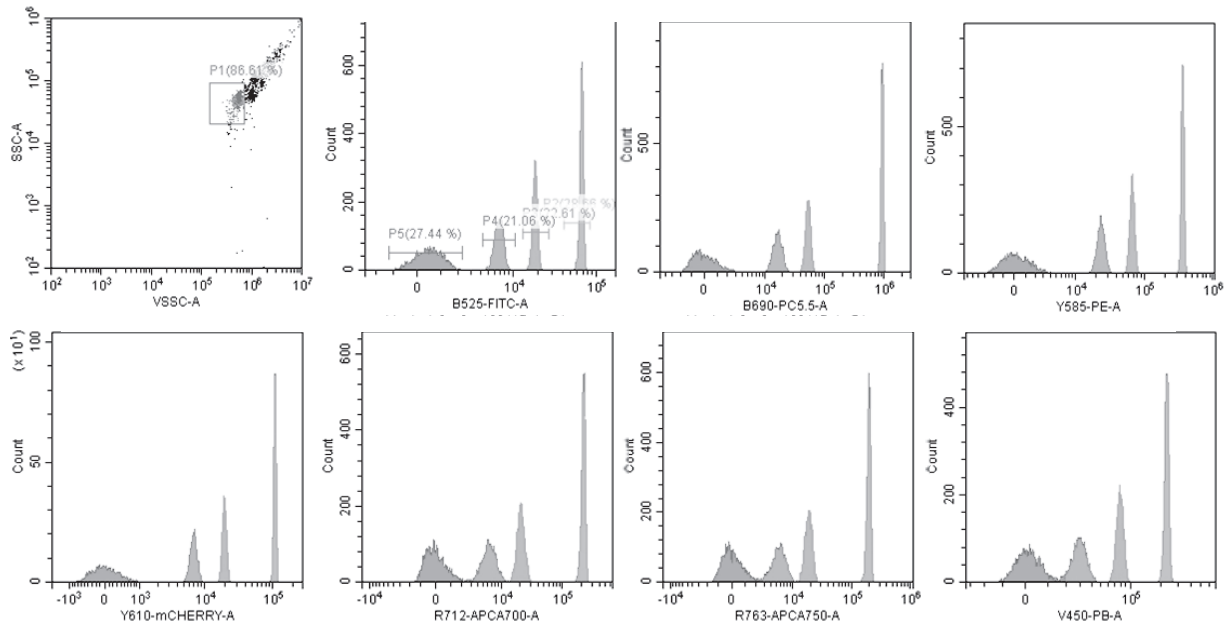
Dot plot and histogram of RCP-30-5A from a BD Bioscience LSRFortessa™ X-20

Selected Reference:

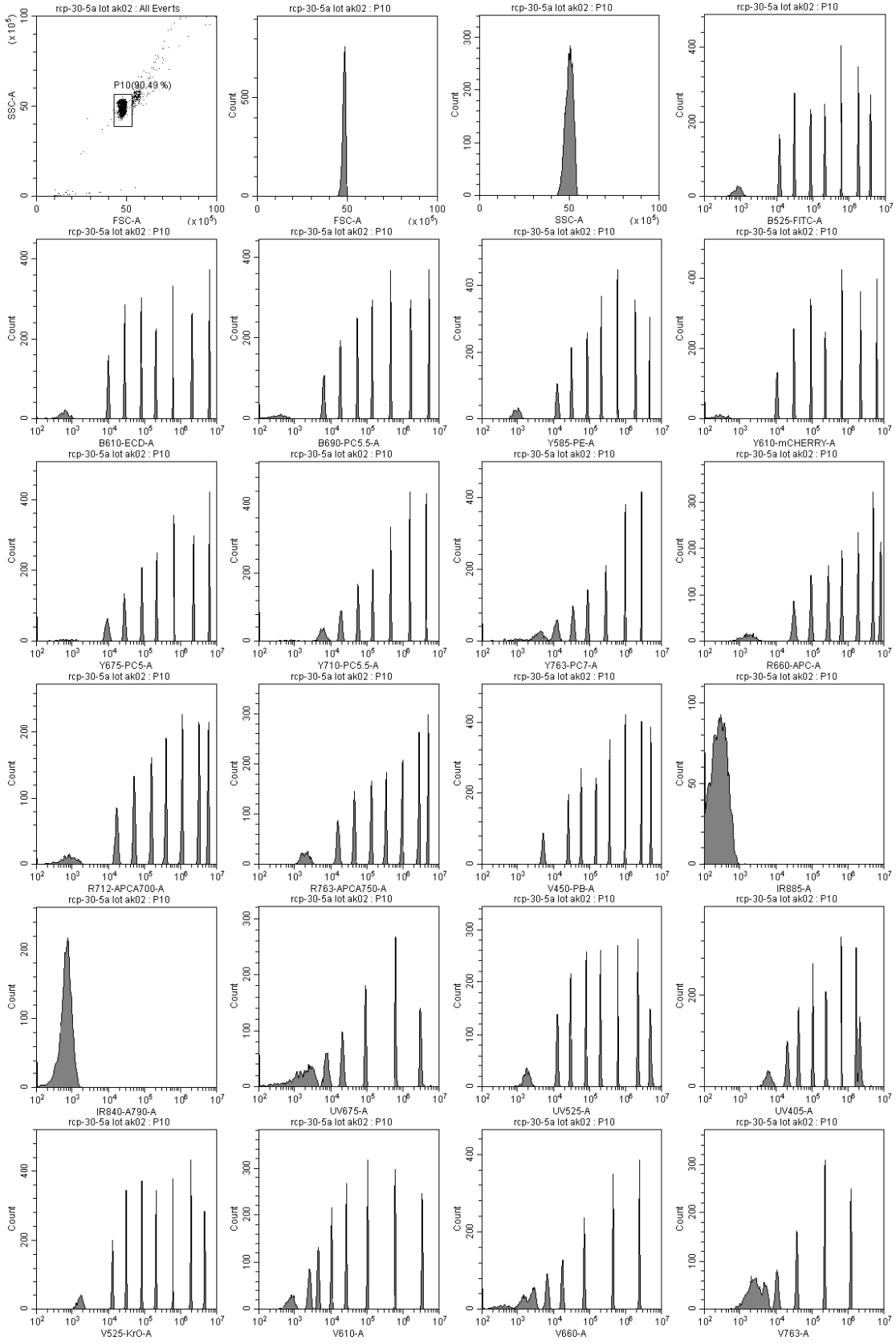
- *Vera S. Donnenberg, Albert D. Donnenberg, Coping with artifact in the analysis of flow cytometric data, Methods, Volume 82, 1 July 2015, Pages 3-11, ISSN 1046-2023, http://dx.doi.org/10.1016/j.ymeth.2015.03.012.(http://www.sciencedirect.com/science/article/pii/S1046202315001188) - Using RCP-30-5A to calibrate the flow cytometer to predetermined photomultiplier target channels prior to each use*



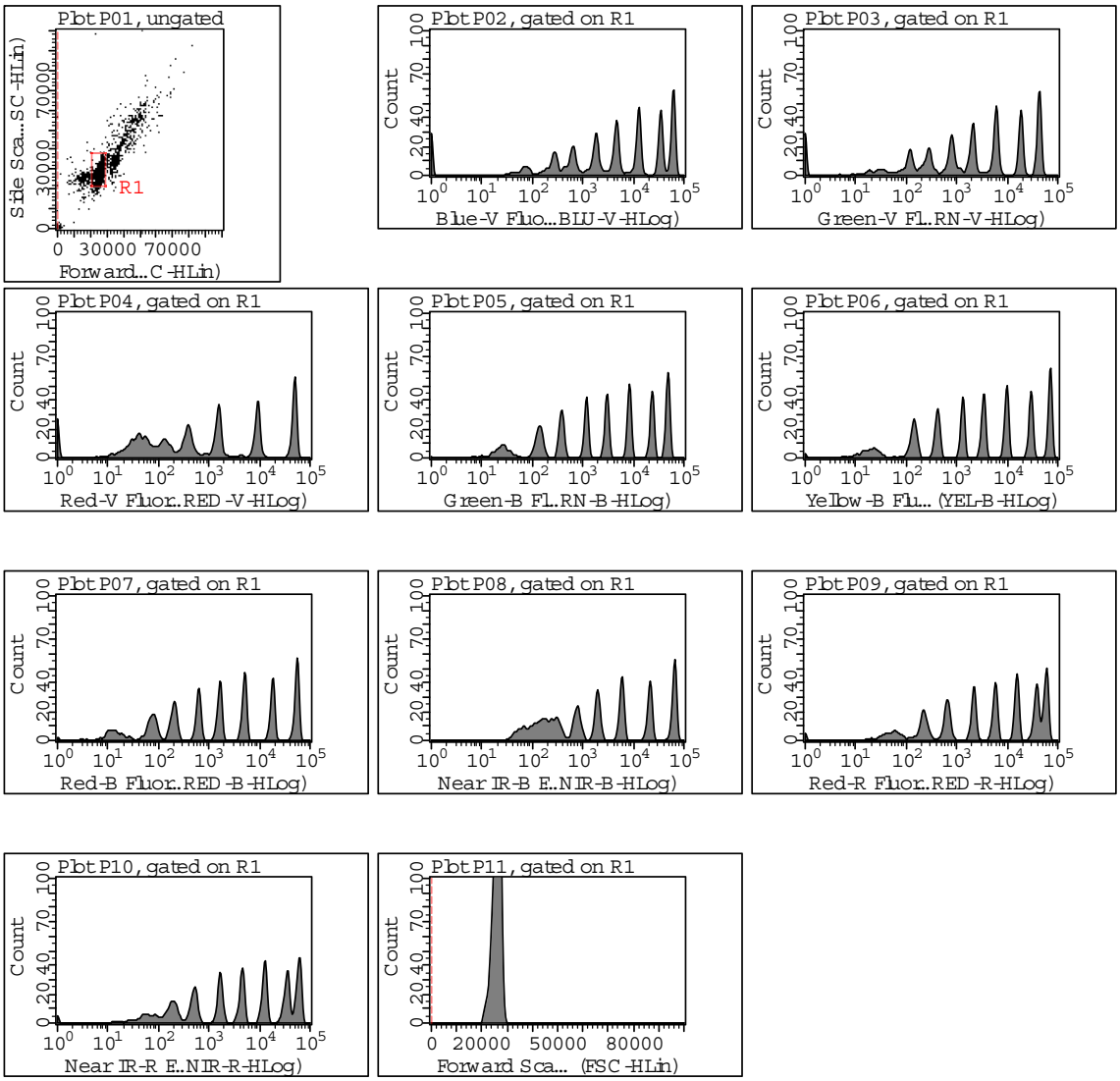
Dot plot and histogram of RCP-30-5 from a BD Fortessa X-20



Dot plot and histogram of RCP-05-5 from a Beckman Coulter CytoFLEX LX



Dot plot and histogram of RCP-30-5A from a Beckman Coulter CytoFLEX LX

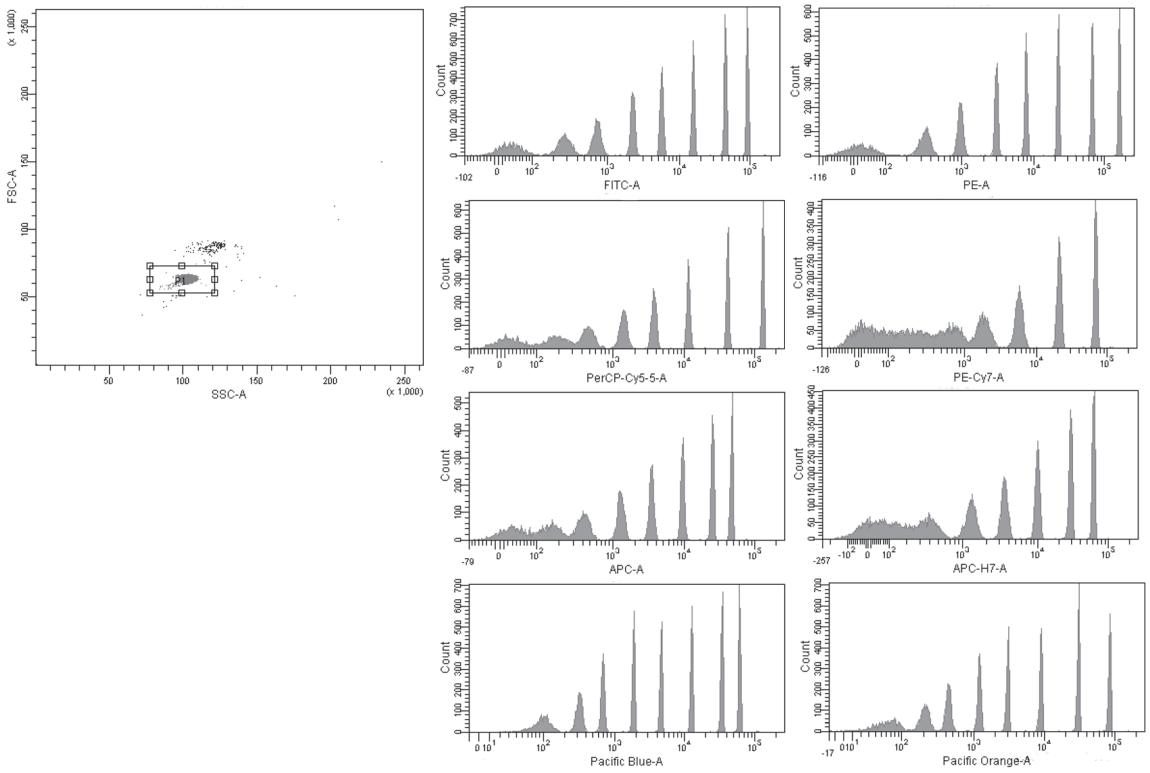


Dot plot and histogram of RCP-30-5A from a Guava easyCyte 12

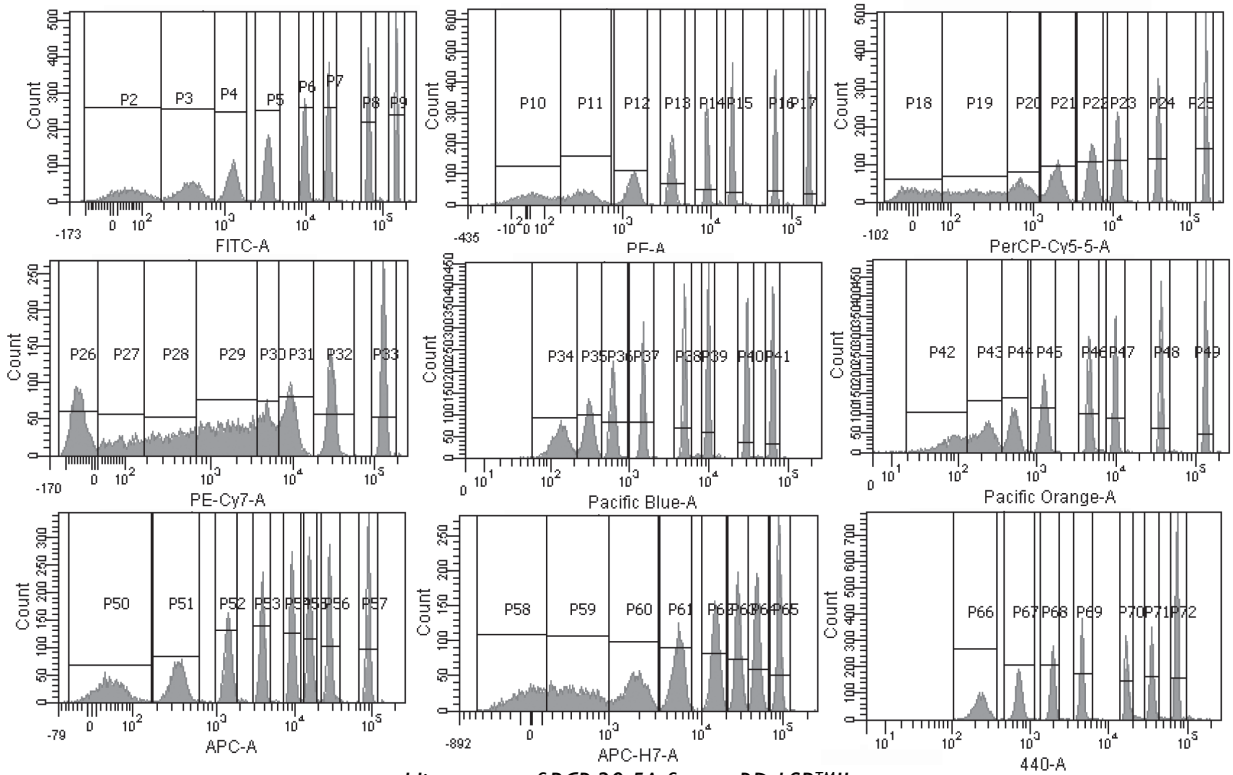
Selected Reference:

- *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.*

- *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.*



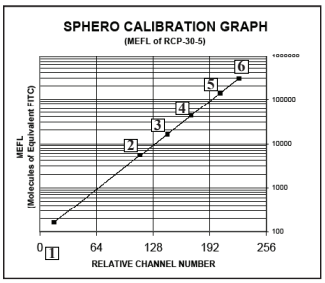
Dot plot and histograms of RCP-30-5A from a BD FacsCanto™II



Histograms of RCP-30-5A from a BD LSR™II

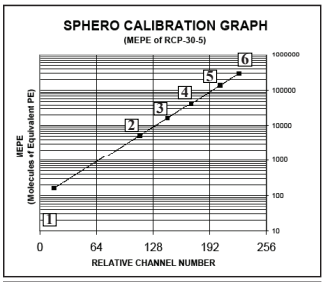
* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

The relative number of fluorophores per particles has been determined for every peak of RCP-30-5 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.



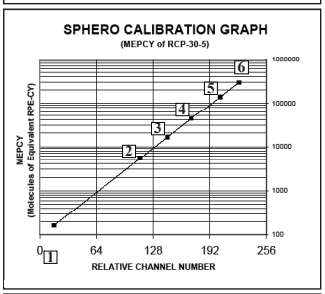
FITC Channel

PEAK	CH#	MEFL
1	26	-------
2	142	4447
3	166	14227
4	196	46322
5	227	133924
6	248	276897



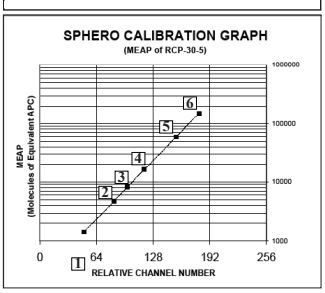
PE Channel

PEAK	CH#	MEPE
1	13	-------
2	137	3236
3	162	10754
4	193	34842
5	225	104483
6	249	245894



PE-CY5 Channel

PEAK	CH#	MECY
1	17	--------
2	112	8737
3	139	28177
4	171	93996
5	208	334087
6	242	1023447



APC Channel

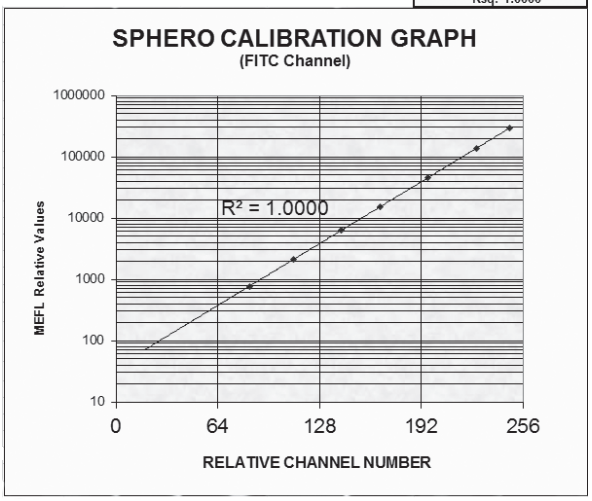
PEAK	CH#	MEAP
1	23	--------
2	137	2395
3	162	8273
4	193	27652
5	223	75669
6	237	145428

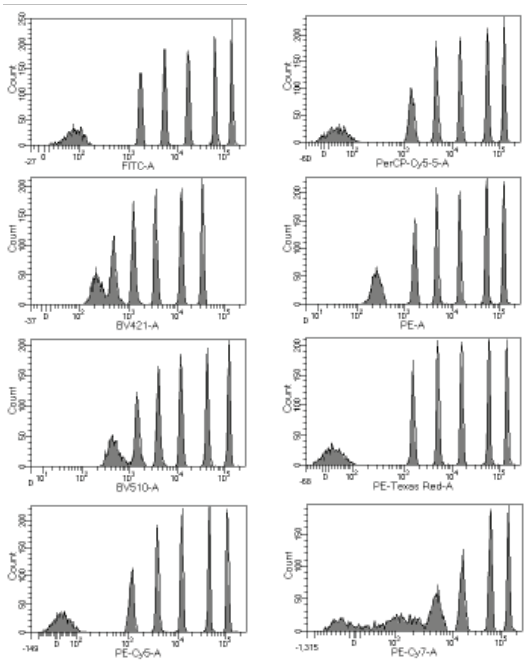
A Template for MS Excel files, as shown below, is available free of charge upon request. The template will allow the user to check and report the linearity of PMT in all channels easily by using RCP-30-5, RCP-30-5A, RCP-60-5, URCP-38-2K, URCP-50-2K, or ACP-30-5K.

PMT LINEARITY QC RECORD

PEAK #	CH #	MEFL	MEFL LOG	CALC.	RESIDUAL	CALC. MEFL
1	18.28			1.852		71
2	83.97	771	2.887	2.887	0.00%	771
3	111.66	2106	3.324	3.324	0.00%	2106
4	141.70	6262	3.797	3.797	0.00%	6262
5	166.11	15183	4.181	4.181	0.00%	15183
6	196.24	45292	4.656	4.656	0.00%	45292
7	226.60	136258	5.134	5.134	0.00%	136258
8	247.52	291042	5.464	5.464	0.00%	291042
			Ave Residual		0.00%	

Slope: 0.0158
Intercept: 1.5643
Rsq: 1.0000





Histogram of RCP-60-5
from a BD Bioscience LSRFortessa™ X-20

SPHERO™ Rainbow Calibration Particles for EuroFlow™ Standardization Guidelines

Rainbow Calibration Particles, 8 peaks Cat. No. RCP-30-5A (EuroFlow) are used in EuroFlow™ standardization guidelines throughout the study of initial PMT characterization, to set target MFI values and daily performance tracking of the flow cytometers.

SPHERO™ Rainbow Calibration Particles, EuroFlow™

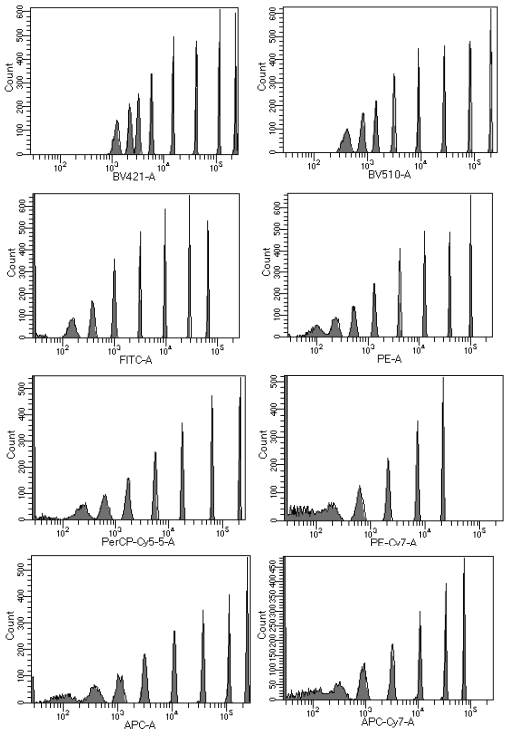
Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Calibration, 8 peaks, 10^7/mL	3.0-3.4	RCP-30-5A (EuroFlow)	5 mL

- RCP-30-5A (EuroFlow) provide target mean fluorescence intensities (MFI) to aid in the full standardization in 8-color flow cytometric immunophenotyping panel of normal and malignant leukocytes in bone marrow and blood.

Selected Reference:

Kalina T, Flores-Montero J, van der Velden VHJ, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia. 2012;26(9):1986-2010. doi:10.1038/leu.2012.122.

The detailed EuroFlow SOP for instrument setup is available at the EuroFlow website (www.euroflow.org).



Histogram of RCP-30-5A (EuroFlow) from a BD Bioscience FACSCanto™ II at peak 7 MFI target values

FACSCanto™ Fluorescence Intensity Target Values for RCP-30-5A-7 (Peak 7)

Channel	Lower MFI (-15%)	Target Value	Upper MFI (+15)
Pacific Blue	90,855	106,888	122,922
Pacific Orange	72,500	85,294	98,088
FITC	23,292	27,402	31,513
PE	32,666	38,430	44,195
PerCP-Cy5.5	57,874	68,087	78,301
PE-Cy7	6,774	7,969	9,165
APC	95,180	111,976	128,773

Molecules of Equivalent Fluorochrome (MEF) for RCP-30-5A (EuroFlow™)

Peak #	MEFL	MEPE	MEPTR	MECY	MEAP
1	N/A	N/A	N/A	N/A	N/A
2	789	443	187	1,137	736
3	1,896	1,245	543	3,041	1,892
4	4,872	3,415	1,536	7,960	4,804
5	15,619	11,299	5,423	25,995	14,248
6	47,116	35,875	17,825	82,663	42,425
7	143,912	112,460	63,989	294,040	113,026
8	333,068	287,758	207,649	973,175	227,044