

## Spherotech, Inc.

27845 Irma Lee Circle, Unit 101, Lake Forest, IL 60045

### 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

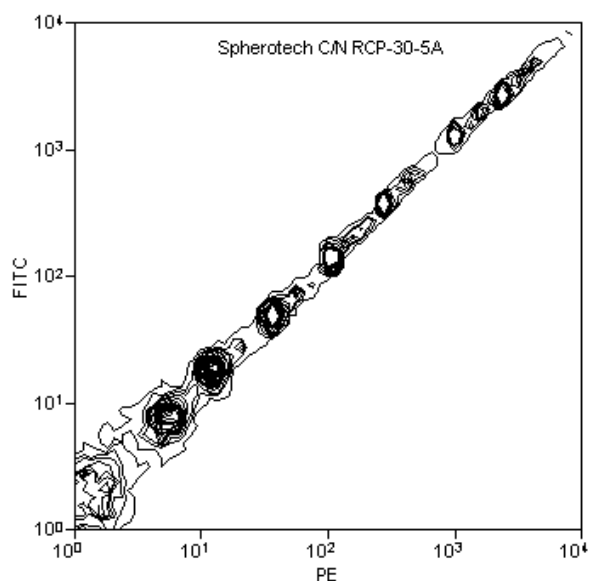
The **Rainbow Calibration Particles (RCPs)** contain a mixture of several similar size particles with different fluorescence intensities. Every particle contains a mixture of fluorophores that allows excitation at any wavelength from 365 to 650 nm. As a result, all channels in the flow cytometer can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

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. They are packaged in a dropper bottle to facilitate the 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, 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

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



Due to the stability of SPHERO™ Rainbow Calibration Particles, these particles are very useful for long term tracking of instrument performance as well as for routine calibration of the flow cytometer. Advantages of using water insoluble fluorescent dyes to obtain fluorescent particles as compared to surface labeled microparticles are listed below.

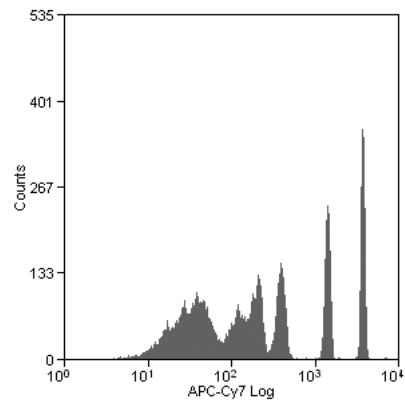
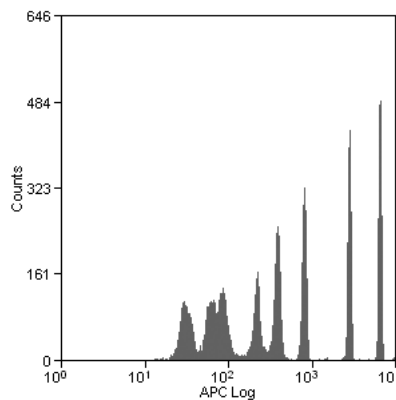
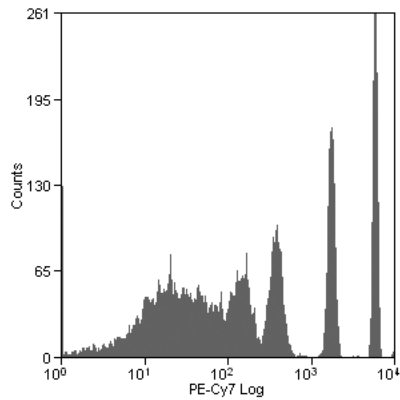
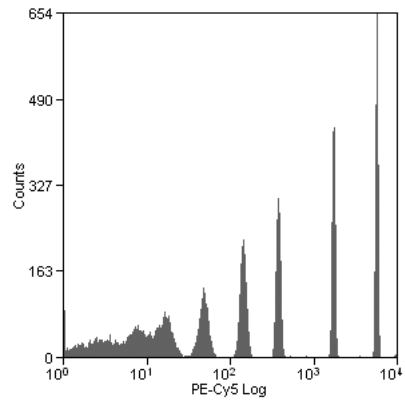
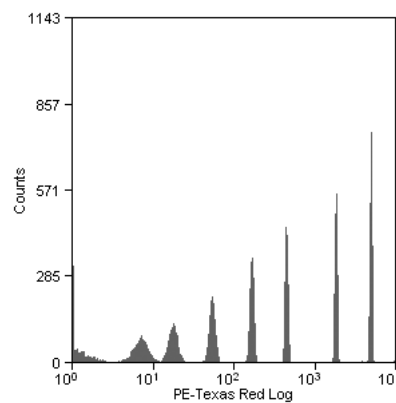
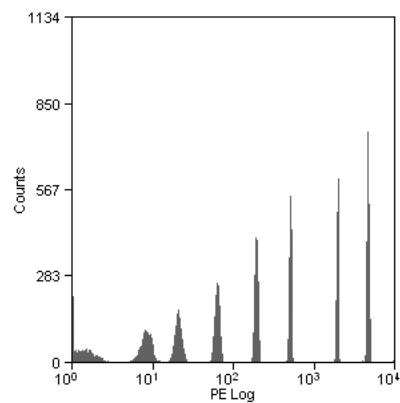
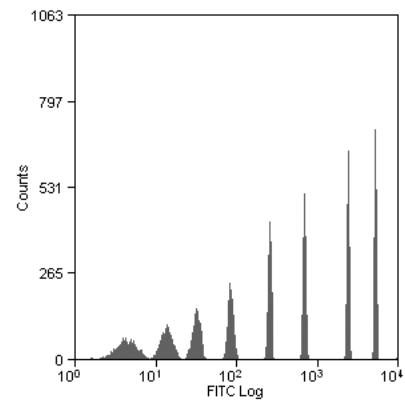
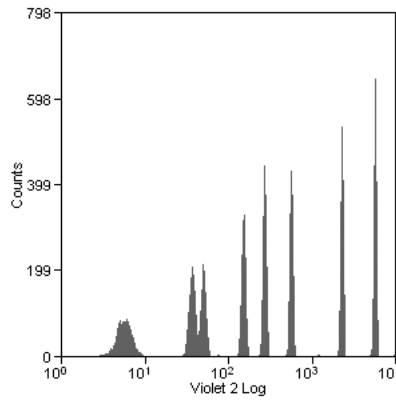
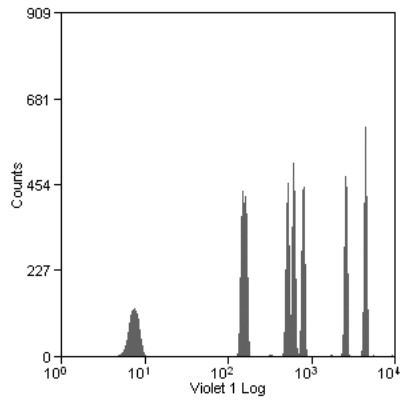
The SPHERO™ Fluorescent Particles and Calibration Particles:

- Stable for several years when stored properly.
- Withstand freeze-thaw cycles; diluted particles can be stored frozen for later use.
- Contains multiple fluorophores incorporated in the same particle to be used in different channels of the flow cytometer.
- Available with different fluorescent intensities on the same size particles.
- Can be sanitized by treating with 70% ethanol or other antibiotic agents.

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Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog # RCP-30-5A) are shown below.



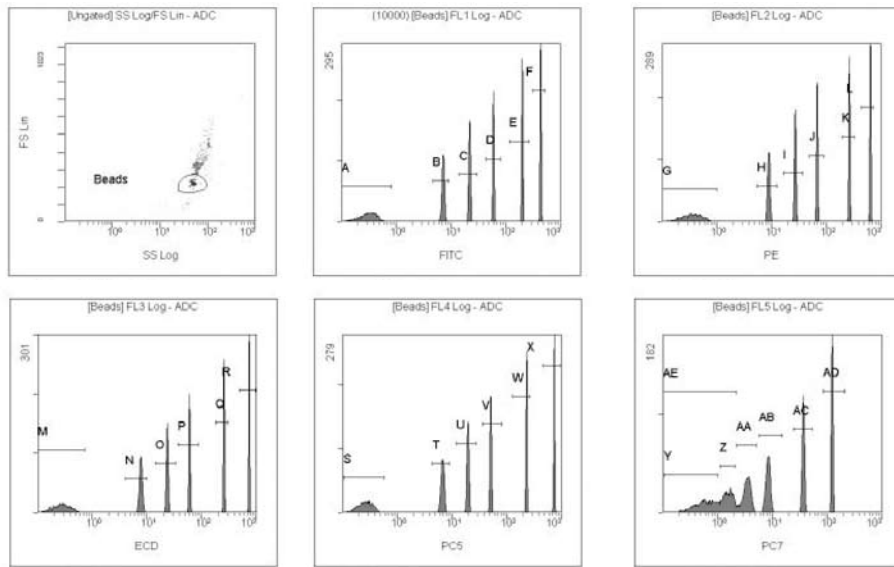
Tel.: 800-368 0822 or 847-680 8922; Fax: 847-680 8927; E-Mail: [service@spherotech.com](mailto:service@spherotech.com)

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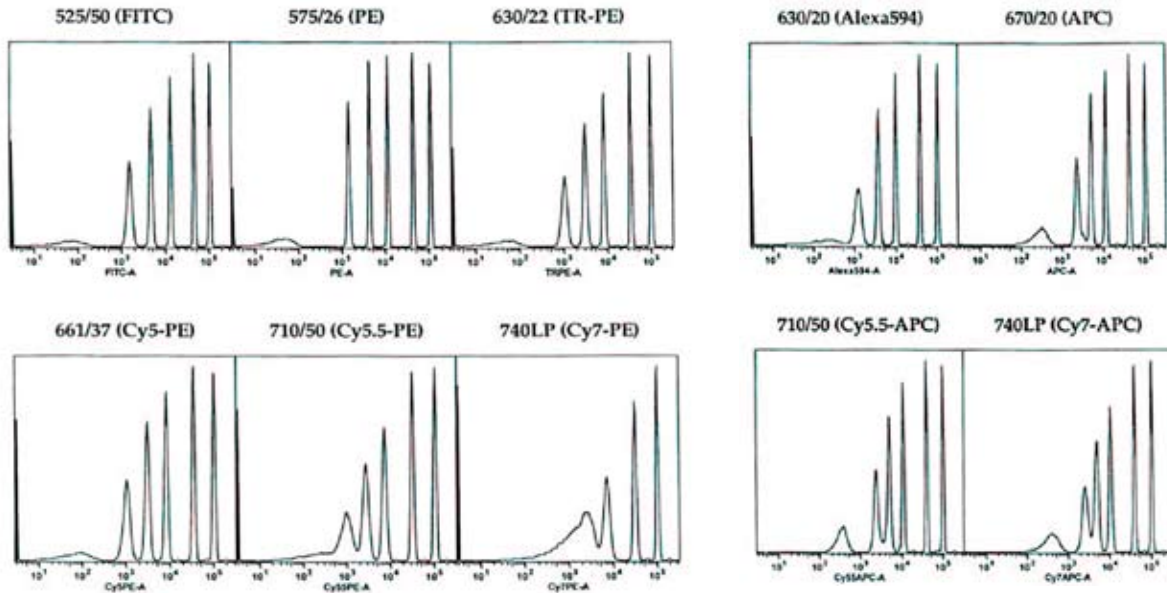
## The histograms of RCP-30-5 (6 peaks) obtained from a FC500 in six channels



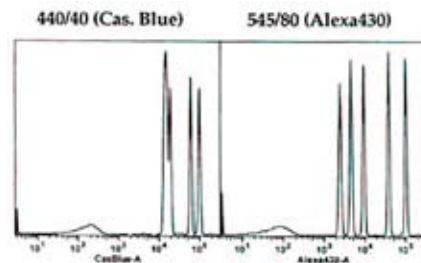
## The histograms of RCP-30-5 (6 peaks) obtained from a modified FACS Vantage with 3 lasers

### 488 nm Excitation

### 595 nm Excitation



### 406 nm Excitation



\* FC500 is a Trademark of Beckman Coulter

\* FACS Vantage is a Trademark of BD Biosciences

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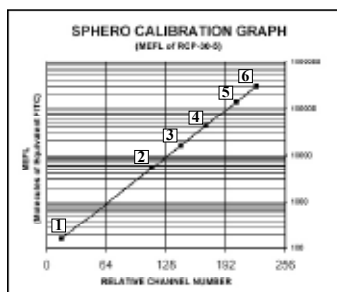
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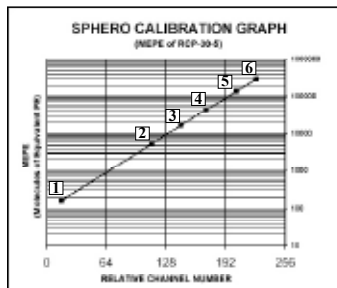
The relative number of fluorophores per particles has been determined for every peak of RCP-30-5 in FL1 (FITC, MEFL), FL2 (RPE, MEPE), FL3 (RPE-Cy5, MEPCY) and FL4 (APC, MEAP) channels of flow cytometer to plot the calibration graph as shown below. The calibration graph is used to check the linearity 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 16. 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 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.



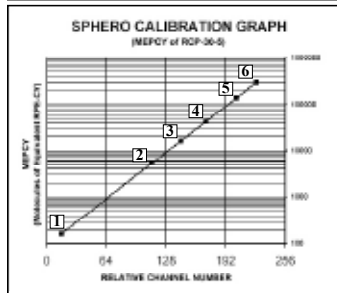
### FITC Channel

PEAK	CH#	MEFL
1	16	-----
2	113	4700
3	144	15000
4	171	40000
5	204	140000
6	226	330000



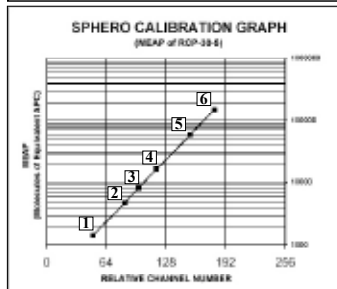
### PE Channel

PEAK	CH#	MEPE
1	11	-----
2	109	3800
3	140	12000
4	167	34000
5	203	124000
6	228	300000



### PE-CY5 Channel

PEAK	CH#	MEPCY
1	21	-----
2	91	1880
3	118	5070
4	148	14450
5	190	63800
6	221	209200



### APC Channel

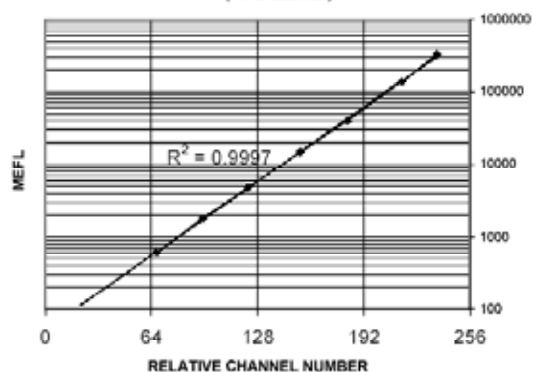
PEAK	CH#	MEAP
1	50	-----
2	84	4760
3	99	8160
4	118	16170
5	154	59050
6	180	150470

## PMT LINEARITY QC RECORD

PEAK #	CH #	MEFL	MEFL LOG	CALC.	RESIDUAL	CALC. MEFL
1	21			2.000		112
2	47	600	2.778	2.786	2.97%	612
3	95	1800	3.255	3.235	6.28%	1718
4	122	4700	3.672	3.667	1.26%	4680
5	154	15000	4.176	4.180	0.95%	15138
6	180	40000	4.602	4.629	5.73%	42510
7	215	140000	5.145	5.157	2.15%	143618
8	238	330000	5.519	5.494	4.54%	311593

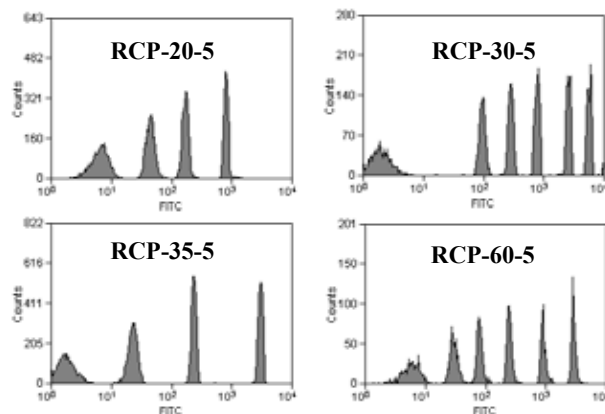
Slope: 0.0140  
Intercept: 1.7132  
Rsq: 0.9997

## SPHERO CALIBRATION GRAPH (FITC Channel)



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

The Rainbow Calibration Particles are available in sizes of 2 to 6  $\mu\text{m}$  as shown below to suit various applications.



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