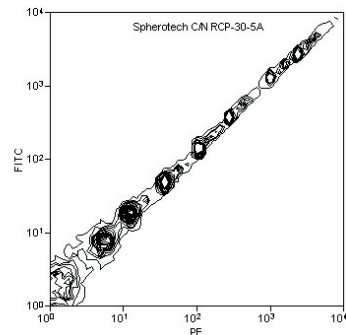


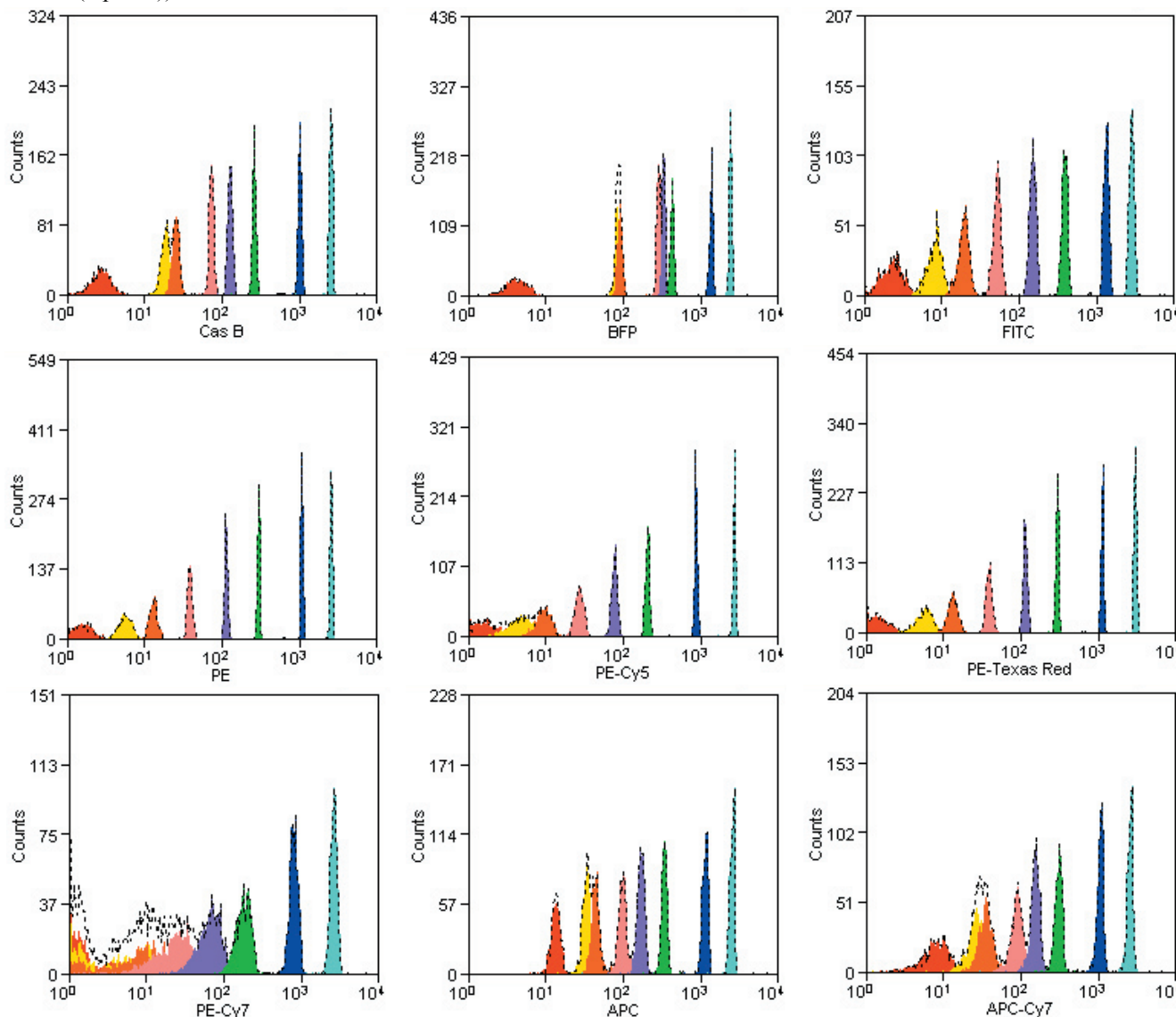
SPHERO™ Rainbow Calibration Particles

The **Rainbow Calibration Particles** 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. It enables the calibration of all channels in the flow cytometer with the same set of particles. The fluorophores used are very stable but non-spectral matching to the commonly used fluorophores such as FITC, PE or PE-Cy5. 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 repeat freezing and thawing. Diluted particle suspension can be stored in the freezer and reused later.

The contour plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A (8 peaks)).

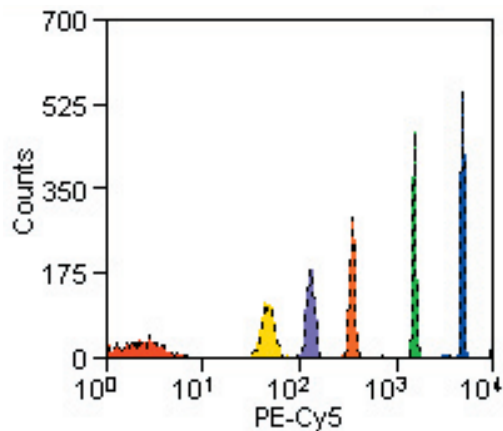
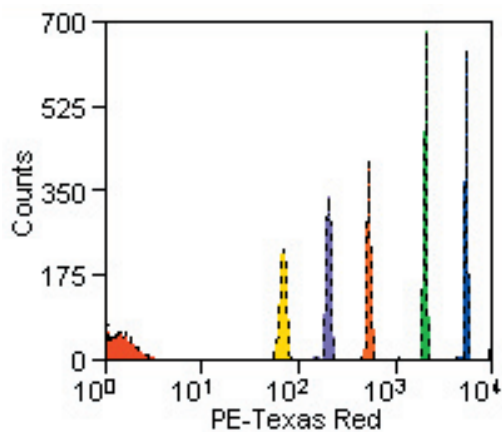
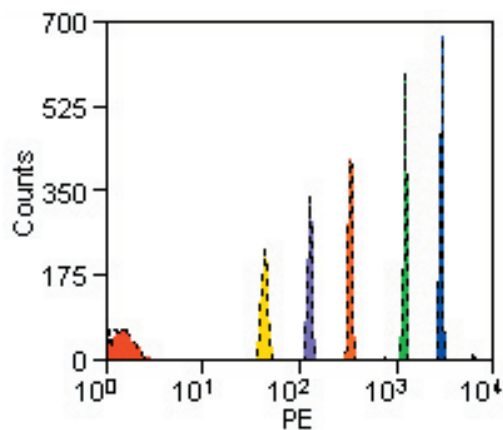
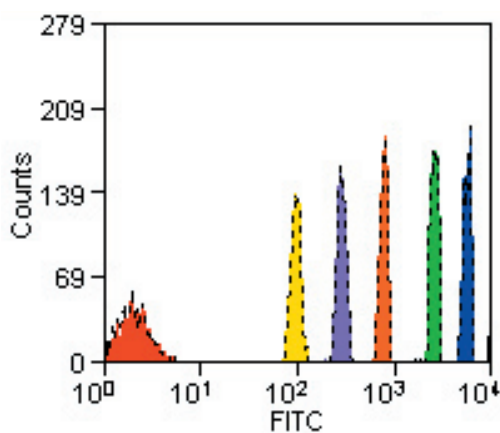
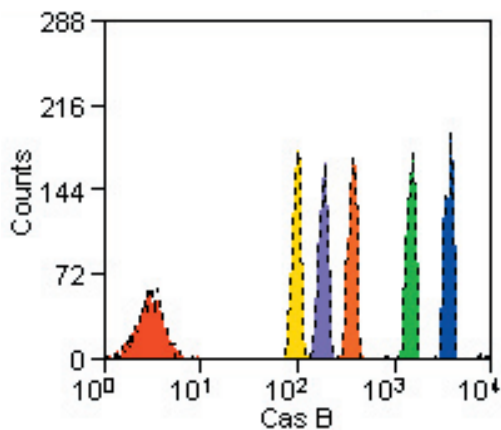


Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Cat. No. RCP-30-5A (8 peaks)) are shown below.



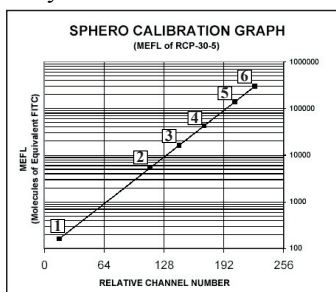
SPHERO™ Rainbow Calibration Particles

Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Cat. No. RCP-30-5 (6 peaks)) are shown below.

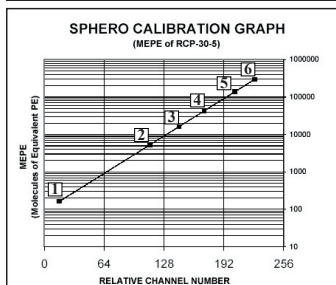


SPHERO™ Rainbow Calibration Particles

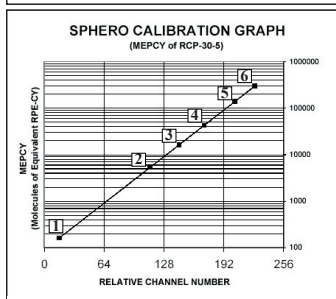
The **relative number of fluorophores per particles** has been determined for every peak of RCP-30-5 (6 peaks) in FL1 (FITC, MEFL), FL2 (RPE, MEPE), FL3 (RPE-Cy5, MEPCY) and FL4 (APC, MEAP) channels of flow cytometer to plot the calibration graph as shown below. The calibration graph is used to check the linearity of the PMT in each channel. 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 (8 peaks), which is identical to RCP-30-5 (6 peaks) with the exception of two additional peaks between the blank and the dimmest peak of RCP-30-5 (6 peaks) to give a total of 8 peaks is shown on the previous page. The RCP-30-5A (8 peaks) is very useful in checking the sensitivity and resolution of the flow cytometer.



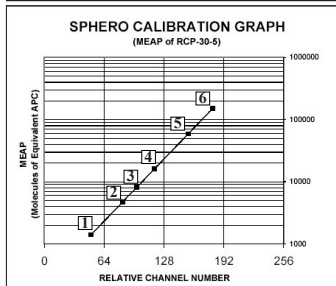
PEAK	CH#	MEFL
1	16	100
2	113	4700
3	144	15000
4	171	40000
5	204	140000
6	226	330000



PEAK	CH#	MEPE
1	11	70
2	109	3800
3	140	12000
4	167	34000
5	203	124000
6	228	300000

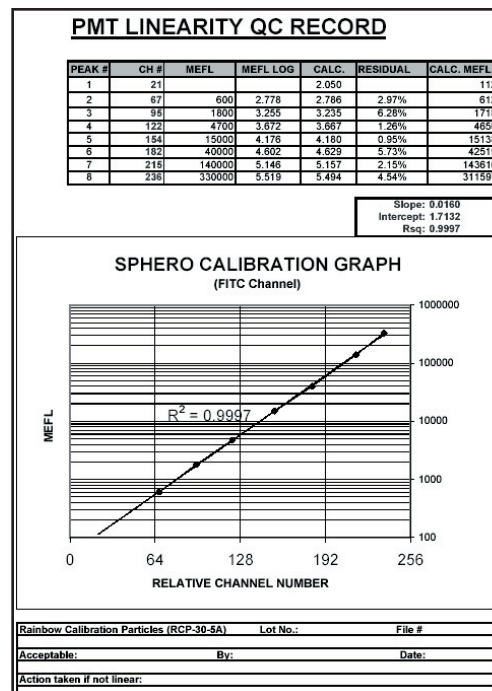


PEAK	CH#	MEPCY
1	21	150
2	91	1880
3	118	5070
4	148	14450
5	190	63800
6	221	209200

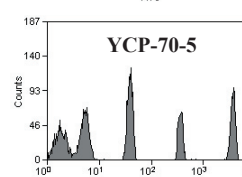
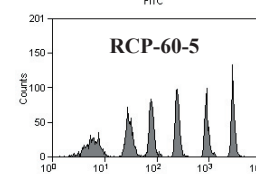
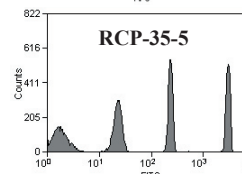
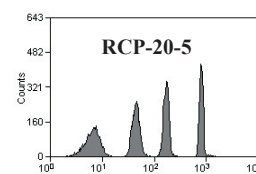
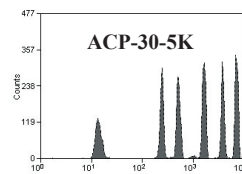


PEAK	CH#	MEAP
1	50	1400
2	84	4760
3	99	8160
4	118	16170
5	154	59050
6	180	150470

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 (6 peaks), RCP-30-5A (8 peaks), RQC-4K or ACP-30-5K.



The Rainbow Calibration Particles are available in sizes of 2 to 6.3 μm to suit various applications.



Product	Size, μm	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 (6 peaks)	5 mL
Rainbow Calibration, 8 peaks, $10^7/\text{mL}$	3.0-3.4	RCP-30-5A (8 peaks)	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