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## TECHNICAL DATA

**PRODUCT:** SPHERO™ Rainbow Calibration Particles, 3.0–3.4  $\mu\text{m}$ .

**CAT. NO.:** RCP-30-5

**LOT NO.:** U01

**SIZE:** 5 mL in water containing 0.01% NP40

**CONC.:**  $\sim 10 \times 10^6$  particles/mL

**PRESERVATIVE:** 0.02% Sodium Azide\*

**STORAGE:** Store at 2 to 8° C.

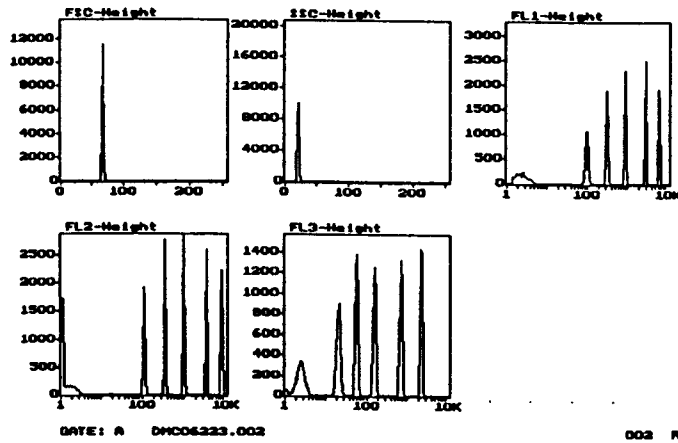
**DESCRIPTION:** This product contains a mixture of 3.0  $\mu\text{m}$ . Rainbow particles in six different fluorescent intensities. Every Rainbow particle contains a mixture of fluorophores that enable the Rainbow particles to be excited at any wavelength from 365 to 650 nm.

**USE:** Routine calibration of Flow cytometers. Dilution of 3 - 5 drops to 1 mL will provide adequate number of particles for flow cytometry.

**NOTE:** Shake vigorously or vortex briefly before use. Inclusion of small amount of detergent in the diluent will help to increase the number of singles. Diluted particles can be stored in the refrigerator for future use.

**\*WARNING:** Sodium Azide can react with Cu and Pb in plumbing to form explosive metal azides. Flush this reagent down drains with copious amounts of water.

**NOTE: FOR RESEARCH APPLICATIONS ONLY. NOT FOR DIAGNOSTIC USE.  
EXPIRES ONE YEAR AFTER OPENING. PLEASE RECORD OPENING  
DATE ON THE BOTTLE.**

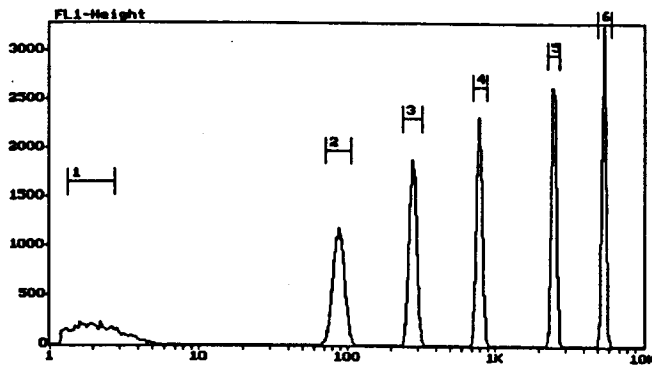


### Distribution of Singlet Beads

Gated on singlet beads.

Resolution of all populations for all three spectral ranges, green, yellow, and red is excellent.

The resolution of particle size is excellent. Forward scatter distribution is less than 1.6%CV.

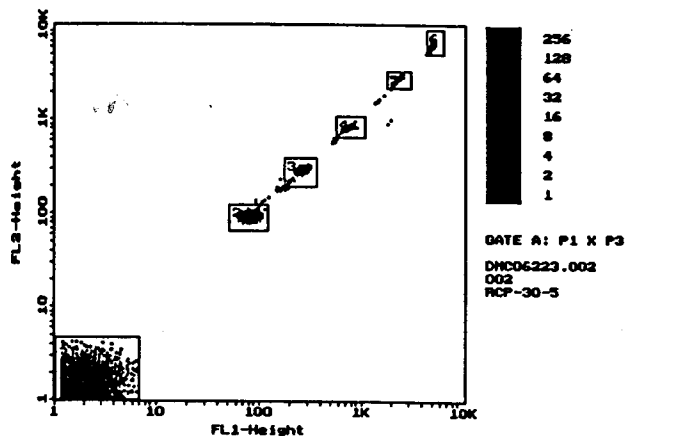


### Green Distribution of Singlet Beads

Gated to include only singlet beads. All populations are very well resolved, and the relative brightness differences are well spaced in the distribution. The brightness differences match the FITC equivalents quite well.

DATE: A DMC06223.002 002 RCP-30

R	K1	K2	COUNT	%TOTAL	PEAK	MEDIAN	MEAN	SD	%CV
1	1.33	2.73	3836	9.27	2.21	1.98	1.91	5.5	32.2
2	71.0	105	6948	16.79	86.6	86.6	83.5	2.2	1.8
3	237	321	7207	17.42	273	273	273	1.4	0.9
4	710	881	6987	16.89	777	777	749	1.4	0.8
5	2287	2738	6998	16.92	2458	2458	2458	0.6	0.4
6	4958	6152	7133	17.24	5424	5424	5232	1.0	0.4



### Dot Plot of FL1 vs. FL2

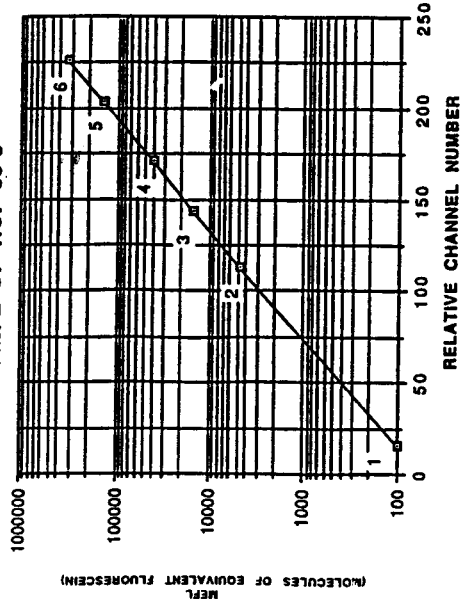
Gated on singlet beads the dot plot shows the easily resolved populations of all five medium to bright beads and the near negative dim population.

The percentage of each region shows that all beads in the mix are present at nearly the same number. (The dimmest beads are present at a lower frequency since some were gated out.)

DATE: A: P1 X P3  
DMC06223.002  
002  
RCP-30-S

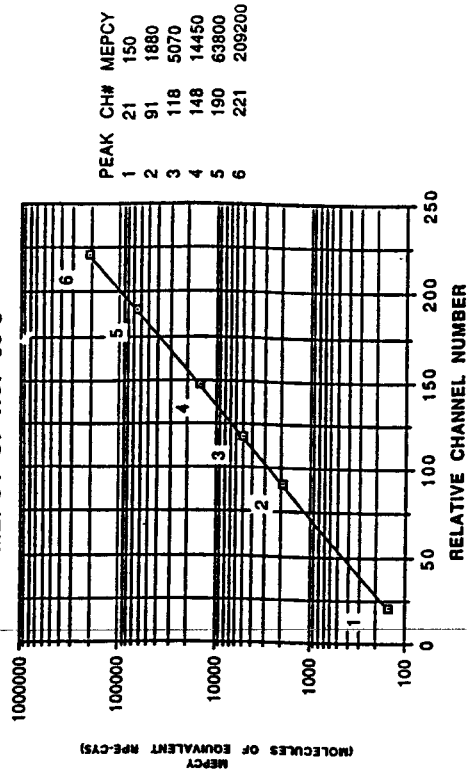
R	X1	Y1	K2	Y2	COUNT	%TOTAL	PEAK X	PEAK Y	PEAK H	NH X	NH Y
1	1.00	1.00	7.03	4.40	5543	13.40	2.14	1.00	83	1.99	1.38
2	33.1	63.7	121	117	7046	17.03	85.0	98.2	327	82.0	98.2
3	188	195	360	360	7271	17.58	270	311	778	270	300
4	576	642	1104	1027	7135	17.25	769	889	1151	742	889
5	1831	2274	3148	3383	7164	17.32	2444	3036	1713	2444	3036
6	4833	5411	6721	9303	7141	17.26	5411	7224	1527	5219	7224

SPHERO CALIBRATION GRAPH  
MEFL OF RCP-30-5

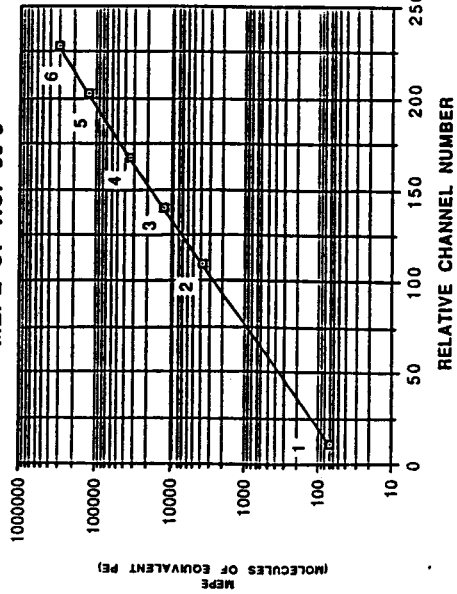


DR CODERS DATA  
30-MEFL-DC-04198A

SPHERO CALIBRATION GRAPH  
MEPCY OF RCP-30-5

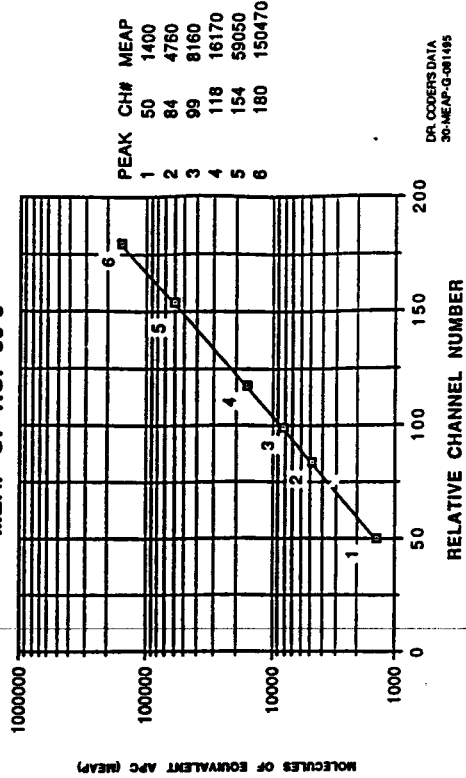


SPHERO CALIBRATION GRAPH  
MEPE OF RCP-30-5



DR CODERS DATA  
30-MEPE-DC-04198A

SPHERO CALIBRATION GRAPH  
MEAP OF RCP-30-5



DR CODERS DATA  
30-MEAP-DC-041985

FLOWCYTOMETRY DATA WAS OBTAINED BY DR. DAVID M. CODER  
(UNIVERSITY OF WASHINGTON)

# FACStarPLUS Data

## Distribution of Singlet Beads

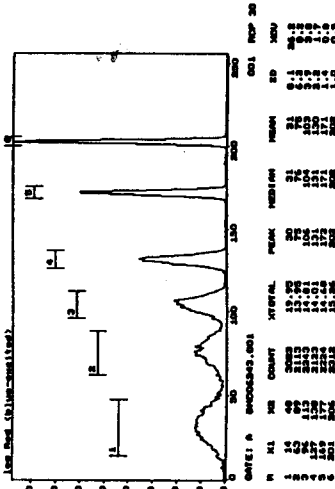
Gated on singlet beads.

Resolution of all populations for all three spectral ranges, green, yellow, and red is excellent to very good; the blue fluorescence (uv-excited) shows less resolution.

The resolution of particle size is excellent. Forward scatter distribution is less than 2.6%CV.

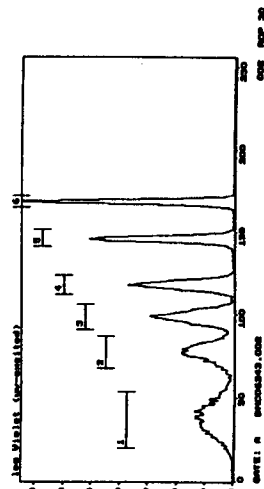
## Red Distribution of Singlet Beads

All populations are well resolved; the brightest have 96%v's well below other particles used. (Red is after 610SPDC and a 660BP20 filters; FL3/2)



## Violet Distribution of Singlet Beads

All populations are easily resolved, though differences in absolute brightness diminishes with uv-excited fluorochrome. (Violet is after 450SPDC and a 395BP20 filter; the same as used for indo-1 calcium-bound fluorescence.)



## Blue Distribution of Singlet Beads

All six beads are well resolved although the three intermediate beads are slightly closer in relative intensity. (Blue fluorescence is reflected through a 435SPDC and passing through a 485BP15; the filter used for indo-1 calcium-free fluorescence.)

