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TECHNICAL DATA

PRODUCT: SPHERO™ Rainbow Calibration

Particles, 8 Peaks, 3.0-3.4 µm

CAT. NO.: RCP-30-5A (EuroFlowTM)

LOT NO.: EAP01

SIZE: 5 mL

CONCENTRATION: I X 10⁷ particles/mL

STORAGE BUFFER: Deionized water with 0.02%

Sodium Azide and 0.01% NP40

STORAGE TEMP: Stable at room temperature.

Store between 2 and 8° C

after first use.

<u>CAUTION</u>: Do not freeze.

Protect from light.

<u>DESCRIPTION</u>: This product contains a mixture of 3.4 µm Rainbow Particles in **eight** 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.

NOTE: Shake vigorously or vortex briefly before use. Inclusion of a 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. Expires one year after opening. Please record opening date on bottle.

INSTRUCTIONS FOR USE:

RCP-30-5A (EuroFlow[™]) are used with the EuroFlow[™] standardization guidelines to study the initial PMT characterization, to set target MFI values, and to perform daily performance tracking of flow cytometers.

A. Preparation of Particles

I. Vortex the particles vigorously.

2. Dilute 3-5 drops to 1 mL of deionized water.

B. For the placement of PMT voltages for fluorescence measurements or for the determination of the EuroFlowTM SOP instrument settings visit:

T Kalina, et al. on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708) (2012). EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*, 26: 1986-2010. Related link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3437409/

- C. For linearity assessment visit SPHERO[™] Technical Note #8 Calibration and Performance Tracking of Flow Cytometer Using SPHERO[™] Calibration Particles at https://www.spherotech.com/tech_SpheroTech_Note_8.html
- D. For the determination of the flow cytometer sensitivity visit SPHERO $^{\text{TM}}$ Technical Note #17 Determination of a Flow Cytometer's Sensitivity Using Detection Efficiency (Q) and the Background Light Level (B) at https://www.spherotech.com/tech_SpheroTech_Note_17.html.

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	I,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

Dot Plot and Histograms for RCP-30-5A (EuroFlow™) on a BD Biosciences LSRFortessa™ X-20 using Assigned 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
APC-H7	28,594	33,64 I	38,687

