

CONTROLLO QUALITA' (QC): LINEARITA'

a cura della Dott.ssa Maria Carollo

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

- 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.

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 are also 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.

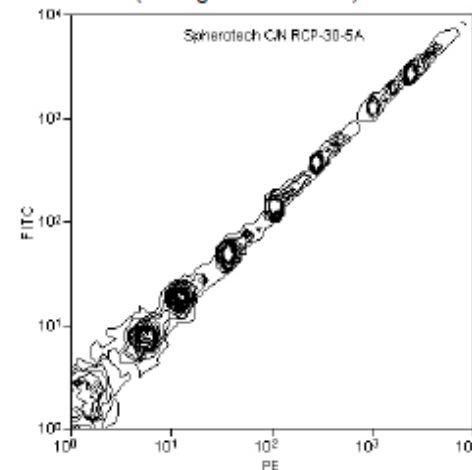
The RCPs provide a reliable and reproducible particle mixture for calibrating flow cytometers. They are very stable since the fluorochromes are entrapped within the particles instead of being located on the surface. In addition, Spherotech uses fluorophores that are non-spectral matching to the commonly 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 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, 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	5 mL
Rainbow Calibration, 8 peaks, $10^7/\text{mL}$	3.0-3.4	RCP-30-5A	5 mL
Rainbow Calibration, 6 peaks, $10^7/\text{mL}$	3.2 (+/-0.1)	RCP-32-5	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

IN USO

Figure 1 The contour plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A).



QC: LINEARITA' 8 Peaks Rainbow

(#Lotto RCP-30-5A, Spherotech)

LASER BLUE

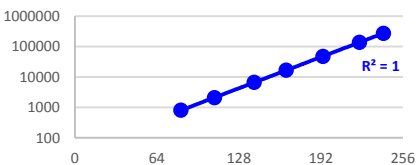
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEFL
1	23,98	
2	82,63	792
3	108,85	2079
4	139,92	6588
5	164,94	16471
6	193,56	47497
7	222,14	137049
8	240,75	271647
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEPE
1	11,27	
2	71,08	531
3	99,60	1504
4	131,08	4819
5	156,92	12506
6	185,76	36159
7	215,87	109588
8	238,47	250892
R ² =		1

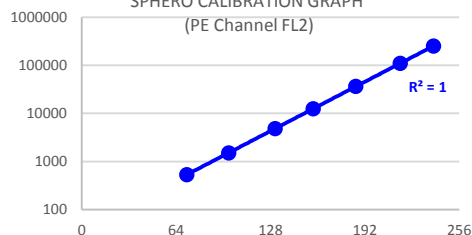
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEPTR
1	5,07	
2	67,86	233
3	95,23	669
4	126,55	2179
5	152,86	5929
6	182,39	18219
7	215,27	63944
8	243,62	188785
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MECY
1	7,51	
2	62,84	1614
3	87,37	4035
4	117,83	12025
5	144,96	31896
6	175,52	95682
7	211,83	353225
8	242,79	1077421
R ² =		1

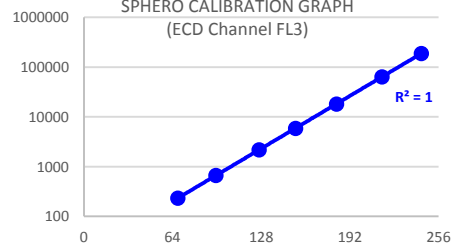
SPHERO CALIBRATION GRAPH
(FITC Channel FL1)



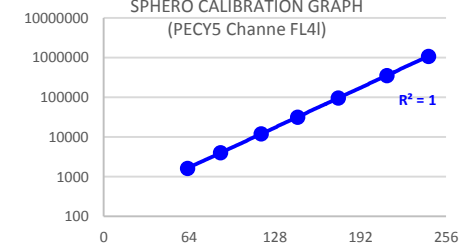
SPHERO CALIBRATION GRAPH
(PE Channel FL2)



SPHERO CALIBRATION GRAPH
(ECD Channel FL3)

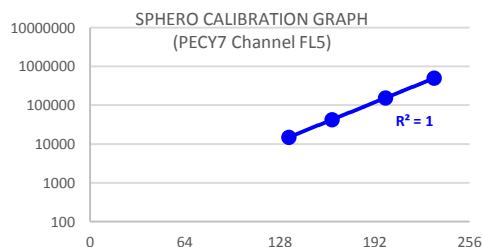


SPHERO CALIBRATION GRAPH
(PECY5 Channe FL4)



PMT LINEARITY QC RECORD

RIFERIMENTO		
PEAK #	CH #	MEPCY7
1		
2		
3		
4		
5	134,08	14916
6	163,25	42336
7	199,32	153840
8	231,96	494263
R ² =		1



QC: LINEARITA' 8 Peaks Rainbow (#Lotto RCP-30-5A, Spherotech)

LASER ROSSO

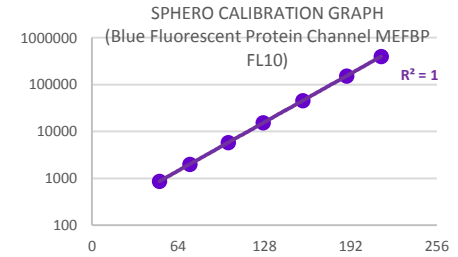
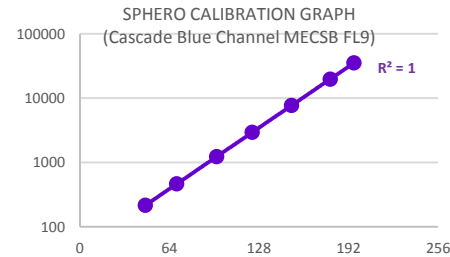
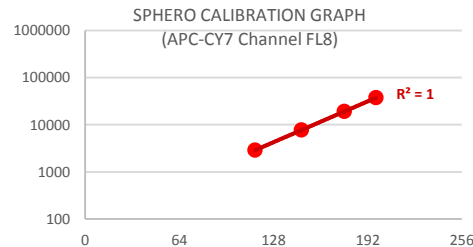
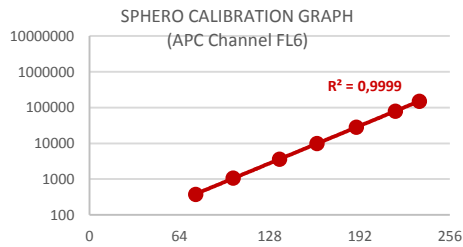
LASER VIOLA

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEAP
1	10,33	
2	75,49	373
3	102,13	1079
4	135,05	3633
5	161,59	9896
6	189,60	28189
7	217,33	79831
8	234,36	151008
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEAPCY7
1		
2		
3		
4		
5	115,43	2864
6	146,89	7644
7	176,21	19081
8	197,65	37258
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MECSB
1	11,27	
2	46,77	216
3	69,14	464
4	97,70	1232
5	123,12	2940
6	151,17	7669
7	178,92	19812
8	195,96	35474
R ² =		1

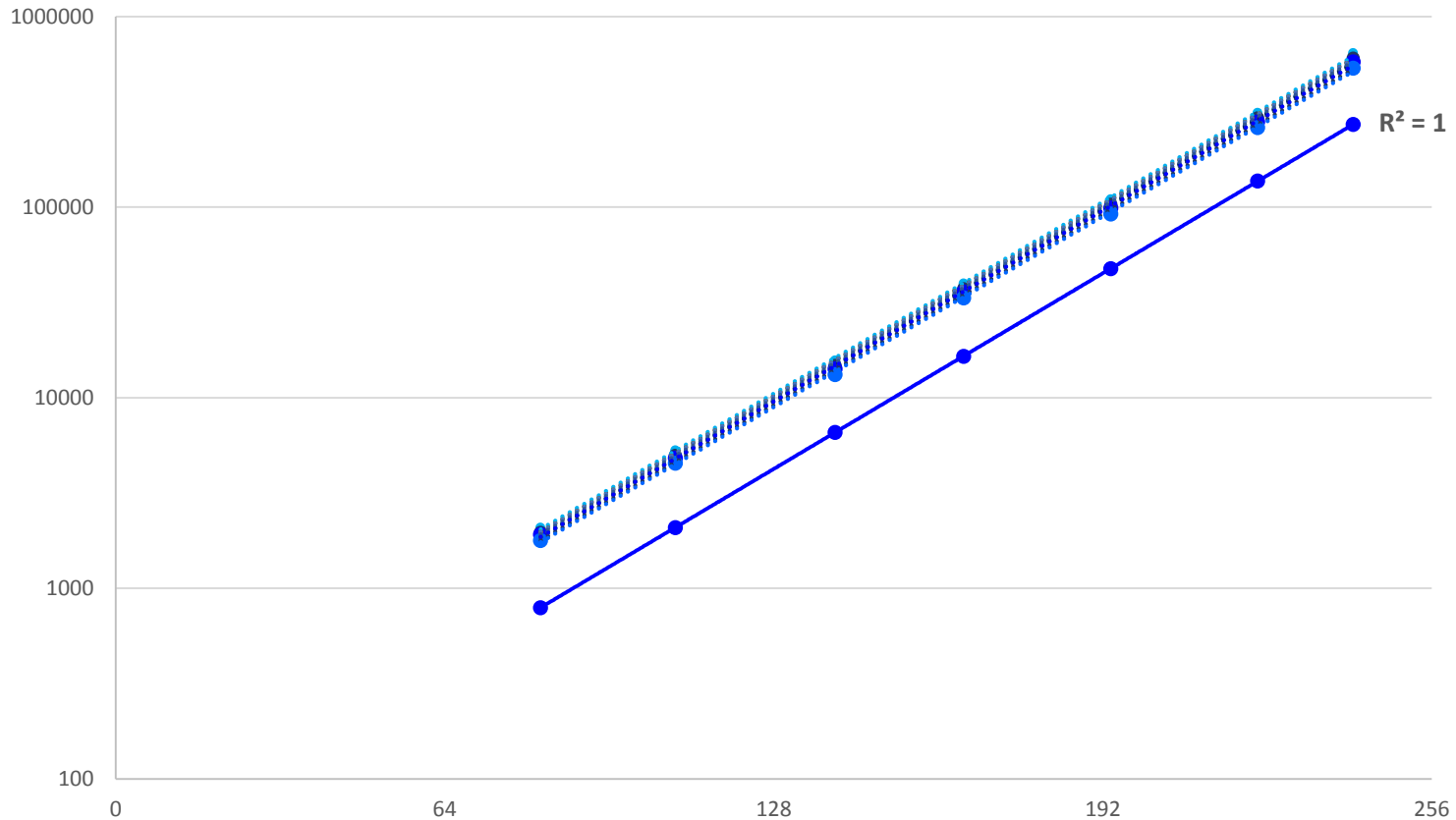
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEBFP
1	14,09	
2	50,03	861
3	72,61	1997
4	101,11	5776
5	127,13	15233
6	156,43	45389
7	188,97	152562
8	214,62	396759
R ² =		1



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEFL												
1	23,98		292	298	293	285	287	290	293	324	339	305	314	296
2	82,63	792	2037	1912	1835	1886	1899	1905	1899	2084	2012	1967	1913	1785
3	108,85	2079	5203	4856	4607	4791	4867	4900	4851	5301	5118	4991	4809	4530
4	139,92	6588	15429	14258	13548	14122	14401	14587	14389	15712	15216	14764	14175	13239
5	164,94	16471	39007	36170	34260	35741	36471	36844	36384	39784	38478	37356	35848	33439
6	193,56	47497	107931	100078	94739	98987	100989	102206	100802	110104	106380	103548	99044	91907
7	222,14	137049	305942	283735	268461	280252	286122	289154	285993	312289	301948	293774	280383	261634
8	240,75	271647	629408	582514	550297	576010	587151	593704	588897	642656	621651	604314	577116	536314
	R² =	1	0,9999	0,9999	0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9998	0,9998

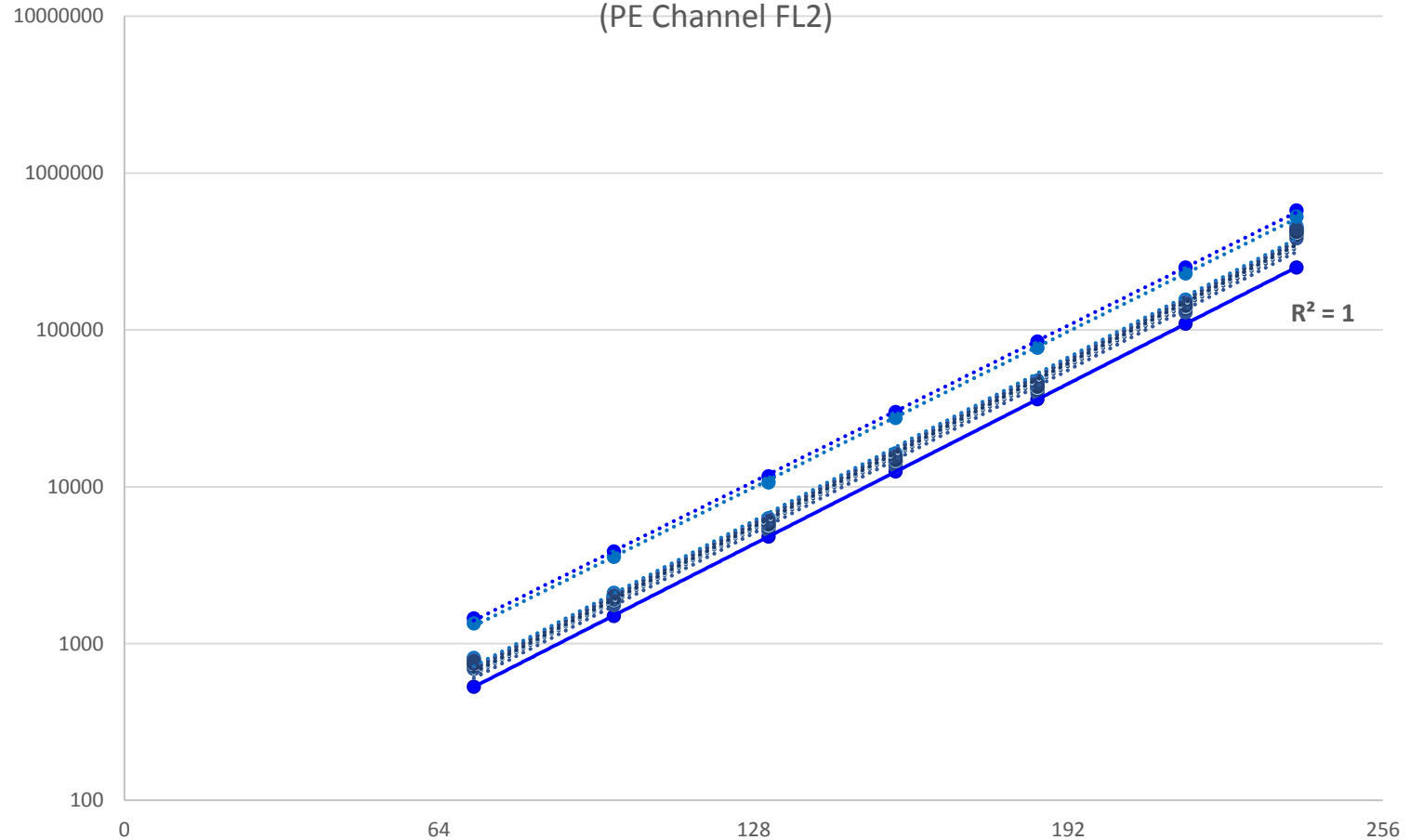
SPHERO CALIBRATION GRAPH
(FITC Channel FL1)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

RIFERIMENTO			25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEPE												
1	11,27		204	212	198	220	223	221	215	234	222	216	297	295
2	71,08	531	795	733	690	728	736	745	751	812	790	776	1451	1344
3	99,60	1504	2043	1882	1757	1864	1896	1918	1941	2111	2046	2011	3878	3575
4	131,08	4819	6113	5558	5224	5554	5659	5759	5808	6315	6158	6038	11675	10684
5	156,92	12506	15778	14397	13488	14318	14623	14813	14974	16332	15836	15575	29980	27436
6	185,76	36159	45874	42005	39377	41825	42721	43390	43736	47604	46256	45495	84591	76941
7	215,87	109588	150524	138236	129475	137261	140382	142710	143693	156229	152287	148920	250574	229894
8	238,47	250892	444498	409911	383358	406100	416464	424018	422111	458601	447217	437888	579145	529303
	R² =	1	0,9998	0,9997	0,9997	0,9998	0,9998	0,9998	0,9998	0,9998	0,9998	0,9998	0,9999	0,9999

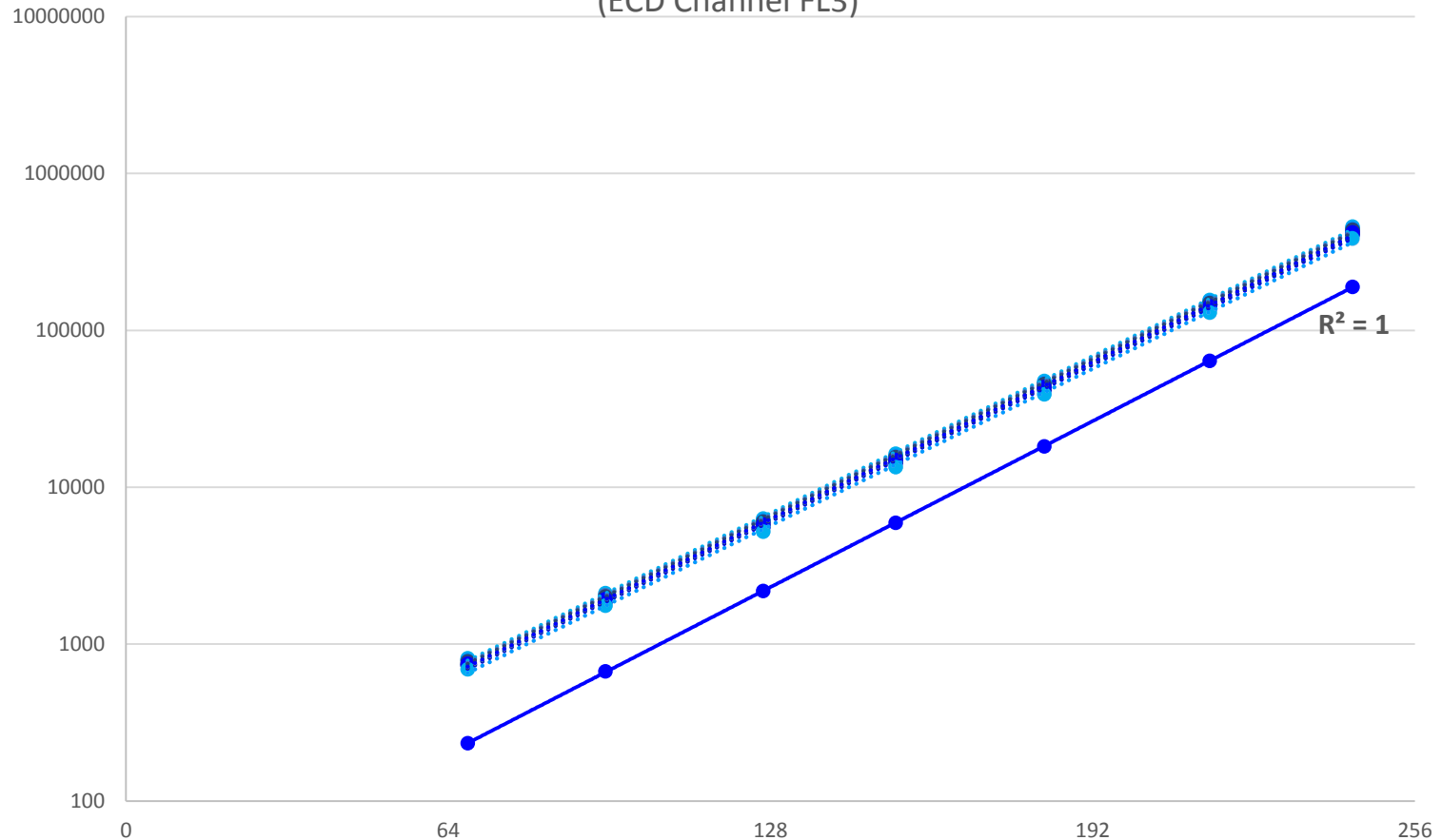
SPERO CALIBRATION GRAPH
(PE Channel FL2)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEPTR												
1	5,07		204	212	198	220	223	221	215	234	222	216	208	202
2	67,86	233	795	733	690	728	736	745	751	812	790	776	748	691
3	95,23	669	2043	1882	1757	1864	1896	1918	1941	2111	2046	2011	1930	1761
4	126,55	2179	6113	5558	5224	5554	5659	5759	5808	6315	6158	6038	5770	5217
5	152,86	5929	15778	14397	13488	14318	14623	14813	14974	16332	15836	15575	14895	13447
6	182,39	18219	45874	42005	39377	41825	42721	43390	43736	47604	46256	45495	43470	39047
7	215,27	63944	150524	138236	129475	137261	140382	142710	143693	156229	152287	148920	142319	130013
8	243,62	188785	444498	409911	383358	406100	416464	424018	422111	458601	447217	437888	420560	384916
	R² =	1	0,9998	0,9997	0,9997	0,9998	0,9998	0,9998	0,9998	0,9998	0,9998	0,9998	0,9998	0,9997

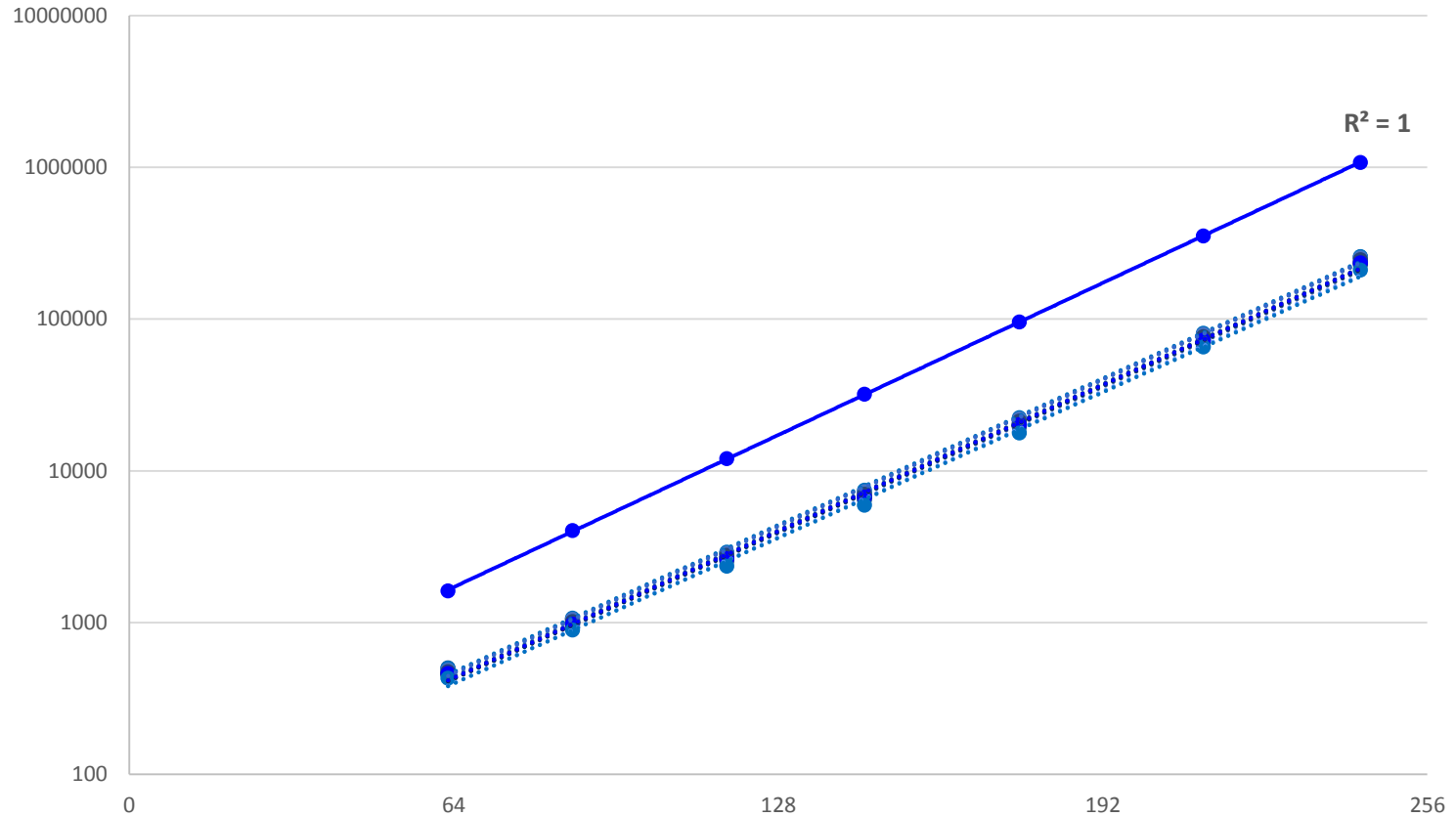
SPHERO CALIBRATION GRAPH
(ECD Channel FL3)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MECY												
1	7,51		156	162	157	156	153	163	157	172	161	161	156	163
2	62,84	1614	502	458	433	458	456	471	453	500	485	474	460	430
3	87,37	4035	1054	968	896	960	962	991	986	1070	1049	1016	976	890
4	117,83	12025	2856	2575	2380	2545	2590	2679	2652	2913	2850	2766	2634	2353
5	144,96	31896	7194	6469	6005	6446	6573	6734	6737	7422	7250	6992	6691	5911
6	175,52	95682	21743	19512	18089	19469	19843	20409	20305	22318	21922	21187	20169	17729
7	211,83	353225	79086	71197	66165	70564	71979	73784	72764	80476	78897	76371	72617	65243
8	242,79	1077421	256223	231518	214770	228692	232304	237255	233145	257776	252956	245507	234363	210153
	R² =	1	0,999	0,9988	0,9987	0,9988	0,999	0,999	0,9993	0,9992	0,9993	0,9992	0,9991	0,9986

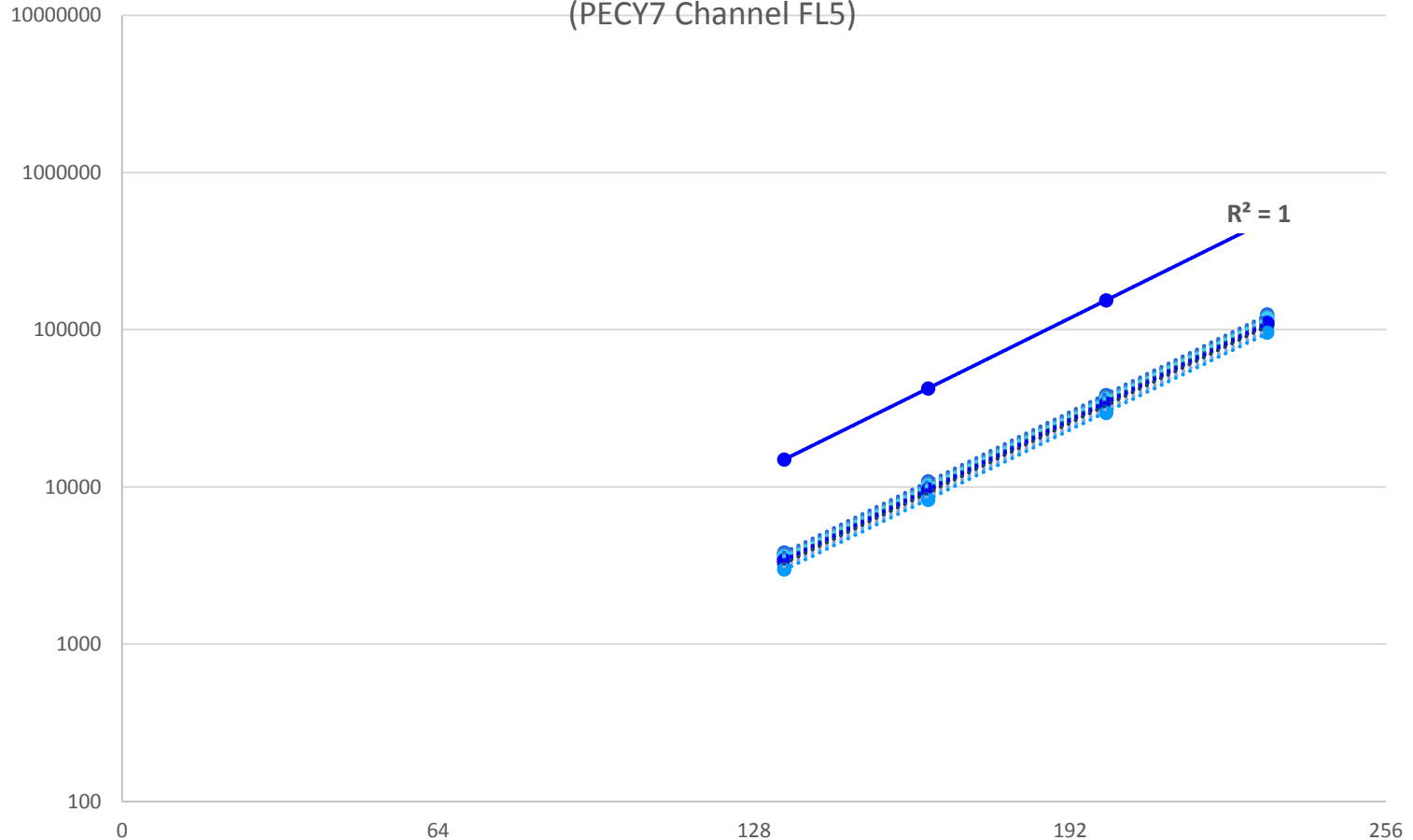
SPHERO CALIBRATION GRAPH
(PECY5 Channe FL4)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

RIFERIMENTO			25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEPCY7												
1			342	351	347	339	352	358	355	381	359	375	353	372
2			557	538	521	519	524	553	531	575	556	565	535	525
3			808	782	745	741	762	783	776	837	818	812	778	733
4			1677	1525	1445	1488	1530	1593	1591	1701	1686	1611	1553	1392
5	134,08	14916	3703	3326	3122	3281	3374	3497	3519	3828	3744	3621	3414	2990
6	163,25	42336	10367	9314	8683	9138	9400	9899	9943	10840	10596	10274	9646	8270
7	199,32	153840	37033	33042	30620	32395	33429	34865	35054	38386	37565	36346	33944	29526
8	231,96	494263	121549	108396	100408	106573	109391	113791	114465	125123	122639	118609	110843	96076
H² =			1,0000	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	1,0000	1,0000	1,0000	0,9999	0,9999

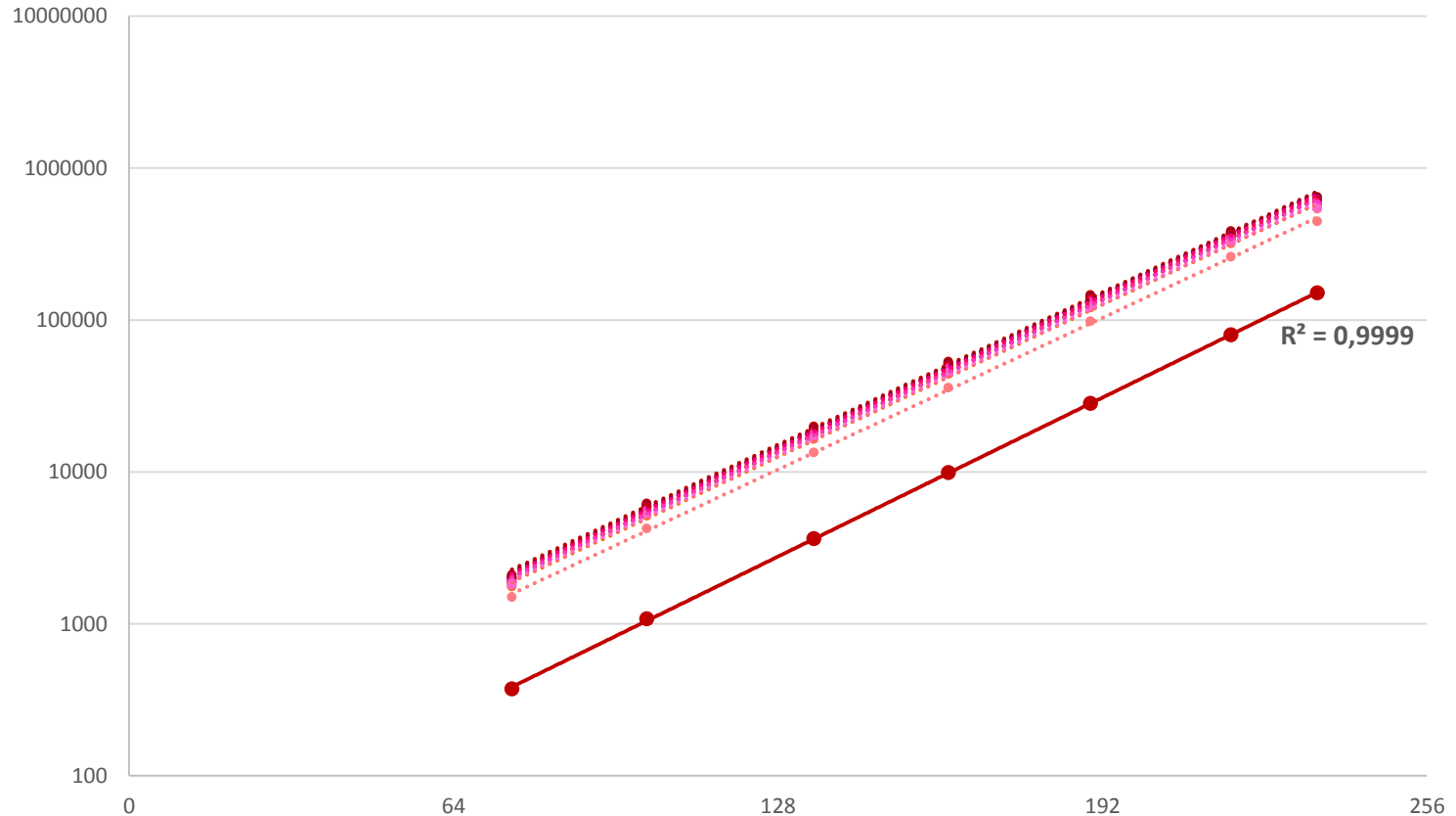
SPHERO CALIBRATION GRAPH
(PECY7 Channel FL5)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEAP												
1	10,33		136	139	138	131	136	170	134	141	138	132	132	134
2	75,49	373	2031	2095	1918	1884	1763	1496	1856	2018	1979	1968	1878	1800
3	102,13	1079	6011	6189	5590	5535	5131	4225	5457	5945	5865	5823	5525	5225
4	135,05	3633	19329	19897	18019	17820	16433	13400	17537	19133	18880	18741	17742	16681
5	161,59	9896	51751	53215	48335	47739	44063	35818	47019	51280	50522	50150	47578	44782
6	189,60	28189	142326	146158	132721	131334	120693	98080	128979	140804	138671	137932	130280	122287
7	217,33	79831	375182	384656	350195	347007	319304	260317	340860	371826	366326	362882	342144	324299
8	234,36	151008	631045	643459	586065	586602	540803	445927	584328	632619	621692	611764	574531	543189
	R ² =	1	0,9992	0,9993	0,9993	0,9994	0,9994	0,9996	0,9994	0,9994	0,9993	0,9993	0,9993	0,9994

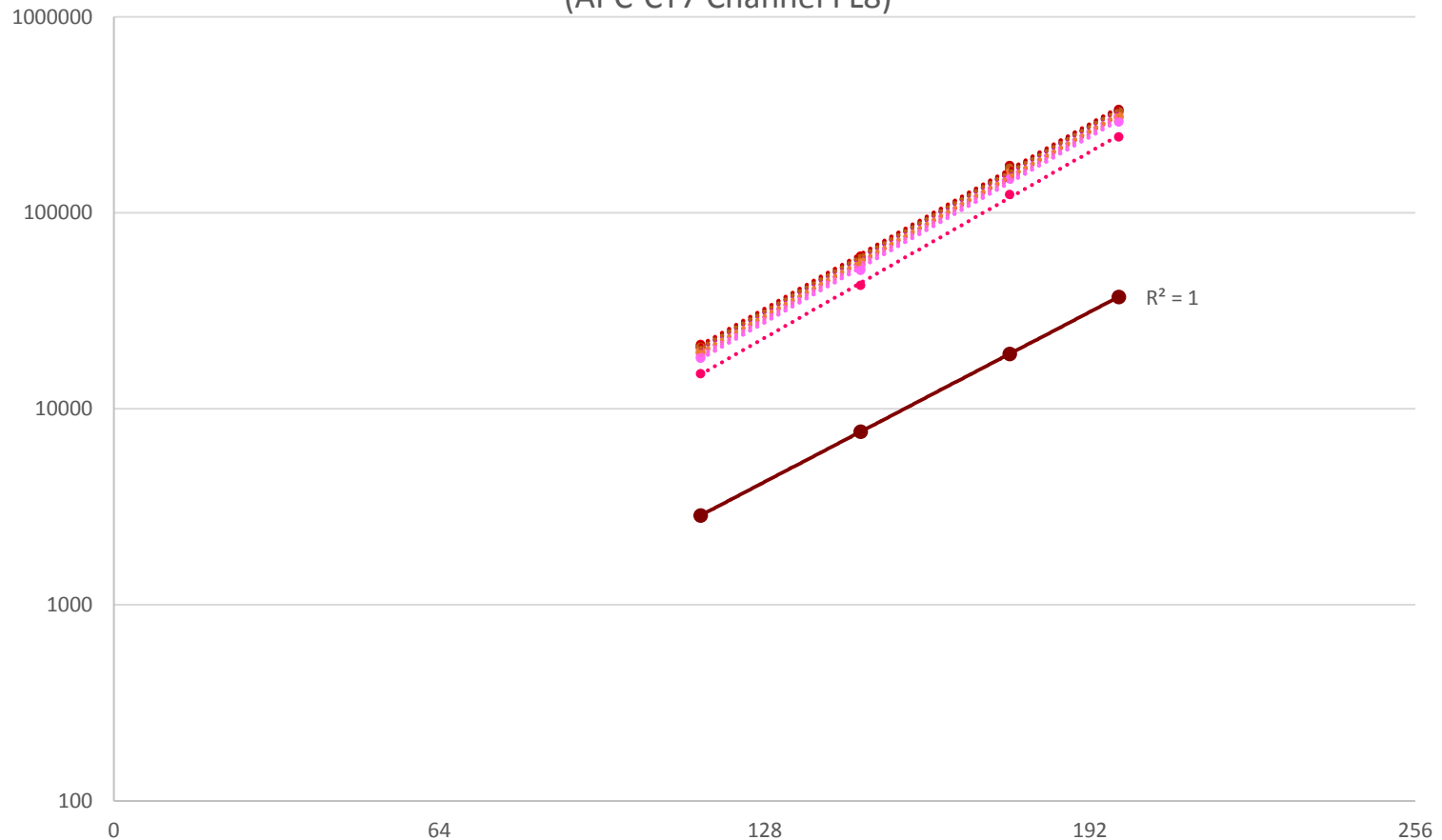
SPHERO CALIBRATION GRAPH
(APC Channel FL6)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

RIFERIMENTO			25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEAPCY7												
1			505	510	496	493	501	540	497	524	510	511	479	491
2			1088	1083	1015	995	967	933	985	1057	1043	1052	1003	987
3			2658	2639	2443	2416	2301	2019	2350	2593	2548	2585	2471	2318
4			8014	7908	7267	7244	6836	5772	7043	7748	7702	7777	7409	6839
5	115,43	2864	21284	21119	19348	19323	18181	15118	18738	20638	20363	20663	19658	18134
6	146,89	7644	60099	59527	54336	54568	51344	42640	52687	58118	57398	58279	55386	50836
7	176,21	19081	174242	173488	158561	159591	149761	124092	152733	168706	166621	168817	160289	149180
8	197,65	37258	336975	336180	307606	313559	292688	244324	303416	331733	326669	328402	310222	290452
	R² =	1	0,9993	0,9992	0,9992	0,9994	0,9993	0,9994	0,9995	0,9994	0,9994	0,9993	0,9993	0,9992

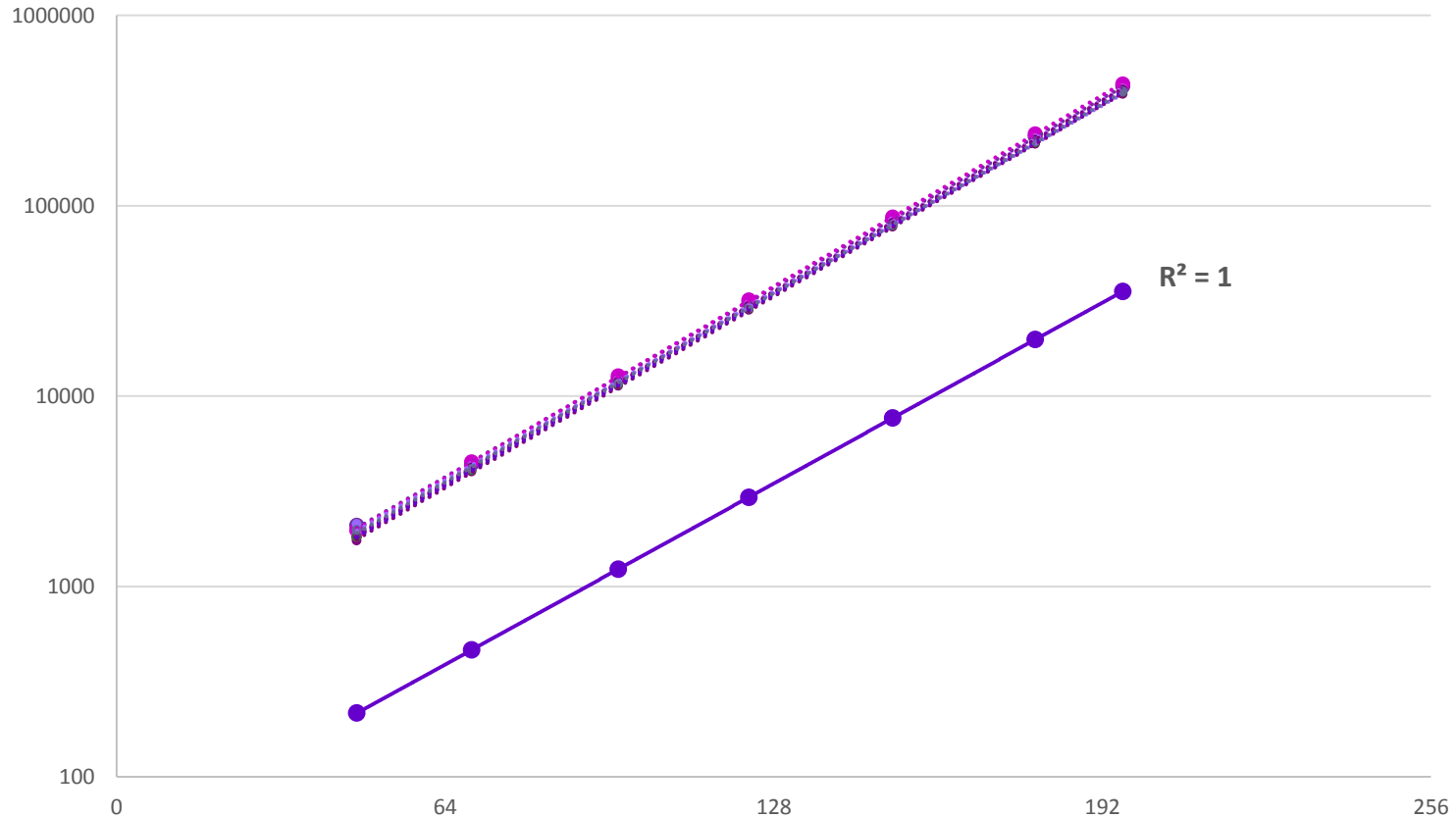
SPHERO CALIBRATION GRAPH
(APC-CY7 Channel FL8)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH#	MECSB												
1	11,27		201	217	209	204	204	1010	194	203	199	198	194	199
2	46,77	216	2097	1968	1864	1839	1829	2127	1739	1811	1818	1798	1813	1979
3	69,14	464	4389	4515	4269	4236	4199	4099	4009	4180	4196	4126	4065	4123
4	97,70	1232	12399	12740	12037	11937	11860	11535	11288	11787	11854	11646	11463	11597
5	123,12	2940	31064	31912	30153	29898	29689	28867	28322	29514	29704	29212	28745	29012
6	151,17	7669	84983	87253	82468	81711	81150	78896	77350	80728	81114	79806	78569	79106
7	178,92	19812	231175	237733	224633	222405	221250	215241	210872	220220	221237	217746	214525	216570
8	195,96	35474	423875	435169	411511	407365	406106	395046	386439	403696	406187	399975	394451	398114
	R ² =	1	0,9999	1	1	1	1	0,9995	1	1	1	1	1	0,9999

SPHERO CALIBRATION GRAPH
(Cascade Blue Channel MECSB FL9)



QC: LINEARITA' GENNAIO 2019 – DICEMBRE 2019

	RIFERIMENTO		25/01/19	19/02/19	01/03/19	05/04/19	10/05/19	03/06/19	25/07/19	19/08/19	12/09/19	14/10/19	29/11/19	30/12/19
PEAK #	CH #	MEFBP												
1	14,09		132	140	141	134	122	183	118	123	124	123	121	128
2	50,03	861	854	833	815	788	773	773	702	726	728	727	723	780
3	72,61	1997	1719	1741	1698	1642	1622	1557	1476	1521	1522	1513	1510	1553
4	101,11	5776	4619	4682	4544	4412	4360	4192	3971	4097	4096	4072	4047	4157
5	127,13	15233	11720	11879	11533	11195	11056	10638	10100	10393	10392	10344	10287	10515
6	156,43	45389	35386	35743	34681	33669	33242	31996	30371	31284	31244	31122	30967	31623
7	188,97	152562	126111	128250	124436	120877	119272	114842	109115	112522	112364	111994	111522	114890
8	214,62	396759	374280	380595	369812	359504	354125	340535	324342	334618	324717	334263	333000	342235
	R ² =	1	0,9982	0,9984	0,9984	0,9984	0,9985	0,9982	0,9985	0,9984	0,9986	0,9984	0,9983	0,9978

SPHERO CALIBRATION GRAPH
(Blue Fluorescent Protein Channel MEFBP FL10)

