

如何选择流式细胞仪测定常用的荧光染料

流式细胞仪测定常用的荧光染料有很多种,我们如何来选择流式细胞仪测定的常用荧光染料呢?

首先要考虑,他们分子结构不同,激发光谱和发射光谱也不同,选择荧光染料时,必须依据流式细胞仪所配备的激光光源的发射光波长做为重点考虑。

发射光波长,如:氩离子气体激光管,它的发射光波 488nm;

氦氖离子气体激光管发射光波长 633nm;

488nm 激光光源常用的荧光染料有 FITC (异硫氰酸荧光素);

PE (藻红蛋白)

PI (碘化丙啶)

CY5 (化青素)

preCP (叶绿素蛋白)

ECD (藻红蛋白-得克萨斯红)等。

激发光和发射光波长,如下:

	激发光波长 (nm)	发射光峰值 (nm)
FITC	488	525 (绿)
PE	488	575 (橙红)
PI	488	630 (橙红)
ECD	488	610 (红)
CY5	488	675 (深红)
PreCP	488	675 (深红)

流式细胞仪中常用的荧光染料光学特征以及需要的激光器

名 称	Ex 波长 (nm)	Em 波长 (nm)	备 注	激光器	
Hoechst 33342	355	465	DNA 染色 核酸标记	351nm 紫外激光器	
Hoechst 33258	365	465			
DAPI	372	456			
Indo-1	350	405	细胞内钙离子 标记	488nm 氩 离 子 激 光 器	
FITC	490	520	标记抗体		
R_PE	480	578			
PE_CY5	480	670			
PE_CY5.5	480	695			
PerCP	490	675			
Alexa Fluor 488	494	517			
PE_Texas Red	480	613			
Rhodamine 123	500	540			线粒体标记
Fluo-3	506	526			细胞内钙离子 标记
DiOC6(3)	480	501			内质网标记
YOYO-1	490	510	DNA 染色 核酸标记		
Propidium Iodide	530	615			
Acridine Orange	490	640			
APC	650	660	标记抗体		633nm 氦氖激光器
Alexa Fluor 647	650	668			
CY5	650	667			

流式·细胞仪交流

论坛: <http://bbs.bbiox.com/forum-181-1.html>

荧光染料谱

染料名称	Excitation(nm)	Emission(nm)	分子量	备注信息
Reactive and conjugated probes				
Hydroxycoumarin	325	386	133	Succinimidyl ester
Aminocoumarin	350	445	330	Succinimidyl ester
Methoxycoumarin	360	410	317	Succinimidyl ester
Cascade Blue	(375);401	423	596	Hydrazide
Pacific Blue	403	455	406	Maleimide
Pacific Orange	403	551		
Lucifer yellow	425	528		
NBD	466	539	294	NBD-X
R-Phycoerythrin (PE)	480;565	578	240 k	
PE-Cy5 conjugates	480;565;650	670		aka Cychrome, R670, Tri-Color, Quantum Red
PE-Cy7 conjugates	480;565;743	767		
Red 613	480;565	613		PE-Texas Red
PerCP	490	675		Peridinin chlorophyll protein
TruRed	490,675	695		PerCP-Cy5.5 conjugate

FluorX	494	520	7	58	GE Healthcare
Fluorescein	495	519	9	38	FITC; pH sensitive
BODIPY-FL	503	512			
TRITC	547	572	4	44	TRITC
X-Rhodamine	570	576	8	54	XRITC
Lissamine Rhodamine B	570	590			
Texas Red	589	615	5	62	Sulfonyl chloride
Allophycocyanin (APC)	650	660	4 k	10	
APC-Cy7 conjugates	650;755	767			PharRed
Alexa Fluor 系列荧光染料					
Alexa Fluor 350	343	442	0	41	
Alexa Fluor 405	401	421	28	10	
Alexa Fluor 430	434	540	2	70	
Alexa Fluor 488	499	519	3	64	QY 0.92
Alexa Fluor 500	503	525	0	70	
Alexa Fluor 514	517	542	4	71	
Alexa Fluor 532	530	555	4	72	QY 0.61
Alexa Fluor 546	561	572		10	QY 0.79

			79	
Alexa Fluor 555	553	568	50	12 QY 0.1
Alexa Fluor 568	579	603	2	79 QY 0.69
Alexa Fluor 594	591	618	0	82 QY 0.66
Alexa Fluor 610	610	629	85	12
Alexa Fluor 633	632	648	00	12
Alexa Fluor 647	652	668	00	13 QY 0.33
Alexa Fluor 660	663	691	00	11
Alexa Fluor 680	680	702	50	11
Alexa Fluor 700	696	719	00	14
Alexa Fluor 750	752	776	00	13
Alexa Fluor 790	782	804	50	17
Cy 系列荧光染料				
Cy2	489	506	4	71 QY 0.12
Cy3	(512);550	570;(615)	7	76 QY 0.15
Cy3B	558	572;(620)	8	65 QY 0.67
Cy3.5	581	594;(640)	02	11 QY 0.15

Cy5	(625);650	670	2	79	QY 0.28
Cy5.5	675	694	28	11	QY 0.23
Cy7	743	767	8	81	QY 0.28
核酸相关探针染料					
Hoechst 33342	343	483	6	61	AT-selective
DAPI	345	455			AT-selective
Hoechst 33258	345	478	4	62	AT-selective
SYTOX Blue	431	480	00	~4	DNA
Chromomycin A3	445	575			CG-selective
Mithramycin	445	575			
YOYO-1	491	509	71	12	
Ethidium Bromide	493	620	4	39	
Acridine Orange	503	530/640			DNA/RNA
SYTOX Green	504	523	00	~6	DNA
TOTO-1, TO-PRO-1	509	533			Vital stain, TOTO: Cyanine Dimer TO-PRO: Cyanine Monomer
Thiazole Orange	510	530			
Propidium Iodide (PI)	536	617	8.4	66	
LDS 751	543;590	712;607	2	47	DNA (543ex/712em), RNA (590ex/607em)

7-AAD	546	647			7-aminoactinomycin D, CG-selective			
SYTOX Orange	547	570	00	~5	DNA			
TOTO-3, TO-PRO-3	642	661						
DRAQ5	647	681,697	3	41	usable excitation down to 488			
细胞功能探针								
Indo-1	361/330	490/405	10	10	AM ester. Low/High Ca ⁺⁺ ,			
Fluo-3	506	526	5	85	AM ester. pH > 6			
DCFH	505	535	9	52	2'7'Dichlorodihydrofluorescein, oxidized form			
DHR	505	534	6	34	Dihydrorhodamine 123, oxidized form, light catalyzes oxidation			
SNARF	548/579	587/635			pH 6/9			
荧光蛋白								
					QY	BR	PS	
Y66H	360	442						
Y66F	360	508						
EBFP	380	440			0.18	0.27		monomer
EBFP2	383	448				0.20		monomer
Azurite	383	447				0.15		monomer
GFPuv	385	508						
T-Sapphire	399	511			0.60	0.26	0.25	weak dimer
Cerulean	433	475			0.62	0.27	0.26	weak dimer

mCFP	433	475		40	0.3	14	6	monomer
ECFP	434	477		15	0.3			
CyPet	435	477		51	0.8	19	5	weak dimer
Y66W	436	485						
mKeima-Red	440	620		24	0.3			monomer
TagCFP	458	480			0.9	2		dimer
AmCyan1	458	489		75	0.9	2		tetramer
mTFP1	462	492				4	5	dimer
S65A	471	504						
Midoriishi Cyan	472	495		9	0.5	2		dimer
Wild Type GFP	396,475	508	k	26	0.77			
S65C	479	507						
TurboGFP	482	502	k	26	0.53	3		dimer
TagGFP	482	505				4	3	monomer
S65L	484	510						
Emerald	487	509		68	0.9	3	0.69	weak dimer; (Invitrogen)
S65T	488	511						
EGFP	488	507	k	26	0.60	4	1	74 weak dimer

Azami Green	492	505		74	0.	4		monomer
ZsGreen1	493	505	10 5k	91	0.	4		tetramer
TagYFP	508	524			7	4		monomer
EYFP	514	527	26 k	61	0.	5	6	weak dimer
Topaz	514	527			7	5		monomer
Venus	515	528		57	0.	5	1	weak dimer
mCitrine	516	529		76	0.	5	4	monomer
YPet	517	530		77	0.	8	4	weak dimer
TurboYFP	525	538	26 k	53	0.	1.		dimer
ZsYellow1	529	539		65	0.	1		tetramer
Kusabira Orange	548	559		60	0.	3		monomer
mOrange	548	562		69	0.	4	9	monomer
mKO	548	559		60	0.	3	1	monomer
TurboRFP	553	574	26 k	67	0.	6		dimer
tdTomato	554	581		69	0.	9	9	tandem dimer
TagRFP	555	584			0	5		monomer

DsRed monomer	556	586	~2 8k	0. 1	3. 5	1 6	monomer
DsRed2 ("RFP")	563	582	~1 10k	0. 55	2 4		
mStrawberry	574	596		0. 29	2 6	1 5	monomer
TurboFP602	574	602	26 k	0. 35	2 6		dimer
AsRed2	576	592	~1 10k	0. 21	1 3		tetramer
mRFP1	584	607	~3 0k	0. 25			monomer
J-Red	584	610		0. 20	8. 8	1 3	dimer
mCherry	587	610		0. 22	1 6	9 6	monomer
HcRed1	588	618	~5 2k	0. 03	0. 6		dimer
Katusha	588	635			2 3		dimer
mKate (TagFP635)	588	635			1 5		monomer
TurboFP635	588	635	26 k	0. 34	2 2		dimer
mPlum	590	649		0. 10	4. 1	5 3	
mRaspberry	598	625		0. 15	1 3		monomer; faster photobleach than mPlum
其他探针							
Monochlorobimane	380	461	22 6	Glutathione probe			

Calcein	496	517	3	62	pH > 5
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