DiD，DiO，DiI，DiR和DiS细胞膜染料

产品描述

DiD，DiO，DiI，DiR和DiS染料 是一族亲脂性的荧光染料，可以用来染细胞膜和其它脂溶性生物结构。当与细胞膜结合后其荧光强度大大增强，这类染料有着很高的淬灭常数和激发态寿命。一旦对细胞染色，这类染料在整个细胞膜上扩散，最佳浓度时可以使整个细胞膜染色。它们的荧光颜色区分明显：DiI（橙色荧光），DiO（绿色荧光），DiD（红色荧光）和 DiR （深红色荧光）这使得他们可以用来对活细胞进行多色成像和流式分析。DiI和DiO可以分别用标准的 FITC和TRITC的滤光片。DiD可以用 633 nm He－Ne 激光器激发，有着比DiI更长的激发波长和发射波长，在细胞和组织染色中更有价值。 DiR的红外荧光可以穿透细胞和组织，在活体成像中用来示踪。

使用方法

1. DiD， DiO， DiI， DiR和DiS细胞膜染色液制备

（1） 配置储存液用DMSO或EtOH配置浓度1～5 mM。 注意：未使用的储存液保存在-20℃，避免反复冻融。

（2） 工作液制备：用合适的缓冲液（如：无血清培养基，HBSS或PBS）稀释储存液，配制浓度为1～5 μM的工作液。注意：工作液的最终浓度是根据不同细胞和实验的经验来配制。可以从推荐浓度的十倍以上寻找最佳条件。

2. 悬浮细胞染色

（1） 悬浮细胞密度为 1 × 10 /mL加入到工作液中。

（2） 在37 ℃培养细胞 2～20分钟，不同的细胞最佳培养时间不同。

（3） 染色细胞试管在1000～1500转离心5分钟。

（4） 倾倒上清液，再次缓慢加入预温37℃的培养液。

（5） 重复（3），（4）步骤两次以上。

3. 粘壁细胞的染色

（1） 使粘壁的细胞在无菌实验室培养。

（2） 从培养基中移走盖玻片，吸走过量培养液，将盖玻片放在潮湿的环境中。

（3） 在盖玻片的一角加入100μL的染料工作液，轻轻晃动使染料均匀覆盖所有细胞。

（4） 在37 ℃培养细胞 2～20分钟，不同的细胞最佳培养时间不同。

（5） 吸干染料工作液，用培养液洗盖玻片2～3次，每次用预温的培养基覆盖所有细胞，培养5～10分钟，然后吸干培养基。

4. 显微镜检测

（1） DiD，DiO，DiI，DiR和DiS滤光器的选择参见表1。

（2） 多色染料的同时检测，滤光器按照以下设定： a) DiI和DiO＝Omega XF52，Chroma 51004；b) DiI和DiD＝Omega XF92，Chroma 51007；c) DiI，DiO和DiD＝Omega XF93，Chroma 61005

5. 流式细胞仪的检测

DiD，DiO，DiI，DiR和DiS　　染色的细胞可以分别用经典的FL1，FL2，FL3和FL4流式细胞仪检测。

储存条件：－20℃干燥避光保存，有效期一年。

表1.　DiD，DiO，DiI，DiR，DiS的化学性质

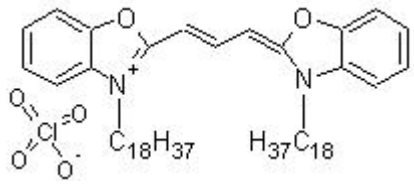
目录号	产品名称	规格	分子量	Ex/Em	推荐滤光器*
DiD					
22033	DiD labeling solution	10 mL	959.91	644/663 nm	XF47-Omega, 31023—Chroma
DiO					
22038	DiOC2(3) iodide	100 mg	881.7	484/501 nm	XF23-Omega, 31001—Chroma
22039	DiOC3(3) iodide	100 mg	488.32	482/497 nm	XF23-Omega, 31001—Chroma
22040	DiOC7(3) iodide	100 mg	600.57	482/504 nm	XF23-Omega, 31001—Chroma
22042	DiOC16(3) perchlorate	25 mg	825.6	484/501 nm	XF23-Omega, 31001—Chroma
22045	DiOC5(3) iodide	100 mg	544.47	482/504 nm	XF23-Omega, 31001—Chroma
22046	DiOC6(3) iodide	100 mg	572.52	482/504 nm	XF23-Omega, 31001-Chroma
22066	DiO perchlorate	100 mg	881.7	484/501nm	XF23-Omega, 31001—Chroma
DiI					
22035	DiIC12(3) perchlorate	100 mg	765.55	549/565 nm	XF32-Omega, 31002—Chroma
22044	DiIC16(3) perchlorate	100 mg	877.76	549/565 nm	XF32-Omega, 31002—Chroma
22052	DiIC18(3)—DS	50 mg	993.53	555/570 nm	XF32-Omega, 31002—Chroma
22054	DiIC18(5)—DS	50 mg	1019.57	650/670 nm	XF47-Omega, 31023—Chroma
22056	DiIC1(5) iodide	100 mg	510.45	638/658 nm	XF47-Omega, 31023—Chroma
22101	DiI iodide	100 mg	961.32	549/565 nm	XF32-Omega, 31002—Chroma
22102	DiI perchlorate	100 mg	933.87	549/565 nm	XF32-Omega, 31002—Chroma
22103	DiI triflate	100 mg	983.48	549/565 nm	XF32-Omega, 31002—Chroma
DiR**					
22070	DiR iodide	10 mg	1013.39	748/780 nm	XF112—Omega, 41009—Chroma
DiS					
22073	DiSC2(3)	25 mg	492.44	560/571 nm	XF32-Omega, 31002—Chroma
22076	DiSC3(5)	100 mg	546.53	660/675 nm	XF47-Omega, 31023—Chroma

*　　Omega滤光器由Omega公司提供（www.　omegafilters.com）；Chroma滤光器由Chroma公司提供（www.chroma.com）。

**DiR的发射波长肉眼不可见，所以需要配备CCD镜头或其他的近红外检测仪器

DiO perchlorate [3,3-Dioctadecyloxacarbocyanine perchlorate]

Size	Price	MW	Abs	Em	Soluble in	Storage
10 mg	$95	881.7	484 nm	501 nm	DMSO	F/D/L



Features and Biological Applications

DiI, DiO, DiD and DiR dyes are a family of lipophilic fluorescent stains for labeling membranes and other hydrophobic structures. The fluorescence of these environment-sensitive dyes is greatly enhanced when incorporated into membranes or bound to lipophilic biomolecules such as proteins although they are weakly fluorescent in water. They have high extinction coefficients, polarity-dependent fluorescence and short excited-state lifetimes. Once applied to cells, these dyes diffuse laterally within the cellular plasma membranes, resulting in even staining of the entire cell at their optimal concentrations. The distinct fluorescence colors of DiI (orange fluorescence), DiO (green fluorescence), DiD (red fluorescence) and DiR (deep red fluorescent) provide a convenient tool for multicolor imaging and flow cytometric analysis of live cells. DiO and DiI can be used with standard FITC and TRITC filters respectively. Among them DiD is well excited by the 633 nm He–Ne laser, and has much longer excitation and emission wavelengths than those of DiI, providing a valuable alternative for labeling cells and tissues that have significant intrinsic fluorescence. DiR might be useful for in vivo imaging or tracing due to the effective transmission of infrared light through cells and tissues and low level of autofluorescence in the infrared range.

References

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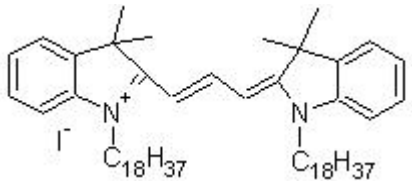
9. Levai O, Strotmann J. (2003) Projection pattern of nerve fibers from the septal organ: DiI-tracing studies with transgenic OMP mice. Histochem Cell Biol, 120, 483.

DiI iodide [1,1-Dioctadecyl-3,3,3,3- tetramethylindocarbocyanine iodide]

Size	Price	MW	Abs	Em	Soluble in	Storage
10mg	$95	961.32	549 nm	565 nm	DMSO	F/D/L



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References

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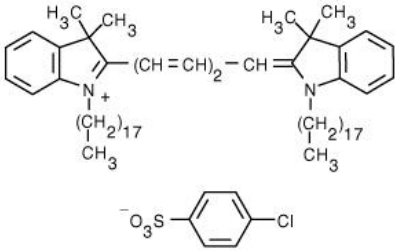
DiD [1,1-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine]

Size	Price	MW	Abs	Em	Soluble in	Storage
10 mg	$95	959.91	644 nm	643 nm	DMSO	F/D/L



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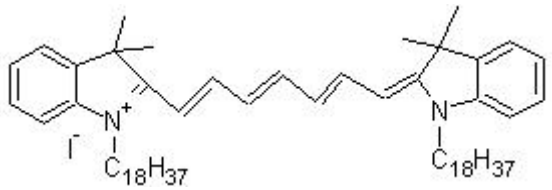
DiR iodide [1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide]

Size	Price	MW	Abs	Em	Soluble in	Storage
10 mg	$95	1013.39	748 nm	780 nm	DMSO	F/D/L



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