

FT-JO6660

Advion Interchim

Maleimide CY_{anine} labels

Maleimide-CY_{anine} agents for fluorescent labeling of biomolecules via their Sulfhydryl groups.

Introduction

• CY_{anine} maleimide reagents can be used to attach fluorophore to proteins and peptides containing cysteine residues, as well as to other thiolated molecules (such as thiol-containing oligonucleotides). Cystines should be reduced with TCEP (tris-carboxyethylphosphine) prior to the labeling. Labeling with CY_{anine} maleimide is selective, and efficient at neutral pH.

• Dye information:

 $CY_{anine}3$: An orange-to-red fluorescence-emitting dye. Excitation/emission maximum 555/567 nm Can replace Tetramethylrhodamine (TRITC). $Cy_{anine}3$ is one of the most broadly used fluorophores which can be detected by various fluorometers, imagers, and microscopes. Due to inherently high extinction coefficient, this dye is also easily detected by naked eye on gels, and in solution. See also alternative superior dye: <u>FluoProbes547H</u>. $CY_{anine}3.5$ can replace SulfoRhodamine 101.

See also alternative superior dye: FluoProbes594.

CY_{anine}5 : A far-red fluorescence-emitting dye. Excitation/emission maximum 647/657 nm

can replace far red red fluorescent dyes. CY_{anine}5 flurophore has become an incredibly popular label in life science research and diagnostics. Fluorophore emission has maximum in red region, where many CCD detectors have maximum sensitivity, and biological objects have low background. Dye color is very intense, therefore quantity as small as 1 nanomol can be detected in gel electrophoresis by naked eye. See also alternative superior dye: <u>FluoProbes647H</u>

 CY_{anine} 5.5 : A near-infrared (IR) fluorescence-emitting dye. Excitation/emission maximum 678/694 nm

can replace near infrared fluorescent dyes. See also alternative superior dye: FluoProbes682.

CY_{anine}7: A near-IR fluor that is invisible to the naked eye. Excitation/emission maximum 750/776 nm

It is used in in vivo imaging applications. See also alternative superior dye: FluoProbes752.

CY_{anine}7.5 is a near infrared red fluorophores used for *in vivo* imaging applications.

See also alternative superior dye: FluoProbes800.

Main fluorescent features are displayed in below table.

Product Information

Product name	MW	$\lambda_{exc} \setminus \lambda_{em}$.	mol. abs.	Comment
cat.number	$(g \cdot mol^{-1})$	max.(nm)	$(M^{-1}cm^{-1})$	
CY _{anine} 3 – maleimide	615.20	555 / 570	150 000	
FP-OO2030, 1 mg FP-OO2031, 5 mg FP-OO2032, 25 mg			QY: 0.31	
FP-OO2033, 50 mg FP-OO2034, 100 mg				HN N
Soluble in organic solvents (DMF, DMSO, dichloromethane), insoluble in water				o″
DiSulfo-CY _{anine} 3 – Maleimide	776.96	548 / 563	162 000	-0₃S, SO₃-K⁺
FP-SJI050, 1mg FP-SJI051, 5mg FP-SJI052, 25mg FP-SJI053, 50mg FP-SJI054, 100mg	K ⁺ salt		QY: 0.1	HN N



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Product name	MW	$\lambda_{exc} \setminus \lambda_{em}$	mol. abs.	Comment
cat.number	(g·mol⁻¹)	max.(nm)	$(M^{-1}cm^{-1})$	
DiSulfo-CY _{anine} 3 – Maleimide , TFA ⁻ salt FP-LQV010, 1mg	938.0 CF ₃ CO ₂ salt	555/ 565		$\begin{array}{c} HO_3S \\ H_3C \\ CF_3CO_2 \\ $
CY _{anine} 3 – PEG – Maleimide 2000DaFP-Inquire, 5mg 3400DaFP-Inquire, 5mg	~2500 ~3900 ~5500	649/665	"	Cy3 NH-PEG-CH2-C-NHCH2-CH2-N
5000DaFP-1L1000, 5mg				0 0
Tetra-Sulfo-CY _{anine} 3.5 – Maleimide FP-LQV350, 1mg	1198.24 CF ₃ CO ₂ salt	581 / 596		bears different substituents (4 sulfo, ethyl)
CY _{anine} 5 – maleimide FP-JO6660, 1 mg FP-JO6661, 5 mg FP-JO6662, 25 mg FP-JO6663, 50 mg FP-JO6664, 100 mg	641.24	646 / 662	250 000 QY : 0.2	r + r + r + r + r + r + r + r + r + r +
DiSulfo-CY _{anine} 5 – Maleimide (M) FP-1N8961, 1mg FP-1N8962, 5mg FP-1N8963, 25mg FP-1N8964, 50mg FP-1N8965, 100mg	803	649 / 665		o ₃ s
DiSulfo-CY _{anine} 5 – Maleimide , TFA salt ^(M) FP-LQV070, 1mg	892.87	649 / 665		HO ₃ S H ₃ C CH ₃ H ₃ C CH ₃ H ₃ C CH ₃ SO ₃ H CF ₃ CO ₂ . N ⁺ CF ₃ CO ₂ $\overset{N^+}{C_2H_5}$ $\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}$
Tri-Sulfo-CY _{anine} 5 – Maleimide FP-1I1940, 1mg FP-1I1941, 5mg	873,04 (protonated) 1074.50 (triethylammo nium salt)	649 / 670		bears 3 sulfo groups ^(2 sulfo + 1 buthyl-Sulfo)



FT-J06660	-	-		
Product name	MW	$\lambda_{exc} \backslash \lambda_{em^{\bullet}}$	mol. abs.	Comment
cat.number	(g·mol ⁻¹)	max.(nm)	$(M^{-1}cm^{-1})$	
CY _{anine} 5 – PEG – Maleimide 2000DaFP-Inquire, 5mg 3400DaFP-Inquire, 5mg 5000DaFP-1L1000, 5mg	~2500 ~3900 ~5500	649/665	"	о N-CH ₂ CH ₂ -C-NH-(OCH ₂ CH ₂) ₃ -CH ₂ CH ₂ -Cy5
CY _{anine} 5.5 - Maleimide	741.36	673 / 707	209 000	\bigcirc \bigcirc
FP-KV6770, 1 mg FP-KV6771, 5 mg			QY : 0.2	
Tetra-Sulfo-CY _{anine} 5.5 –	1153.20	678 / 701		ОН ОН О=\$=0 О=\$=0
Maleimide FP-LQV280, 1mg				$F_{3}C \rightarrow C$
Tetra-Sulfo-CY _{anine} 5.5 – Maleimide	1139.43	673 / 691	211 000 QY : 0.21	SO ₃ K KO ₃ S
FP-AS29TA, 1mg				
FP-AS29TB, 5mg			CF_{260} : 0.09	
FP-AS29TC, 25mg			CI ² 280. 0.11	N N +
FP-AS29TD, 50mg				
FP-AS29TE, 100mg				
CY _{anine} 7 – Maleimide , Cl salt FP-SJH930, 1mg FP-SJH931, 5mg FP-SJH932, 25mg FP-SJH933, 50mg FP-SJH934, 100mg	707.34	750 / 773	199 000 QY : 0.3	
DiSulfo-CY _{anine} 7 – Maleimide	869.10	750 / 773		KO3S SO3
FP-0B8290, 1mg FP-0B8291, 5mg FP-0B8293, 25mg FP-0B8294, 50mg FP-0B8295, 100mg				
DiSulfo-CY _{anine} 7 – Maleimide , CF ₃ CO ₂ ⁻ salt FP-LQV230, 1mg	918.99	749 / 776		$\begin{array}{c} HO_3S \\ H_3C \\ CH_3 \\ CF_3CO_2^- \\ C_2H_5 \\ O \\ $

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FT-JO6660				
Product name	MW	$\lambda_{exc} \lambda_{em}$	mol. abs.	Comment
cat.number	(g·mol ⁻¹)	max.(nm)	$(M^{-1}cm^{-1})$	
DiSulfo-CY _{anine} 7.5 – Maleimide	807.46	788 / 808		
FP-1C4390, 1mg				
FP-1C4391, 5mg				
FP-1C4393, 25mg				- Co
FP-1C4394, 50mg				HN_ Î
FP-1C4395, 100mg				
				U

Storage: –20°C, protected from light ^(M)

Directions for use

Handling and Storage

CY_{anine} maleimide is supplied as dry powder and is stable for at least one year. It is readily soluble in organic solvents (DCM, chloroform, DMF, DMSO, MeCN, alcohols), poorly in water.

Mother solutions can be stored frozen for months, provided the solvent is dry and the tube is closed properly with minimal air.

Labeling of proteins

The **maleimide group** reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7. The reaction is rapid (a few minutes for cystein), but in the absence of –SH, maleimide stay well stable. In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein. The technical sheet give a standard labeling protocol for proteins, but this can be adaptated for any SH containing molecule.

- 1. Dissolve the protein at 50-100 μM in suitable buffer pH7-7.5 (NaCl 150mM, phosphate 20mM pH7.5, Tris, HEPES).
- 2. Reduction of disulfide bonds: add 10-fold molar excess of DTT or TCEP, and incubate for 30min.
- 3. Remove the excess reducer prior to introducing the reactive dye, i.e. by dialysis or gelfiltration. *Note* : work in oxygen-free environment because thiols can be oxidized to disulfides with buffers deoxygenated. It is not necessary to remove excess TCEP during conjugation.
- 4. Prepare a 10 mM stock solution of the reactive dye in DMSO. Protect all stock solutions from light. *Note:* Maleimide-Cy can be dissolved in other organic solvents as dimethylformamide (DMF).
- 5. Add approximately 10-20 moles of reagent for each mole of protein. Add the reagent dropwise to the protein solution as it is stirring. Allow the reaction to proceed for 2 hours at room temperature or overnight at 4°C. Protect from light. *Note :* Upon completion of the reaction with the protein, an excess of glutathione, mercaptoethanol or other soluble low molecular weight thiol can be added to consume excess thiol-reactive reagent, thus ensuring that no reactive species are present during the purification step.
- 6. Desalt the conjugate by dialysis or gel filtration in PBS.

Other applications

Please ask application notices for fluorescent labeling of proteins to analyze in electrophoresis (2D-gel PAGE).

Related products

* <u>CY_{anine} Dyes</u> (Cy3, Cy5, Cy5.5, Cy7...) functionalized by NHS (<u>FT-BB7493</u>), Maleimide (<u>FT-JO6660</u>), Azide (<u>FT-HO7250</u>), Alkyne (<u>FT-1A6320</u>), Hydrazide (<u>LQV050</u>), DBCO (<u>FT-DQP790</u>: CycloAlkynes, for strain-promoted Click reactions), Amino group (<u>CY3AM0</u>), Carboxyl group (<u>CY3CA0</u>). 3Dye 2D DI GE (CY2/CY3/CY5) labeling kit (<u>EV0870</u>)

* Superior FluoProbes fluorescent dyes

*CY_{anine}-labeled probes

- Labeled lectins, i.e. ConA-CY_{anine}3 #FT-WT868.
- Labeled secondary antibodies
- Labeled tags, i.e. CY_{anine}3-polylysine #FT-WT8550

*Buffers



FT-JO6660

- Reducers: DTT #UP284250, TCEP #UP242214
- Desalting: UptiSpin filters; Gelfiltration G-25 columns # 84874
- FluoProbes[®] Protein labeling Kits

Ordering information

Catalog size quantities and prices may be found at <u>www.interchim.com/</u>. Please inquire for bigger quantities and for any information, please ask : FluoProbes[®] / Interchim; Hotline : +33(0)4 70 03 73 06 **Disclaimer :** Materials from FluoProbes[®] are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use. FluoProbes[®] is not liable for any damage resulting from handling or contact with this product.