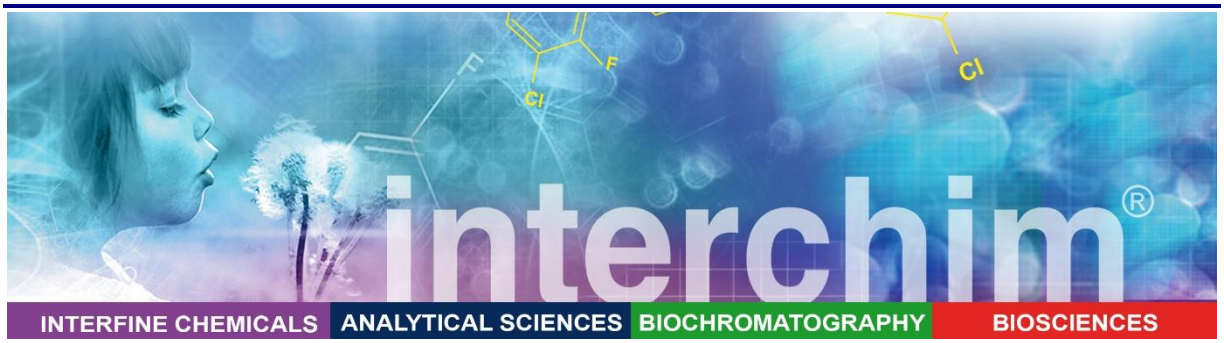
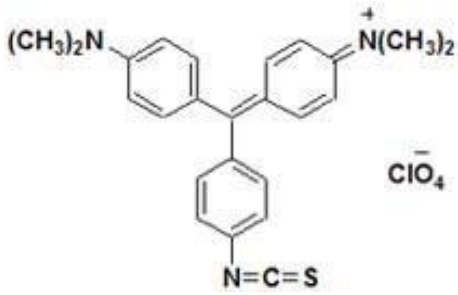


Malachite green isothiocyanate

Amine-reactive nonfluorescent photosensitizer probe

Product Description

Name :	**Malachite green ITC (MGITC)**
	Methanaminium, N-[4-[[4-(dimethylamino)phenyl](4-isothiocyanatophenyl)methylene]-2,5- cyclohexadien-1-ylidene]-N-methyl-, chloride CAS: 147492-82-8
Catalog Number :	FP-98782A, 10mg
Molecular Weight :	MW= 485.98
Fluorescence:	$C_{24}H_{24}ClN_3O_4S$
Solubility	$\lambda_{exc}\backslash\lambda_{em}$ (CH_3CN) = 628nm/none
Purity	DMSO, DMF, CH_3CN
	≥ 98%



Storage: -20°C (J) . Stable for at least one year. Protect from light and moisture

Technical information

Chromophore-Assisted Laser Inactivation (CALI) uses a 620nm pulsed laser to photoactivate malachite green (MG) dye molecules, causing local damage to a protein of interest by the subsequent generation of hydroxyl radicals (Jay, 1988). An antibody against the protein of interest is labeled with this chromophore (6-10 dyes per antibody is optimal (Beerman & Jay, 1994), which needs to be positioned within 15 Å of the protein of interest in order for the radicals to reach it due to their short lifetimes (10^{-9} to 10^{-12}s). Thus any unbound MG-labelled antibodies cause no damage, due to the short penetration distance of the radicals, and only the bound proteins which fall within the diameter of the laser spot are inactivated. In this way a non-function-blocking antibody is converted into an inactivating reagent with high spatial and temporal resolution.

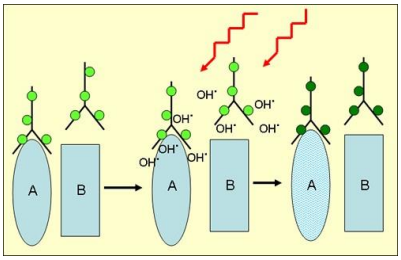


Figure 2. Antibody binds specifically to protein A and is armed with the covalently bound dye (green circles) which when irradiated by the laser light (red arrows) causes the dye to generate reactive hydroxyl radicals. The proximity of the antibody to its binding partner enables the short half-life, highly reactive radicals to damage protein A while leaving other proteins in the mixture (e.g. B) unaffected.

CALI therefore allows the inactivation (within minutes) of a specific protein in a living cell. Recovery requires new synthesis of the protein, and so can take hours or days. Thus the dependence of cellular processes on this protein can be investigated *in situ*, within this window of time.

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Advantages of CALI are a) high spatial and temporal resolution for deactivation of a protein, b) it can be used to study the function of proteins whose absence causes embryonic lethality, since knockout methods are of no use for investigating processes involving these proteins at later stages of development. c) there is less likelihood of genetic compensation occurring d) CALI's photochemical mechanism of protein inactivation is much simpler to perform than other target validation strategies for functional genomics, such as mouse or invertebrate knockouts and genetic screening. Antibody library screening for proteins which are involved in particular cellular processes could also be greatly enhanced by the function-perturbing effect of CALI.

Directions to label proteins

- Prepare a 20 mg/ml stock solution of malachite green isothiocyanate in dimethyl sulfoxide
- Add aliquots 5 µ1 of malachite green ITC stock solution to protein dissolved in 500 mM $NaHCO_3$ (pH 9.8) at 5-min intervals until a reagent/protein molar ratio of 100 is attained.
- After 4 hr of incubation on ice, separate free label from the labeled protein by gel filtration over a desalting column into 150 mM NaCl/50 mM NaPi, pH 7.3 (some precipitate had to be spun down before the buffer change).
- Determine the ratio of labeling by measuring the optical density at 620 nm of a solution of known protein concentration and calculate the dye concentration by using a molar absorptivity E = 150,000 $M^{-1}cm^{-1}$.

References

- **Beermann A. & Jay D.**, Chromophore-assisted laser inactivation of cellular proteins, *Methods Cell Biol.* 44:715-32 (1994) Abstract
- **Grate D. & Wilson C.**, Laser-mediated, site-specific inactivation of RNA transcripts, *PNAS*, Vol. 96, Issue 11, 6131-6136 (1999)
- **Jay D.**, Selective Destruction of Protein Function by Chromophore-Assisted Laser Inactivation, *PNAS*, 85: 5454 - 5458 (1988) Article
- **Koester M**. *et al*., Adenomatous Polyposis Coli Is Differentially Distributed in Growth Cones and Modulates Their Steering, *The Journal of Neuroscience*, 27(46):12590-12600 (2007) Abstract
- **Schmucker D**. *et al*., Chromophore-Assisted Laser Inactivation of Patched Protein Switches Cell Fate in the Larval Visual System of Drosophila, *Proceedings of the National Academy of Sciences*, Vol 91, 26642668 (1994) Abstract
- **Surrey T**. *al*., Chromophore-assisted light inactivation and self-organization of microtubules and motors, *PNAS*, 95: 4293 - 4298 (1998) Article

Related / associated products and documents

• **Malachite Green Oxalate salt** FP-N1219A

Synonym: N,N,N′,N′-Tetramethyl-4,4′-diaminotriphenylcarbenium oxalate, Basic Green 4
CAS: 2437-29-8 ; CE: 219-441-7 ; C.I.42000 ; $C_{23}H_{25}N_2{\bullet}C_2HO_4{\bullet}_{0.5}C_2H_2O_4$ - MW:463.5 (L)
A biological counterstain to fuchsin and safranin

• Molybdic acid ammonium salt tetrahydrate, N12150
• FITC, 017396
• HCl, 11439F

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