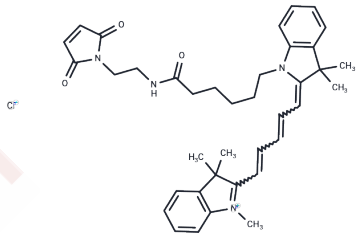
# Data Sheet (Cat.No.T74120)

TargetMol

## Cy5 maleimide

### Chemical Properties



CAS No. : 1437796-65-0

Formula: C38H45ClN4O3

Molecular Weight: 641.24

Storage: Keep away from direct sunlight,Keep away from moisture,Store at low temperature
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

### Biological Description

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| Description | Cy5 maleimide (Cyanine5 maleimide) is a highly sensitive anatase dye for the detection of free thiols in natural protein extracts. |
| Targets(IC50) | Autophagy |
| Cell Research | Instructions for use<br>I. Solution preparation<br>1. Stock solution: Dissolve Cy5 Maleimide in an appropriate solvent, such as dimethylsulfonamide (DMSO) or dimethylformamide (DMF). Common stock solution concentration: 1-10 mM. Store at -20°C and protect from light to prevent photodegradation.<br>2. Working solution: Dilute the stock solution to the required concentration (e.g. 1-50 μM) using an appropriate buffer (e.g. PBS or HEPES buffer, pH 7. Prepare freshly when used to ensure optimal labeling.<br>II. Thiol labeling steps using Cy5 Maleimide<br>1. Protein sample preparation:<br>1) Extract proteins from cells or tissues using an appropriate protein extraction buffer (e.g. RIPA buffer or PBS).<br>2) Ensure that there are no other groups in the protein sample that may react with Cy5 Maleimide (e.g. amino or carboxyl groups) to avoid interfering with the labeling process.<br>2. Labeling reaction:<br>1) Mix Cy5 Maleimide with protein sample in an appropriate buffer (e.g., PBS, pH 7.4).<br>2) Incubate at room temperature for 30 min to 1 h to allow the dye to react with free thiol groups in the protein.<br>3) The typical molar ratio of Cy5 Maleimide to protein is 1:1 to 10:1, depending on the desired labeling level.<br>3. Termination and purification:<br>1) After the reaction is complete, terminate the labeling reaction by adding a thiol-containing reagent such as dithiothreitol (DTT) or tris(2-aminoethyl)phosphine (TCEP).<br>2) Purify the labeled protein to remove excess dye using methods such as dialysis, size exclusion chromatography, or affinity purification.<br>4. Cell and imaging applications |

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| Cell Research | Cell imaging:<br>(1) Labeling of live or fixed cells by using biomolecules labeled with Cy5 Maleimide or directly in SPAAC/CuAAC labeling.<br>(2) Wash cells with PBS to remove unbound dye.<br>(3) Analyze fluorescence signals using confocal or fluorescence microscopy, and select a filter set suitable for Cy5 (excitation wavelength: 646 nm, emission wavelength: 662 nm).<br>Tissue imaging:<br>(1) Stain tissue sections with Cy5 Maleimide-labeled probes.<br>(2) Wash sections thoroughly to remove background fluorescence.<br>(3) Analyze using a fluorescence microscope or whole-slice imaging system.<br>In vivo imaging:<br>(1) Inject Cy5 Maleimide-labeled probes into animal models.<br>(2) Perform near-infrared fluorescence imaging using an in vivo imaging system (such as IVIS) or similar platform.<br>(3) Monitor the distribution, targeting, and clearance of labeled probes.<br>Notes<br>1. Photosensitivity: Cy5 Maleimide is light-sensitive and should be avoided from long-term exposure to light and stored in a light-proof environment.<br>2. Reaction optimization: Optimize dye concentration, reaction time, and buffer conditions according to experimental conditions to achieve efficient labeling and reduce background.<br>3. Copper toxicity (for CuAAC): Copper ions may affect biological samples, so the copper concentration should be carefully controlled, or SPAAC reaction should be selected due to copper toxicity issues.<br>4. Stability: The stock solution should be aliquoted to avoid repeated freezing and thawing to maintain the activity of the solution.<br><br>The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands. |

## Solubility Information

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| Solubility | DMSO: 100 mg/mL (155.95 mM),Sonication is recommended.<br>(< 1 mg/ml refers to the product slightly soluble or insoluble) |
| In vivo Formulation | 10% DMSO+90% Saline: 3.3 mg/mL (5.15 mM),Sonication is recommended.<br>*Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.* |

Preparing Stock Solutions

| | 1mg | 5mg | 10mg |
|---|---|---|---|
| 1 mM | 1.5595 mL | 7.7974 mL | 15.5948 mL |
| 5 mM | 0.3119 mL | 1.5595 mL | 3.119 mL |
| 10 mM | 0.1559 mL | 0.7797 mL | 1.5595 mL |
| 50 mM | 0.0312 mL | 0.1559 mL | 0.3119 mL |

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Requejo R,et al. Quantification and identification of mitochondrial proteins containing vicinal dithiols. Arch Biochem Biophys. 2010 Dec 15;504(2):228-35.
Maeda K, Finnie C, Svensson B. Cy5 maleimide labelling for sensitive detection of free thiols in native protein extracts: identification of seed proteins targeted by barley thioredoxin h isoforms. Biochem J. 2004 Mar 1;378(Pt 2): 497-507.
Shindy, H. A. (2017). Fundamentals in the chemistry of cyanine dyes: A review. Dyes and Pigments, 145, 505-513.