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### ThioGlo® Reagents for Labeling Active Thiols

	<u>Catalog No.</u>	<u>Excitation max</u>	<u>Emission max</u>
ThioGlo®1	T-001	379 nm	513 nm
ThioGlo®3	T-003	378 nm	446 nm
ThioGlo®5	T-005	365 nm	536 nm

### Introduction:

ThioGlo® reagents are maleimide derivatives of naphthopyranone fluorophores. Hence, general protocols for the use of maleimide dyes can be used to prepare highly fluorescent adducts of protein, peptide or other active SH substituents. However, ThioGlo® reagents have several advantages over other available fluorescent SH probes:

1. They have a low quantum yield before reacting.
2. They have a high quantum yield after conjugation to SH, giving higher sensitivity (at least 5 to 28 times more sensitive than 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM), depending on the reagent used).
3. The hydrolysis rates for the ThioGlo® reagents are slower than most other maleimide reagents (for example,  $t_{1/2}$  for ThioGlo® 1 is 8.5 hours in buffer at 25°C, pH 7.0 vs. 2-5 h for most other maleimide reagents).
4. They all hydrolyze to an SH inactive product with even lower quantum yield.
5. They all have large Stokes shifts of 80-170.
6. Stock solutions in acetonitrile, DMF or DMSO are quite stable at room temperature. However, storage at 4°C is recommended for long-term use.
7. Reaction with glutathione, reduced, (GSH) or other simple thiol peptides takes only seconds in buffered systems at pH 7.0, whereas reactions with terminal amine groups or  $\epsilon$ -amine groups requires higher pH (above 8.0) and make take hours to complete.

In view of the advantages listed above, the reagent need not be separated from the fluorescent adduct before quantitation of the active thiol compound. Many other maleimide (M) thiol probes require separation of the dye because of significant fluorescence of the reagent (i.e., Eosin-M, tetramethylrhodamine-M) or of its thiol inactive hydrolysis product (i.e., pyrene-M, fluorescein-M or CPM).

### General Instructions:

To avoid hydrolysis of the maleimide ring prior to reaction with SH, it is necessary to prepare aqueous solutions of the dye just before use. Concentrated stock solutions in anhydrous acetonitrile, dimethylformamide or DMSO should be kept at 4°C for long-term storage and diluted with the appropriate buffer (pH 7.0 to 7.4) just prior to derivatization. Maximum solubility of ThioGlo®1 in aqueous buffer is 26 $\mu$ M. Solubilities of ThioGlo®5 and ThioGlo®3 are 7.9 and 1.3  $\mu$ M, respectively.

### To derivatize active SH groups on proteins, peptides, or other biomolecules of interest:

1. Use a 5:1 molar ratio of dye to expected active SH, keeping the concentration below 20 $\mu$ M. Add the dye stock solution to the dissolved substrate and allow it to react for 30 min. The reaction is usually completed in less than 2 min; however, some proteins are slower to react. The reaction can be followed by observing the increase in fluorescence at the emission maximum with excitation maximum cited for the specific ThioGlo® reagent being used.
2. Isolate the adduct by passing the solution through a Sephadex G-25 column using phosphate buffered saline (PBS), pH 7.4, as the mobile phase and collecting the front-running fluorescent fractions. Excess dye will

remain near the top of the column. The adduct should be used immediately, or lyophilized and stored in the freezer (-20°C) until used.

For a protein with many non-crosslinked but “buried” cysteine thiols, or for higher degree of labeling, use of higher dye ratios and longer times are recommended. Under these circumstances it would be advantageous to add the concentrated dye stock solution incrementally over time. High absorbance of the product at its absorption maximum accompanied by low fluorescence quantum yield is indicative of non-specific adsorption of the dye on the protein surface.

**NOTE:** Do not increase the pH or you may risk fast hydrolysis of the dye and possible amine derivatization.

#### **Other Uses:**

When used for quantitation of specific thiols, as in post column derivatization for HPLC or in gels, ThioGlo® 1 is at least five times more sensitive than any other maleimide fluorophore currently available. The fluorescence can be enhanced to give even greater sensitivity by the addition of 20 volume percent of EtOH to the post column derivatization reagent.

**NOTE:** ThioGlo®5 should not be used for pre-column derivatization in HPLC because it is a mixture of two isomers having very close  $R_f$  values.

ThioGlo®1 has been used successfully for determination of cysteine groups in proteins. It is about 40 times more sensitive than 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) for this purpose [see Wright, S.K. and Viola, R. E., *Anal. Biochem.*, **265**, 8-14 (1998)].

GSH and other active thiol containing peptides, and proteins such as actin and myosin can be imaged in cells by incubating the cells in 5 mg/ml solution of ThioGlo® reagent in DMSO for 5-10 min. The active thiols in the cell react immediately and the cells can be rinsed and used for imaging without concern that hydrolysis of the internalized reagent by cell esterases will produce non-specific fluorescence.

Determination of the intracellular GSH status of cells can be ascertained by comparing the fluorescence of the ThioGlo® 1 reagent stained cells with similarly stained cells depleted of the intracellular GSH, via incubation with BSO (DL-buthionine-S,R-sulfoximime) for 24 h. The comparison is most easily done in a flow cytometer but can also be done in a fluorimeter or spectrofluorimeter. [“New Thiol Active Fluorophores for Intracellular Thiols and Glutathione Measurements,” M. E. Langmuir, Jun-Rui Yang, K. A. LeCompte and Ralph E. Durand, *Fluorescence Microscopy and Fluorescent Probes*, J. Slavic, ed. Plenum Press, New York, 1996, pp. 229-233].

It is also possible to measure the glutathione-reductase activity of cells by adding the ThioGlo® reagent and measuring the rate of fluorescence increase after the initial large and almost instantaneous increase in fluorescence due to reaction with GSH and other intracellular thiols. NADPH concentrations can also be measured in cells or in solution using oxidized glutathione (GSSG) as a substrate [Storey, B. T., et al., *Mol. Reprod. Dev.*, **49**, 400 (1998)].

#### **Trademarks**

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