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IRDye 800CW Maleimide Labeling Application Guide

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We use this protocol and it's working

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Abstract

IRDye 800CW Maleimide is a functional derivative of infrared dye IRDye 800CW that is reactive toward free–SH (thiol, sulfhydryl) groups. Most molecules that contain free–SH groups can be labeled with maleimide dyes, including IRDye 800CW Maleimide Infrared Dye.

Attachments

PDF

AppNote_800CW_Maleim...

132KB

Guidelines

I. Introduction

IRDye 800CW Maleimide is a functional derivative of infrared dye IRDye 800CW that is reactive toward free-SH (thiol, sulfhydryl) groups. Most molecules that contain free-SH groups can be labeled with maleimide dyes, including IRDye 800CW Maleimide Infrared Dye.

II. Labeling Reactions and Considerations

Maleimide groups react with sulfhydryl groups at pH 6.5-7.5, forming a stable thioether bond. A protein, peptide, or biomolecule containing a reactive sulfhydryl group can be labeled with IRDye 800CW using the maleimide functional group of IRDye 800CW Maleimide.

The following conditions provide good labeling efficiency:

Table 1. Labeling conditions for IRdye 800CW Maleimide.

	Buffer:	Phosphate buffered Saline (PBS), pH 7.2
	Temperature:	Ambient*
	Time:	2 hours
	Dye equivalents per free-SH	2-5

* Ambient temperature is preferred, but 4°C may be used if the protein is not stable during incubation. If 4°C is used for the labeling reaction, an overnight incubation should be performed.

Generally, PBS works well for labeling, but other buffers with pH 6.5 to 7.5 can be used. Reactions above pH 8.0 should be avoided, since unprotonated amines can also react with maleimides. The labeling reaction is usually complete in 2 hours at room temperature, but the reaction can be carried out at 4°C for 16-18 hours. The labeled molecule should be purified by appropriate purification techniques. Dialysis, size exclusion chromatography, desalting spin columns, and HPLC all work well for purification.

Molecules containing disulfide bonds cannot be directly labeled with a maleimide. However, the disulfide bonds can be cleaved with reducing agents such as TCEP, DTT, or 2-Mercaptoethylamine (MEM) to produce free sulfhydryl groups. After reduction, excess reducing agent should be removed prior to the labeling, since it will also react with the maleimide group.

Additional considerations:

If it is not possible to remove the reducing agent after reduction,TCEP is the preferred choice.

Contrary to some claims in the literature, we have observed that TCEP will react with the maleimide group of the dye during labeling reaction. The ratio of TCEP and maleimide dye to the protein or peptide must be optimized for efficient reduction and labeling.

The overall concentrations of the reactants affect the rate of the reaction.

III. Examples

Labeling of Affibody® Molecules with IRDye 800CW Maleimide

Affibody molecules are small proteins with unique binding sites capable of binding to different target proteins (**www.affibody.com**). Commercial Affibody molecules are engineered with a single C-terminal cysteine residue that can be coupled to any fluorescent dye. The Affibody molecules are partially dimerized due to S-S bridges formed by the C-terminal cysteine and must be reduced prior to labeling with IRDye 800CW Maleimide.

See 'STEPS'

Labeling of Small Molecules with IRDye 800CW Maleimide

Glutathione is a small peptide which is available in the reduced form. LI-COR has used glutathione as a model compound to optimize the labeling of small molecules containing free thiols with IRDye 800CW Maleimide. The following procedure should serve as a general guideline for labeling small molecules containing a free-SH group.

See 'STEPS'

Labeling of Antibodies with IRDye 800CW Maleimide

Antibodies can be labeled with IRDye 800CW Maleimide by selective reduction of disulfide bonds in the hinge region using 2-Mercaptoethylamine (MEM). 2-Mercaptoethylamine is a mild reducing agent which selectively reduces the two disulfide bonds in the hinge region of IgG, thereby producing two heavy chain-light chain molecules, each containing one antigen binding site.

See 'STEPS'

For futher questions, please send a detailed inquiry to biohelp@licor.com

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Safety warnings

See SDS (Safety Data Sheet) for warnings and safety hazards.

1 Please choose labeling molecule or antibody.

STEP CASE

Labeling of Affibody® Molecules with IRDye 800CW M 9 steps

Labeling of Affibody® Molecules with IRDye 800CW Maleimide

2 Prepare a fresh 500 mM solution of TCEP in water (47.5 mg/331 µl).

3 Add 1 µl of 500 mM TCEP to 99 µl Affibody molecule in PBS (1 mg/ml) to get a final TCEP concentration of 5 mM (68-fold molar excess over Affibody dimer).

1 µL 500 mM TCEP

4 Incubate overnight at room temperature.

5 Remove excess TCEP by passing the reduced mixture through a 0.5 ml Zeba™ Desalt Spin Column (Pierce) (30-130 µl sample volume, **www.piercenet.com**).

6 Reconstitute IRDye 800CW Maleimide (0.5 mg, MW 1191) in 50 µl DMSO or water to get ~10 mM solution.

7 Add 4 µl of maleimide solution (2.5-fold molar excess of dye over protein) to 100 µl reduced Affibody molecule solution.
The remaining maleimide dye solution can be stored at -20°C protected from light, and used for dye labeling reactions for up to 2 weeks.

4 µL maleimide solution

8 Mix and incubate at room temperature for 2-3 hours, protected from light.

03:00:00

9 Purify the dye labeled Affibody molecule by passing over two 0.5 ml Zeba Desalt Spin Columns consecutively.

10 Store the labeled Affibody molecule protected from light at -20°C.

-20 °C Storage