Amplite™ Fluorimetric Maleimide Quantitation Kit

Green Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #5523 (200 assays)	Keep in -20°C avoid moisture and light	Fluorescence microplate readers

Introduction

A variety of crosslinking reagents with a maleimide group are widely used for crosslinking proteins to proteins or proteins to other biomolecules. There are few reagents or assay kits available for quantifying the number of maleimide groups that are introduced into the first protein. All the commercial kits have tedious protocols. Our Amplite™ Fluorimetric Maleimide Qutitation kit uses a proprietary dye that has enhanced fluorescence upon reacting with a maleimide. The kit provides a sensitive, one-step fluorimetric method to detect as little as 10 picomoles of maleimide in a 100 µL assay volume (100 nM; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by fluorescence microplate reader with Ex/Em = 490 /520 nm.

Kit Key Features

Broad Application:	Can be used for quantifying maleimide group in a variety of molecules such as proteins.
Sensitive:	Detect as low as 10 picomoles of maleimide.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Maleimide Green™	1 vial
Component B: Reaction Buffer	1 vial (500 µL)
Component C: Assay Buffer	1 bottle (25 mL)
Component D: N-ethylmaleimide Standard	1 vial (10 mM, 50 µL)
Component E: DMSO	1 vial (200 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare 20X maleimide reaction mixture (260 µL) → Incubate at room temperature for 30 min-1 h → Prepare maleimide assay mixture (5 mL total, 50 µL/well) → Add maleimide standards or test samples (50 µL) → Incubate at room temperature for 5-30 min → Read fluorescence at Ex/Em = 490/520 nm

Note: Thaw all the kit components to room temperature before starting the experiment.

1. Prepare 500X Maleimide Green™ stock solution:

Prepare Maleimide Green™ stock solution: Add 20 µL of DMSO (Component E) into the Maleimide Green vial (Component A) to make 500X stock solution.

Note: 10 µL of the stock solution is enough for one 96-well plate. The unused Maleimide Green™ stock solution should be divided as single use aliquots and stored at -20°C and avoid from light.

2. Prepare 20X maleimide reaction mixture:

Prepare the 20X Maleimide reaction mixture: Add 10 µL of 500X Maleimide Green™ stock solution (from step 1) into 250 µL Reaction Buffer (from Component B), mixed well. Incubate this 20X maleimide reaction mixture at room temperature for 30 min and protect from light.

Note1: It is very important to incubate the 20X maleimide reaction mixture at room temperature for at least 30 min to maximize the ratio of signal to background.

Note2: You should see the yellow color after adding the Maleimide Green™ stock solution into reaction buffer.

3. Prepare 2X maleimide assay mixture:

Prepare the 2X maleimide reaction mixture: Add whole contents of 20X maleimide reaction mixture (260 µL from step 2) into 5 mL Assay buffer (Component C), mixed well.

Note: This 2X maleimide assay mixture is not stable, use it within 1 hr.

4. Prepare serial N-ethylaleimide (0 to 10 µM) solutions:

4.1 Add 10 µL of 10 mM (10 nmol/µL) N-ethylmaleimide standard stock solution (Component D) to 990 µL assay buffer (Component C) to generate 100 µM (100 pmol/µL) standard.

Note: The unused 10 mM N-ethylmaleimide stock solution should be divided as single use aliquots and stored at -20°C.

4.2 Take 200 µL of 100 µM solution (from step 4.1) to perform 1:3 serial dilutions to get 30, 10, 3, 1, 0.3, 0.1 and 0 µM standard N-ethylmaleimide solutions.

4.3 Add N-ethylmaleimide standards and maleimide-containing test samples into a 96-well solid black microplate as described in Tables 1 and 2

Table 1. Layout of N-ethylmaleimide standards and test samples in a solid black 96-well microplate:

BL	BL	TS	TS	….	….						
MS1	MS1	….	….	….	….						
MS2	MS2										
MS3	MS3										
MS4	MS4										
MS5	MS5										
MS6	MS6										
MS7	MS7										

Note: MS= N-ethylmaleimide Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well:

N-ethylmaleimide Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay buffer: 50 µL	50 µL

**Note: Add the serially diluted N-ethylmaleimide standards from 0.1 µM to100 µM into wells from MS1 to MS7 in duplicate.*

5. Run maleimide assay:

5.1 Add 50 µL of 2X maleimide assay mixture (from step 3) to each well of the N-ethylmaleimide standard, blank control, and test samples (see step 4.3) so that the total maleimide assay volume is 100 µL/well.

Note: For a 384-well plate, add 25 µL sample, and 25 µL of maleimide reaction mixture per well.

5.2 Incubate the reaction mixture for 5 to 30 minutes at room temperature, protected from light.

Note: For best results, the fluorescence intensity should be read within 30 min due to the fluorescence background increases with time.

5.3 Monitor the fluorescence increase with Ex/Em = 490/520 nm by a fluorescence plate reader.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the maleimide reactions. The typical data are shown in Figure 1 (maleimide standard curve).

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

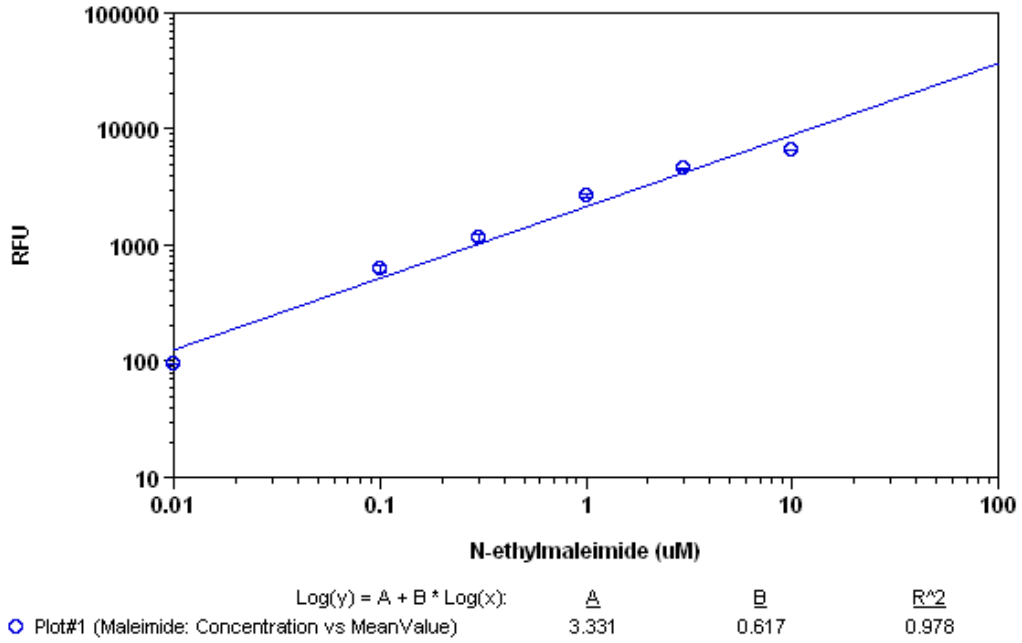

Figure 1. N-ethylmaleimide dose response on 96-well black plate was measured with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit using a NOVOStar microplate reader (BMG Labtech). As low as 0.1 µM (10 picomol/well) of maleimide can be detected with 10 minutes incubation time (n=3).

References:

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3. Fabisiak JP, Sedlov A, Kagan VE. (2002) Quantification of oxidative/nitrosative modification of CYS(34) in human serum albumin using a fluorescence-based SDS-PAGE assay. Antioxid Redox Signal, 4, 855.
4. Ghosh SS, Kao PM, McCue AW, Chappelle HL. (1990) Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonucleotide-enzyme conjugate hybridization probes. Bioconjug Chem, 1, 71.
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6. Wu CW, Yarbrough LR. (1976) N-(1-pyrene)maleimide: a fluorescent cross-linking reagent. Biochemistry, 15, 2863.