Amplite™ Fluorimetric Maleimide Quantitation Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 5523 (200 assays)	Keep at -20 °C Avoid exposure to moisture and light	Fluorescence microplate readers

Introduction

Sensitive assays of maleimide and thiol groups are required for the efficient conjugation of proteins that are expensive and available only in small amounts. 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 without a separation step. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. Compared to kit 5525, this fluorometric assay is more sensitive, and has less interference from biological samples.

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 without a separation step.
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 minutes - 1 hour → 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 minutes → Read fluorescence intensity at Ex/Em = 490/520 nm

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

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1. Prepare 500X 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 into single use aliquots, stored at -20°C and kept from light.

2. Prepare 20X maleimide reaction mixture:

Add 10 µL of 500X Maleimide Green™ stock solution (from Step 1) into 250 µL Reaction Buffer (from Component B), and mix them well. Incubate the 20X maleimide reaction mixture at room temperature for 30 min, protected from light.

Note 1: It is very important to incubate the 20X maleimide reaction mixture at room temperature for at least 30 min to maximize the signal to background ratio.

Note 2: You should see the yellow color after adding the Maleimide Green™ stock solution into reaction buffer.

3. Prepare maleimide assay mixture:

Add the whole contents of 20X maleimide reaction mixture (260 µL from Step 2) into 5 mL of assay buffer (Component C), and mix well.

Note: This maleimide assay mixture is not stable. Use within 1 hour.

4. Prepare serial dilutions of N-ethylaleimide standard (0 to 10 µM):

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

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

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

4.3 Add N-ethylmaleimide standards and maleimide-containing test samples into a solid black 96-well 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 serial dilutions of N-ethylmaleimide standard from 0.1 µM to100 µM into wells from MS1 to MS7 in duplicate.*

5. Run maleimide assay:

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

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

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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 minutes due to the fact that the fluorescence background increases with time.

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

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. A maleimide standard curve is shown in Figure 1.
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.

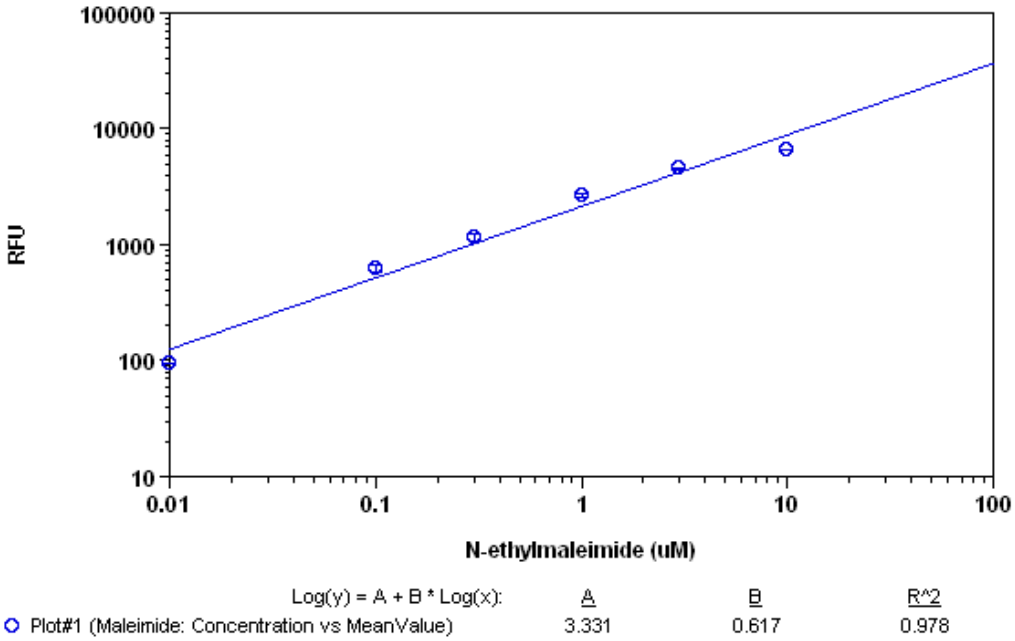

Figure 1. N-ethylmaleimide dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 0.1 µM (10 picomol/well) of maleimide can be detected with 10 minutes incubation time (n=3).

References

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