Amplite® Fluorimetric Maleimide Quantitation Kit *Green Fluorescence*

Catalog number: 5523
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 5523)
Component A: Maleimide Green™	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (500 µL)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)
Component D: N-ethylmaleimide Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (10 mM, 50 µL)
Component E: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

OVERVIEW

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 quantitate the number of maleimide groups that are introduced into the first protein. All the commercial kits have tedious protocols. Our 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 picomole of maleimide in a 100 µL assay volume (100 nM in concentration). The assay is rapid and robust. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation. The kit provides a convenient protocol with all the essential reagents. For the rapid quantification of a protein maleimide group we recommend you use #5526. For the quantification of a nano particle maleimide group we recommend you use #5525.

AT A GLANCE

Protocol Summary

1. Prepare 20X Maleimide reaction mixture (260 µL)
2. Incubate at room temperature for 30 - 60 minutes
3. Prepare N-ethylmaleimide standards or test samples (50 µL)
4. Add Maleimide working solution (50 µL)
5. Incubate at RT for 5 to 30 minutes
6. Monitor the fluorescence increase at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important

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

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Maleimide Green™ stock solution (500X)

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

Note: 10 µL of 500X Maleimide Green™ stock solution is enough for one 96-well plate.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/5523

N-ethylaleimide standard

Add 10 µL of 10 mM (10 nmol/µL) N-ethylmaleimide Standard (Component D) to 990 µL of Assay Buffer (Component C) to generate 100 µM (100 pmol/µL) N-ethylmaleimide standard solution. Take 100 µM (100 pmol/µL) N-ethylaleimide standard solution and dilute to 10 µM. From 10 µM (MS7) standard solution, perform 1:2 serial dilutions to get serially diluted N-ethylmaleimide standards (MS6-MS1) with Assay Buffer (Component C).

PREPARATION OF WORKING SOLUTION

Add 10 µL of 500X Maleimide Green™ stock solution into 250 µL Reaction Buffer (Component B) and mix well to make 20X Maleimide reaction mixture. Incubate 20X Maleimide reaction mixture at room temperature for at least 30 minutes, protected from light.

Note: It is very important to incubate the 20X Maleimide reaction mixture for at least 30 mins to maximize the signal to background ratio.

Note: You should see the yellow color after adding the 500X Maleimide Green™ stock solution into Reaction Buffer (Component B).

Add the whole bottle of 20X Maleimide reaction mixture into 5 mL of Assay Buffer (Component C) and mix well to make Maleimide working solution.

Note: This Maleimide working solution is not stable. Use within 1 hour.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of N-ethylmaleimide standards and test samples in a solid black 96-well microplate. MS = N-ethylmaleimide Standards (MS1 - MS7, 0.1563 µM to 10 µM); BL=Blank Control; TS=Test Samples

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1-SD7	50 µL	Serial Dilutions (0.156 to 10 µM)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

1. Prepare N-ethylmaleimide standards (MS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Maleimide working solution to each well of N-ethylmaleimide standard, blank control and test samples to make the total Maleimide assay volume 100 µL/well. For a 384-well plate, add 25 µL of Maleimide working solution into each well instead, for total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 5 to 30 minutes, protected from light.

   Note: For best results, the fluorescence intenisty should be read within 30 minutes due to the fact that the fluorescence background increases with time.
4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

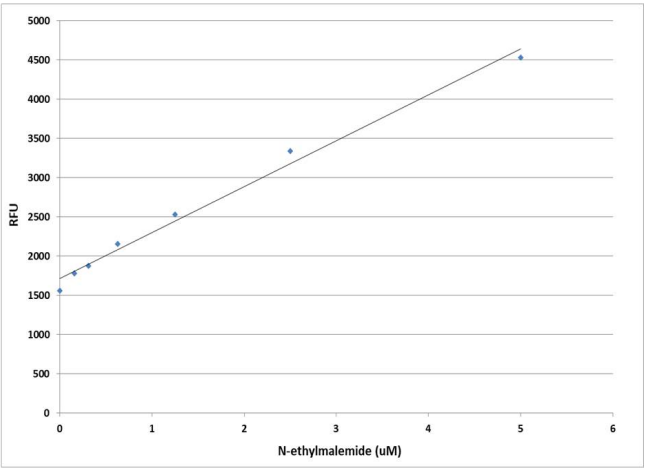

Figure 1. N-ethylmaleimide dose response was measured in a 96-well solid black plate with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech).

DISCLAIMER

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