ab112141

Maleimide Quantification Assay Kit (Fluorometric)

Instructions for Use

For detecting maleimide using our proprietary green fluorescence probe

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 31 January 2019

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1. 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.

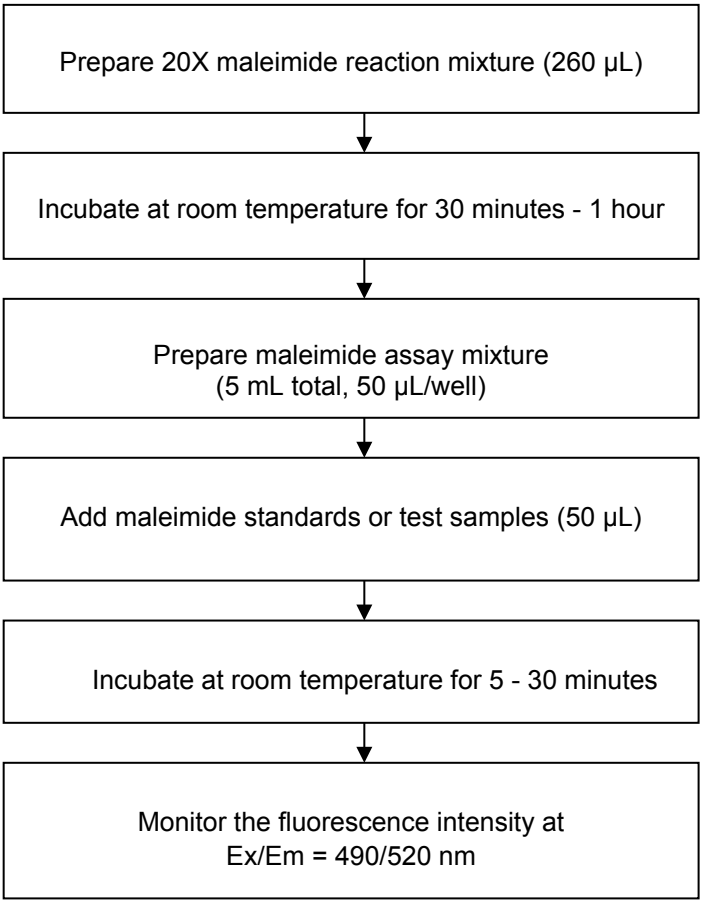
ab112141 uses a proprietary dye that has enhanced fluorescence upon reacting with a maleimide. The kit provides a sensitive, one-step fluorometric method to detect as little as 5 picomoles of free maleimide in a 100 µL assay volume (100 nM; Figure 1). The assay can be performed in a convenient 96-well 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 ab112140, this fluorometric assay is more sensitive, and has less interference from biological samples.

Kit Key Features

- **Broad Application:** Can be used for quantifying free maleimide groups in a variety of molecules such as proteins.
- **Sensitive:** Detects as low as 5 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.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

Components	Amount
Component A: MalemGreen Indicator	1 vial
Component B: Reaction Buffer	500 µL
Component C: Assay Buffer	25 mL
Component D: 10 mM N-ethylmaleimide Standard	50 µL
Component E: DMSO	200 µL

4. Storage and Handling

Keep at -20 °C. Avoid exposure to moisture and light.

5. Assay Protocol

Note: This protocol is for one 96-well plate.

A. Preparation of 500 x MalemGreen Indicator Stock Solution:

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

Note: 10 µL of the stock solution is enough for one 96-well plate. The unused MalemGreen Indicator stock solution should be divided into single use aliquots, stored at -20 °C *and kept from light*.

B. Preparation of 20 x Maleimide Reaction Mixture:

Add 10 µL of 500X MalemGreen Indicator stock solution (from Step A) 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 MalemGreen Indicator stock solution into reaction buffer.

C. Preparation of 2X Maleimide Assay Mixture:

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

Note: This 2X maleimide assay mixture is not stable. Use within 1 hour.

D. Preparation of Serial Dilutions of N-ethylmaleimide Standard (0 to 10 µM):

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*.*

2. Take 200 µL of 100 µM N-ethylmaleimide standard solution (from Step 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.

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

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

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

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

N-ethylmaleimide Standard	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

Table 2. Reagent composition for each well.

**Note: Add the serially diluted N-ethylmaleimide standards from 0.1 µM to 30 µM into wells from MS1 to MS7 in duplicate.*

E. Run Maleimide Assay:

1. Add 50 µL of 2X maleimide assay mixture (from Step C) to each well of the N-ethylmaleimide standard, blank control, and test samples (see Step D.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.

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.

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

6. Data Analysis

The fluorescence in blank wells (with Assay buffer only) is used as a control, and is subtracted from the values for those wells with 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.

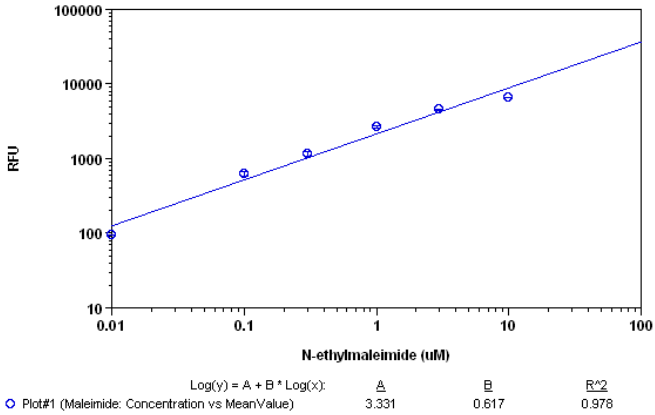


Figure 1. N-ethylmaleimide dose response was measured in a 96-well black plate with ab112141 using a microplate reader. As low as 0.05 µM (5 picomol/well) of maleimide can be detected with 10 minutes incubation time (n=3).

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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