Amplite® Colorimetric Maleimide Quantitation Kit

Catalog number: 5525
Unit size: 100 tests

Component	Storage	Amount
Component A: MEA	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: 4,4'-DTDP	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component D: DMSO	Freeze (< -15 °C)	1 vial (1 mL)

OVERVIEW

Maleimides can be directly assayed spectrophotometrically at 302 nm. However, the small extinction coefficient of 620 M-1cm-1 renders this assay insensitive, and the assay is further complicated by the protein absorbance at the same wavelength. This colorimetric maleimide assay kit quantifies maleimide groups by first reacting a sample with a known amount of thiol present in excess and then assaying the remaining unreacted thiol using 4,4\'-DTDP with a molar extinction coefficient of 19,800 M -1cm-1. The amount of maleimide is calculated as the difference between the initial amount of thiol and the amount of unreacted thiol after the complete reaction of all maleimide groups. This spectrophotometric assay for the determination of maleimide groups is a reverse GSH assay. It takes advantage of the high reactivity of thiols of GSH with the maleimide moiety. Maleimide of the sample is allowed to form a stable thiosuccinimidyl linkage with GSH. After the reaction of the sample is complete, the excess GSH, i.e., the remaining thiols of GSH in the reaction mixture, is estimated by using 4,4\'-DTDP. The amount of GSH reacted with the sample is titrated to determine the extent of maleimide. For more sensitive maleimide quantitation, we recommend that you use our fluorimetric kit # 5523 that has higher sensitivity.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	324 nm
Recommended plate	Clear bottom

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.

1. MEA stock solution (500X)
Add 200 µL of distilled water into the vial of MEA (Component A). **Note:** 10 µL of the 500X MEA stock solution is enough for 50 reactions (0.5 mL/reaction). The unused 500X MEA stock solution should be divided into single use aliquots, stored at -20 ° C and kept from light.

2. 4,4'-DTDP stock solution (50X)
Add 1 mL of DMSO (Component D) into the vial of 4,4'-DTDP (Component B), and mix well. **Note:** 100 µL of the 50X 4,4'-DTDP stock solution is enough for 10 reactions (0.5 mL/reaction). The unused 50X 4,4'-DTDP stock solution should be divided into single use aliquots, stored at -20 ° C and kept from light.

PREPARATION OF WORKING SOLUTION

MEA working solution
Add 10 µL of MEA stock solution (500X) into 5 mL of distilled water, and mix them well. **Note:** The MEA working solution is not stable. We recommend to prepare fresh before use.

SAMPLE EXPERIMENTAL PROTOCOL

The following recommended protocol is for Cuvette.

1. Set up 3 Total SH tubes: Add 400µL of Assay Buffer (Component C) and 100 µL of MEA working solution into each tube and incubate at room temperature for 20 minutes.
2. Set up 3 test tubes for each sample: Add 0.05 mg of test sample and sufficient Assay Buffer (Component C) to make the total volume of 400 µL/tube. Add 100 µLof MEA working solution into each tube and incubate at room temperature for 20 minutes..
3. Measure the absorbance of the Assay Buffer (Component C) as the blank control at 324 nm.
4. Proceed to Total SH determination while tubes are still incubating (from step 1). Add 10µL of 50X 4,4'-DTDP stock solution into each total SH tube and incubate at room temperature for 2 min. *Note* : Do not add 50X 4,4'-DTDP stock solution to the sample containing tubes yet.
5. Measure the absorbance of the 3 Total SH tubes at 324 nm without washing the cuvette. Record the readings and average them to have "OD_{TSH} "
6. Clean the cuvette and read the absorbance of the first sample tube (from Step 2) at 324 nm (OD) before add any 4,4'-DTDP stock solution (50X).
7. Add 10 µL of 4,4'-DTDP stock solution (50X) into the sample cuvette (from Step 6) and mix well. Incubate the sample at room temperature for 2 minutes and read the absorbance at 324 nm (OD). Clean the cuvette, and repeat Steps 6 and 7 for the remaining tubes. Record all readings.

EXAMPLE DATA ANALYSIS AND FIGURES

Calculate the number of maleimide groups for each sample (curvet as an example).

1. Calculate ΔOD for each tube:$\Delta OD = OD_{TSH} - [OD - OD] = OD_{TSH} + OD - OD$

2. Calculate maleimides for each sample:(Moles of Maleimide)/(Conjugate)=([(ΔOD)/(Extinction Coefficient of DTDP at 324 nm)] x Sample Volume (L))/([Conjugate Weight]/[Molecular weight of Conjugate])

=([ΔOD ÷19,800] x 0.51 mL ÷1000)/([Conjugate Weight mg ÷1000]/[Molecular weight of Conjugate])

=([ΔOD] x [Molecular weight of Conjugate])/([Conjugate Weight mg] x 38824)

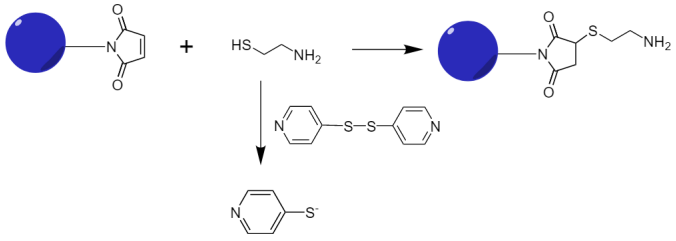

Figure 1. Chemical structure for Amplite™ Colorimetric Maleimide Quantitation Kit.

DISCLAIMER

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