Amplite™ Colorimetric Maleimide Quantitation Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 5525 (100 assays)	Keep at -20 °C Avoid moisture and light	Absorbance readers

Introduction

Maleimides can be directly assayed spectrophotometrically at 302 nm. However, the small extinction coefficient of 620 $M^{-1}cm^{-1}$ 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 $M^{-1}cm^{-1}$. 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.

Kit Components

Components	Amount
Component A: MEA	1 vial
Component B: 4,4'-DTDP	1 vial
Component C: Assay Buffer	1 bottle (50 mL)
Component D: DMSO	1 mL

Assay Protocol for Cuvette

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

1. Prepare 500X MEA stock solution: Add 0.2 mL distilled water into MEA (Component A)

Note: 10μL 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. Prepare MEA working solution: Add 10 μL of the 500X MEA stock solution (from Step 1) into 5mL distilled water**.**

Note: The MEA working solution is not stable. Prepare fresh before use (less than 2 hours at room temperature).

3. Prepare 50X 4,4'-DTDP stock solution: Add 1 mL DMSO (Component D) into the vial of 4,4'-DTDP (Component B) to have 50X 4,4'-DTDP stock solution.

Note: 100μL 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.

4. Run Maleimide assay:

4.1 Set up 3 Total SH tubes: Add 0.4 mL of Assay Buffer (Component C) and 0.1 mL of MEA working solution (from Step 2) into each tube and incubate at room temperature for 20 minutes.

4.2 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 0.4 mL/tube. Add 0.1 mL of MEA working solution (from Step 2) into each tube and incubate at room temperature for 20 minutes.

4.3 Measure the absorbance of the Assay Buffer (Component C) as the blank control at 324 nm.

4.4 Proceed to Total SH determination while tubes are still incubating (from Step 4.1). Add 10µL of 50X 4,4'-DTDP stock solution (from Step 3) 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.*

4.5 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}**".

4.6 Clean the cuvette and read the absorbance of the first sample tube (from Step 4.2) at 324 nm (**OD_0**) **before** add any 50X 4,4'-DTDP stock solution.

4.7 Add 10 µL of 50X 4,4'-DTDP stock solution (from Step 3) into the sample cuvette (from Step 4.6) and mix well. Incubate the sample at room temperature for 2 minutes and read the absorbance at 324 nm (**OD**).

4.8 Clean the cuvette, and repeat Steps 4.6 and 4.7 for the remaining tubes. Record all readings.

Data Analysis

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

1. Calculate ΔOD for each tube:

$$\Delta OD = OD_{TSH} - [OD - OD_0] = OD_{TSH} + OD_o - OD$$

2. Calculate maleimides for each sample:

$$\frac{Moles\ of\ Maleimide}{\text{Conjugate}} = \frac{\left[\frac{\Delta OD}{\text{Extinction Coefficient of DTDP at 324 nm}}\right] \text{ x Sample Volume (L)}}{\text{[Conjugate Weight]/[Molecular weight of Conjugate]}}$$

$$= \frac{[\Delta OD \ \div 19{,}800] \text{ x } 0.51 \text{ mL} \ \div 1000}{\text{[Conjugate Weight mg} \ \div 1000 \text{]/[Molecular weight of Conjugate]}}$$

$$= \frac{[\Delta OD] \text{ x [Molecular weight of Conjugate]}}{\text{[Conjugate Weight mg] x } 38824}$$

References

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