ab112140

Maleimide Quantificat ion Kit - Colorimetric

Instructions for Use

For detecting maleimide in physiology solutions spectrophotometrically.

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

Version 2c, Last updated on 20 August 2024

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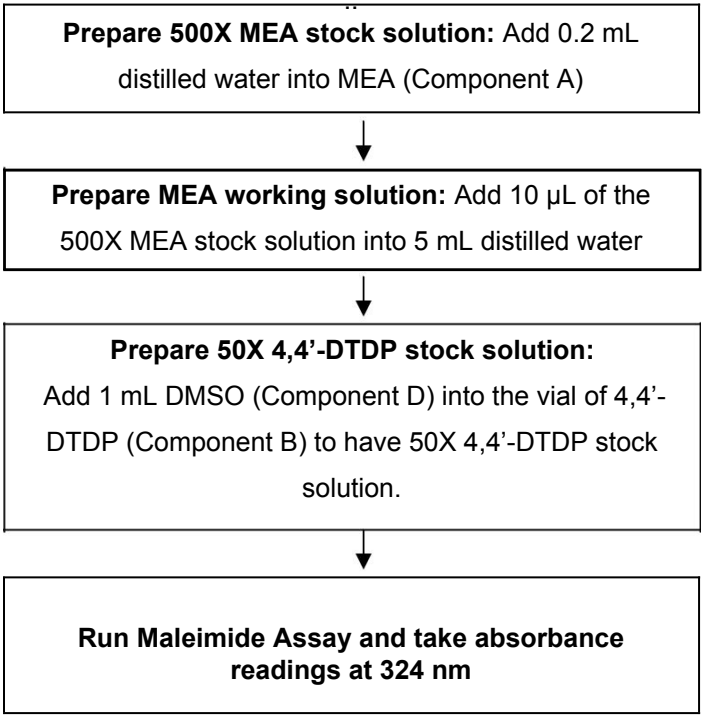
1. Introduction

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.

ab112140 assay kit quantifies maleimide groups by first reacting a sample with a known amount of thiol present in excess and then assaying the remaining un-reacted 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 ab112141 that has higher sensitivity.

2. Protocol Summary



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

3. Kit Contents

Components	Amount
Component A: MEA	1 vial
Component B: 4,4'-DTDP	1 vial
Component C: Assay Buffer	1 x 50 mL
Component D: DSMO	1 mL

4. Storage and Handling

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

5. Assay Protocol

Note: This protocol is for one cuvette. Thaw all the kit components at room temperature before starting the experiment.

A. 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 ^{O}C and kept from light.

B. Prepare MEA Working Solution

Add 10 µL of the 500X MEA stock solution (from step A) into 5mL distilled water.

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

C. 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: 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 ^{O}C and kept from light.

D. Run Maleimide Assay:

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 section B) 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 0.4 mL/tube. Add 0.1 mL of MEA working solution (from section B) 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 (from section C) 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 "$\mathbf{OD_{TSH}}$".

6. Clean the cuvette and read the absorbance of the first sample tube (from step 2) at 324 nm ($\mathbf{OD_0}$) before add any 50X 4,4'-DTDP stock solution

7. Add 10 µL of 50X 4,4'-DTDP stock solution (from section C) 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**).

8. Clean the cuvette, and repeat steps 6 and 7 for the remaining tubes. Record all readings.

6. 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:

$$\frac{Moles\ of\ Maleimade}{Conjugate} =$$

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

$$= \frac{[\Delta OD\ \div 19800] \times 0.51\ mL \div 1000}{[Conjugate\ Weight\ mg\ \div 1000]/[Molecular\ weight\ of\ Conjugate]}$$

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

3. Alternatively, to calculate maleimide in a tested sample:
   Moles of Maleimide

   =([(ΔOD)/(Extinction Coefficient of DTDP at 324 nm)] x Sample Volume (L)

   =([ΔOD ÷19,800] x 0.51 mL)

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 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 consider deproteinizing samples
	Unsuitable sample type	Use recommended sample types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

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 Incorrect mounts used	Refer to datasheet for recommended incubation time and/ or temperature Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)