Amplite™ Rapid Colorimetric Maleimide Quantitation Kit

Catalog number: 5526
Unit size: 2 Tests

Component	Storage	Amount
Component A: Maleimide Blue™	Freeze (<-15 °C), Minimize light exposure	2 vials (One vial is for 50 ~100 µg maleimidelinked antibody or protein)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (15 mL)
Component C: Spin Column	Room temperature	2 columns
Component D: Washing Tube (2 mL)	Room temperature	2 tubes
Component E: Collecting Tube (1.5 mL)	Room temperature	2 tubes

OVERVIEW

A variety of crosslinking reagents with a maleimide group are widely used for crosslinking proteins to proteins, or proteins to other biomolecules. A key challenge in maleimide crosslinking technology has been the quantitation of maleimide linked to a single protein or antibody. 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. AAT Bioquest's Amplite™ Rapid Colorimetric Maleimide Quantitation Kit provides a rapid and accurate method to quantify maleimide using our proprietary maleimide sensor Maleimide Blue™ with the maximum absorbance at ~780nm. The principle of this assay is that Maleimide Blue™ reacts with the maleimide-linked sample, and the resulted product is run through a single spin column to remove the excess sensor. The absorption spectrum of the purified product is measured, and the amount of maleimide to protein ratio can be calculated from the absorbance ratio of 780 nm and 280 nm (for proteins) or 260 nm (for oligos and nucleic acids). This Amplite™ Rapid Maleimide Quantitation kit can be performed in a traditional cuvette, NanoDrop™ Spectrophotometer or a convenient 96-well absorbance plate reader with a UV-transparent plate. This kit has been widely used for the rapid quantification of maleimide group from protein, oligo and nucleic acid samples.

AT A GLANCE

Important Upon receipt, store Maleimide Blue™ (Component A) at -20°C (prefer at -80°C), kept from light and moisture. When stored properly, the kit components should be stable for six months. Do not freeze Spin Column (Component C). Warm all the components before run the required assays. 50 to 100 µg maleimide-linked antibody or protein sample is needed for determining the amount of maleimide.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	900 nm to 250 nm
Recommended plate:	Clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

Prepare sample solution:

1. Use 50 to 100 µg maleimide sample (protein or other polymers).

2. Adjust the volume to 100 µL with Assay Buffer (Component B). *Note:* The maleimide-linked antibody or protein sample should be in pH = 6.0 buffer and without free maleimide.

Run Maleimide Assay:

1. Add the maleimide sample to one vial of Maleimide Blue™ (Component A).

2. Mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

3. Keep the reaction mixture at room temperature and rotate or shake for 30 - 60 minutes.

Prepare Spin Column for Sample Purification:

1. Invert the Spin Column (Component C) several times to resuspend the settled gel and remove any bubbles.

2. Snap off the tip and place the column in the Washing Tube (2 mL, Component D). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube (not provided).

3. Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

4. Apply 1 mL Assay Buffer (Component B) to the column, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3 - 4 times.

5. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the reaction buffer. Discard the buffer.

Note Spin Column (Component C) can fit into 2 mL microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtubes provided with the columns for the initial column equilibration step.

Note Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.

$RCF(g) = (1.12 \times 10^{-5}) \times (RPM)^2 \times r$

RCF = the relative centrifugal force
RPM = the speed of the rotor
r = the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column

Purify Malemide Reaction Product:

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

1. Place the column in a clean Collecting Tube (1.5 mL, Component E). Carefully load the sample (100 µL) directly to the center of the column.

2. After loading the sample, add 10 µL Assay Buffer (Component B) to the top and centrifuge the column for 5 min at 1,000 x g, and collect the solution into the collecting tube.

Run Absorption Spectra with 0.2mL or 0.5 mL Quartz Cuvette:

1. Dilute the maleimide reaction product by 5 - 10 folds with Assay Buffer (Component B) depending on the cuvette size used and the absorbance reading. The dilution factor doesn't affect the final maleimide quantitation result.

2. Measure the absorption spectrum from 900 nm to 250 nm range, or only read the absorbance number at 280 nm and 782 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

For illustrating purpose we use a BSA-maleimide as an example to calculate the number of maleimide groups on maleimide-linked BSA sample with 0.5 mL cuvette measurement.

Sample: BSA-maleimide, 10.75 mg/mL in pH=6.0 Buffer

Procedures:

1. Use 4.7 µL (50 µg) of BSA-maleimide, and then add 95.3 µL Assay Buffer (Component B) to have total volume of 100µL.
2. Add above 100µL solution to Maleimide Blue™ vial (Component A), mix well.
3. Rotate for 60 min at room temperature.
4. Purify with Spin Column (Component C), and collect the product.
5. Take 50 µL of the product, and add 400 µL Assay Buffer (Component B) to the 0.5mL cuvette and measure the absorbance spectra.

Constants needed:

BSA extinction coefficient at 280 nm: 43824 M^{-1} cm^{-1}

Maleimide Blue™ extinction coefficient at maximum absorption (780 ± 3nm): 275,000 M^{-1} cm^{-1}

Correction Factor of Maleimide Blue™ at 280 nm (CF_{280nm}): 0.207

Results:

OD readings obtained with the above BSA-maleimide sample: A_{280nm} = 0.290, A_{782nm} = 1.214

Calculations:

Calculate BSA-maleimide amount with Equation:

(Moles of Maleimide)/(Moles of protein or antibody) = $([A_{782nm}]/\varepsilon_{Maleimide\ Blue™})/[(A_{280nm} - CF_{280nm} \times [A_{782nm}])/\varepsilon_{protein\ or\ antibody\ at\ 280nm}]$

Maleimide Ratio = (1.214/275000)/(0.290 - 0.207 × 1.214)/43824 = 5.00

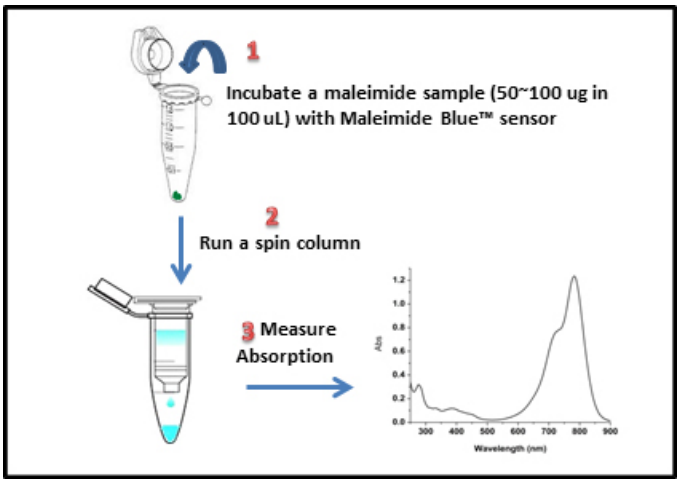

Figure 1.

The Amplite™ Rapid Colorimetric Maleimide Quantitation Assay Principle.

DISCLAIMER