Amplite® Rapid Colorimetric PE-Maleimide Quantitation Kit

Catalog number: 5530
Unit size: 2 Tests

Component	Storage	Amount (Cat No. 5530)
Component A: Maleimide 680™	Freeze (< -15 °C), Minimize light exposure	2 Vials (1 vial is for 100 µg of PE-maleimide)
Component B: Assay Buffer	Refrigerated (2-8 °C)	1 Bottle (15 mL)
Component C: Spin Column	Refrigerated (2-8 °C)	2 Columns

OVERVIEW

Maleimide-based crosslinking reagents are commonly used for conjugating proteins to other proteins or biomolecules. A significant challenge in maleimide chemistry is the precise quantification of maleimide moieties conjugated to proteins. The Amplite® Rapid Colorimetric PE-Maleimide Quantitation Kit facilitates the quantification of maleimide groups on maleimide-activated PE proteins using a proprietary sensor, Maleimide 680™, which has an absorption peak at ~680 nm. The assay involves the reaction of Maleimide 680™ with the maleimide-activated PE, followed by the separation of the reaction mixture via a spin column to remove the unreacted sensor. Subsequently, the absorption spectrum of the isolated product is measured and the amount of maleimide to PE is determined from the absorbance ratios at 680 nm and 566 nm, with the latter being the peak absorbance of PE. This quantitation kit is compatible with various detection systems, including traditional cuvettes, NanoDrop™ Spectrophotometers, and 96-well absorbance plate readers. It offers a robust and adaptable approach for the rapid quantification of maleimide modifications on APC proteins, addressing a critical need in protein biochemistry.

AT A GLANCE

Upon receipt, store the vials of Maleimide 680™ (Component A) at -20 °C (preferred at -80°C), kept from light and moisture. When stored properly, the kit components should be stable for six months.

Note: Do not freeze the Spin Columns (Component C).

Note: Warm all the components before running the required assays. 100 µg PE-maleimide is needed to determine the molar ratio of maleimide to PE.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	900 nm to 250 nm
Recommended plate	Clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

Prepare PE-Maleimide Sample Solution

1. Obtain a 100 µg sample of PE-maleimide.
2. Adjust the volume to 100 µL using Assay Buffer (Component B).

   Note: Make sure the PE-maleimide sample is prepared in a pH 6.0 buffer and without any free maleimide.

Run Maleimide Assay

1. From the 'Prepare PE-Maleimide Sample Solution' section, take the solution prepared in Step 2 and add it to a vial of Maleimide 680™ (Component A). Mix well by repeatedly pipetting a few times or vortexing the vial for a few seconds.
2. Keep the reaction mixture at room temperature and rotate for 30 - 60 minutes.

Prepare the Spin Column for Sample Purification

1. Invert the Spin Column (Component C) several times to suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a 2 mL Washing Tube (not provided). Remove the cap to let the excess packing buffer drain by gravity until it reaches the top of the gel bed. If the column doesn't start to flow, reinsert and remove the cap to initiate drainage. Dispose of the buffer. Place the column back into the Washing Tube. However, if the column is placed into a 12 x 75 mm test tube (not provided), centrifuge immediately.
3. Centrifuge for 1 minute in a swinging bucket centrifuge at 1,000 x g (see Appendix: Centrifugation Notes section) to remove the packing buffer. Discard the buffer.
4. Apply 1 mL of the Assay Buffer (Component B) to the column, let the buffer drain out by gravity, or centrifuge the column for 1 minute 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 Appendix: Centrifugation Notes section) to remove the reaction buffer. Discard the buffer.

Purify Malemide Reaction Product

1. Insert the column from Step 5 of the 'Prepare the Spin Column for Sample Purification' section into a clean 1.5 mL Collecting Tube. Gently pipette the sample (100 µL) directly into the center of the column.
2. After loading the sample, add 10 µL of Assay Buffer (Component B) to the top of the column. Then centrifuge the column for 5 minutes at 1,000 x g, and collect the solution in the collecting tube.

Run Absorption Spectra with Quartz Cuvette or Nanodrop

1. Dilute the maleimide reaction product obtained in Step 2 from

the 'Purify Maleimide Reaction Product' section. Use 5 to 10 times the volume of Assay Buffer (Component B) for this dilution. The exact amount of buffer required will depend on the size of the cuvette and the absorbance measurement obtained.

Note: The dilution factor doesn't affect the final maleimide quantitation result.

Note: Dilution is not needed if using Nanodrop to measure Absorbance

2. Measure the absorption spectrum from 900 nm to 250 nm range, or only read the absorbance number at 566 nm and 675 nm.

Data Analysis

Calculations:

1. Calculate the amount of PE-maleimide

$$\text{Moles of Maleimide/Moles of protein or antibody} = [(A_{675nm}) \div \varepsilon_{\text{Maleimide 680™}}] \div [((A_{566nm} - CF_{566nm}) \div A_{675nm})/\varepsilon_{PE}]$$

Where:

- A_{566} = Absorbance of PE-maleimide sample at 566 nm
- A_{675} = Absorbance of PE-maleimide sample at 675nm
- CF_{566} = 0.08
- $\varepsilon_{\text{Maleimide 680™}}$ = 250,000 $M^{-1}cm^{-1}$
- ε_{PE} = 1,960,000 $M^{-1}cm^{-1}$

APPENDIX

Centrifugation Notes

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.

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 information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, the speed in RPM required to reach the gravitational force of 1,000 x g can be calculated using the following equation:

$$RCF\ (g) = (1.2 \times 10^{-5} \times (RPM)^2 \times r$$

- 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

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