Beadlite™ Rapid Colorimetric Amino Quantitation Kit for Nanoparticles

Catalog number: 5532
Unit size: 2 Tests

Component	Storage	Amount
Component A: Buccutite™ MTA, NHS ester	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA-Dye 650	Freeze (< -15 °C), Minimize light exposure	2 vials
Component C: Reaction Buffer	Freeze (< -15 °C)	1 vial (1 mL)
Component D: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)

OVERVIEW

Surface functionalized micro- and nano-particles have been widely used in the field of nanotechnologies and biological sciences such as bio-separation and purification, assay development, and drug delivery. Among the common functional groups, amine group is one of the most popular groups for modification, and is often the first active group to be introduced. Various biomolecules, either probe molecules or target molecules, can thus be immobilized on the surface of nanoparticles through the reaction with these amine groups. The density of amine groups plays a key role in determining the properties of nanomaterials and controlling their interactions with biomolecules. Therefore, quantitation of amine density on the surface of particles is critical to their applications. Beadlite™ Rapid Colorimetric Amino Quantitation Kit offers a rapid and sensitive absorption-based assay for measuring amine density with high specificity. Upon the selective reaction with the amine group, the Buccutite™ MTA is first immobilized on the surface of particles, and then the modified particles are added to Buccutite™ MTA-Dye 650 solution resulting in a decrease of absorbance in supernatant. The signal can be measured by UV-Vis spectrometer at ~650 nm and the change is proportional to the amount of amine groups on the surface of the nanoparticles.

AT A GLANCE

Protocol summary

1. Wash beads
2. Mix beads and Component A
3. Rotate 1 hour at room temperature
4. Wash beads
5. Prepare Component B solution and measure absorbance at 650 nm as “Original absorbance”(A_O)
6. Mix beads and Component B solution
7. Rotate 1.5-2 hours at room temperature
8. Measure absorbance of supernatant at 650 nm as “Remaining absorbance”(A_R)

Calculate amine density on beads

Important

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

KEY PARAMETERS

NanoDrop

Absorbance	650 nm

Spectrophotometer

Absorbance	650 nm
Recommended plate	Clear

SAMPLE EXPERIMENTAL PROTOCOL

1. Take 1 mg beads that need to be tested. Wash beads with 0.5-1 mL Assay Buffer (Component D) for 3 times and remove all supernatant.
2. Disperse beads in 100 µL Reaction Buffer (Component C). Add this suspension to one vial of Component A and mix well.
3. Rotate the vial (from step 2) at room temperature for 1 hour.
4. Wash beads with 0.5-1 mL Assay Buffer (Component D) for 3 times and remove supernatant.
5. Add 100 µL Assay Buffer (Component D) to one vial of Component B to make Component B stock solution.
6. Take 2 µL Component B stock solution (from step 5) to 198 µL of Assay Buffer (Component D) to have 1 to 100 x dilution. Measure absorbance at 650 nm as “Original absorbance” (A_O).
7. Disperse beads in the rest 98 µL of Component B stock solution.
8. Rotate it (from step 7) at room temperature for 1.5- 2 hours.
9. Separate beads and supernatant.
10. Take 2 µL of supernatant (from step 9) to 198 µL Assay Buffer (Component D) to have 1 to 100 x dilution. Measure absorbance at 650 nm as “Remaining absorbance”(A_R).

Calculate amine density on beads:

Amine density (nmol/mg) = (Original absorbance-Remaining absorbance) * 39.2 nmol/mg

EXAMPLE DATA ANALYSIS AND FIGURES

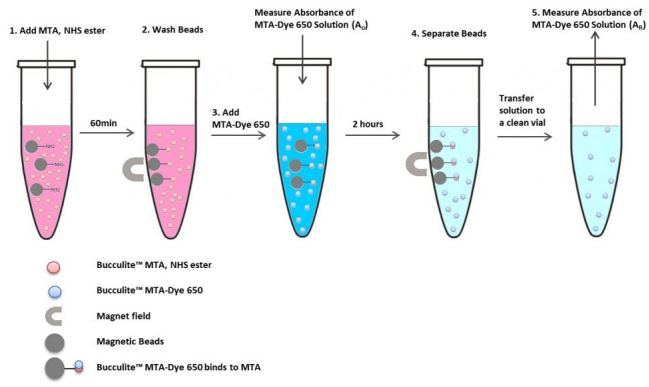

Figure 1. Example of quantitation of surface amino groups on magnetic nanoparticle with Beadlite™ Rapid Colorimetric Amino Quantitation Kit (Cat# 5532).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or

reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.