

Beadlite™ Rapid Colorimetric Thiol Quantitation Kit for Nanoparticles

Catalog number: 5535
Unit size: 100 tests

Component	Storage	Amount (Cat No. 5535)
Component A: Thiolite™ 490	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Assay Buffer (10X)	Freeze (< -15 °C), Minimize light exposure	1 Bottle (10 mL)
Component C: DMSO	Freeze (< -15 °C)	1 Bottle (1.0 mL)

OVERVIEW

Maleimide-thiol chemistry is widely used for preparing nanoparticles (NPs) conjugates. The surface modification of nanoparticles with free sulfhydryl group is a common strategy to activate nanoparticles. A great challenge has been the quantitation of thiol groups on the thiol-modified surface. Beadlite™ Rapid Colorimetric Thiol Quantitation Kit provides a robust method to quantify thiol-functionalized nanoparticle with our proprietary Thiolite™ 490 that has its maximum absorbance at ~490 nm. The kit quantifies sulfhydryl groups by first reacting nanoparticles with a known amount of Thiolite™ 490 present in excess and the remaining unreacted Thiolite™ 490 is measured by its absorption. The amount of sulfhydryl group on the NP surface is calculated to be the difference between the initial amount of Thiolite™ 490 and the amount of unreacted Thiolite™ 490. Beadlite™ Rapid Colorimetric Thiol Quantitation Kit can be performed in a traditional cuvette, NanoDrop™ Spectrophotometer or a convenient 96-well absorbance plate reader. This kit has been widely used for the rapid quantification of sulfhydryl groups on the surface of latex beads, agarose beads, or magnetic nanoparticles.

KEY PARAMETERS

NanoDrop

Absorbance 490 nm

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Thiolite™ 490 Stock Solution

1. Add 250 µL of DMSO (Component C) to dissolve Thiolite™ 490 (Component A).

Note: Store any unused Thiolite™ 490 stock solution at -20°C (preferably at -80°C), protected from light and moisture. Kit components should remain stable for approximately six months when stored properly.

PREPARATION OF WORKING SOLUTION

1X Assay Buffer Working Solution

1. Prepare a 1X Assay Buffer by mixing 1 mL of 10X Assay Buffer (Component B) with 9 mL of ddH₂O.

Note: 10 mL of 1X Assay Buffer is suitable for 10 samples.

Thiolite™ 490 Working Solution

1. Prepare a Thiolite™ 490 working solution by mixing 2 µL of the

Thiolite™ 490 stock solution with 200 µL of the 1X Assay Buffer.

Note: 200 µL is suitable for one assay.

SAMPLE EXPERIMENTAL PROTOCOL

Prepare Sample Solution

1. Obtain a 100 µL sulfhydryl modified nanoparticle (NP-SH) sample.
2. Centrifuge the NP-SH sample to remove the storage buffer, then wash twice with 100 µL of 1X Assay Buffer per wash.

Note: When working with magnetic beads, employ a magnetic holder for washing the beads. Avoid using a centrifuge for this step.

3. After washing, reconstitute the NP-SH sample to a final volume of 100 µL using 1X Assay Buffer.

Run Thiol Assay

1. Add 100 µL of the Thiolite™ 490 working solution to the NP-SH sample. Mix thoroughly by either pipetting up and down several times or vortexing briefly for a few seconds.

Note: The remaining 100 µL of Thiolite™ 490 working solution is used as Total SH.

2. Keep the reaction mixture at room temperature and rotate for 30 to 60 minutes.

Measure Absorbance

1. Measure the absorbance of the Thiolite™ 490 working solution using a Nanodrop (A0).
2. Centrifuge the reaction mixture, and transfer the supernatant into a new clean centrifuge tube.
3. Centrifuge the supernatant at 10,000 rpm for 2 minutes.
4. Transfer the supernatant into a new clean centrifuge tube.
5. Measure the absorbance using a Nanodrop (A1).

Note: If using a UV-VIS spectrometer to measure the absorbance, dilute the Thiolite™ 490 working solution and the supernatant from steps 1 and 4 of the 'Measure Absorbance' section per the recommended guidelines.

DATA ANALYSIS - CALCULATIONS

1. Calculate the change in absorbance at 490 nm:

$$\Delta A_{490} = [(A_0 \times 100) - (A_1 \times 200)]$$

Where:

- A_0 = Absorbance of Thiolite™ 490 working solution at 490 nm
- A_1 = Absorbance of supernatant at 490 nm

2. Calculate the amount of maleimide

$$\mu\text{Moles of Maleimide/mg Agarose} = [\Delta A_{490} \div \epsilon_{\text{Thiolite}^{\text{TM}} 490}] / m$$

Where:

- $\epsilon_{\text{Thiolite}^{\text{TM}} 490} = 80,000 \text{ M}^{-1} \text{ cm}^{-1}$
- m = weight of sample (mg)

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