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Thiol Assay Kit

Product Number: **CM90004**

Product Description

CellMosaic's thiol assay kit is designed to assay the free thiol group content of a biopolymer, such as a partially reduced antibody. CellMosaic routinely uses this kit for its internal bioconjugation-related research.

The assay is based on Ellman's assay using 5'5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) (Ellman, G.L. 1959, Tissue sulfhydryl groups. *Arch Biochem Biophys*. 82, 70–77). Thiol is reacted with DTNB to generate 2-nitro-5-thiobenzoic acid (TNB). TNB is orange in color and has an extinction coefficient of 14,150 $M^{-1}cm^{-1}$ at 412 nm (Riddles, P.W. Blakeley, R. L., and Zerner, B. 1983, Reassessment of Ellman's reagent. *Methods Enzymol.* 91, 49-60). The amount of thiol groups will be calculated based on the amount of TNB generated.

Application of the Product

- Assay the free thiol groups.

Key Feature of the Product

- Less than 30 minutes of preparation and assay time. Fast and easy to use.

Kit Components

Four micro-centrifuge tubes per package. Each package is sufficient for 10 assays (100 µL per assay volume)

Name	Part #	Quantity
Reagent (orange label)	CM13006	1 unit
Blank (yellow label)	CM13007	1 unit
Buffer A (blue label)	CM02018	0.5 mL
Solution A (green label)	CM01003	2 mL

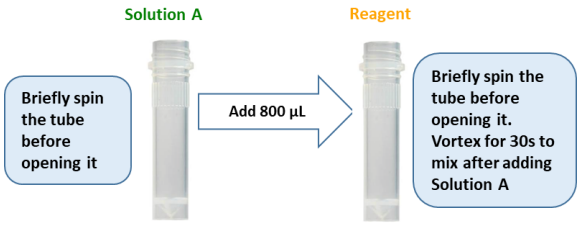
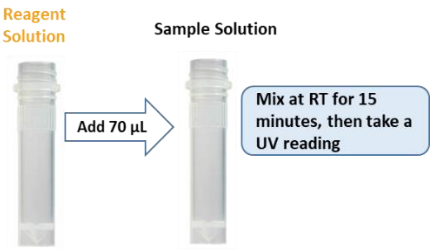
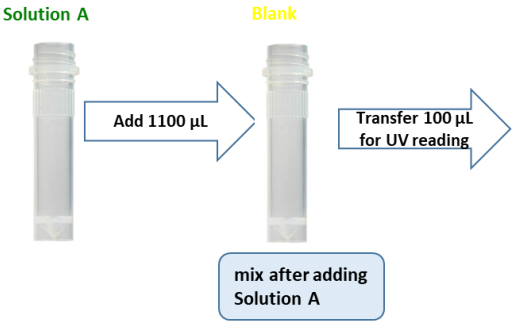
Storage/Stability

Recommended storage of the kit is at 2-8°C. For reagent and blank dissolved in solution A, they can be aliquoted and stored at -20°C up to 1 year without any sign of decomposition.

Equipment (not provided)

1. UV/vis spectrophotometer or micro-plate reader spectrophotometer with pathlength correction capability
2. Ultra-micro UV transparent cuvette: 100 µL (for UV/vis spectrophotometer) or 96-well UV microplate

Protocol

1. Sample preparation: Briefly spin the tube containing **Buffer A** (blue label) before opening it. Dilute the biopolymer thiol in **Buffer A** to a total volume of **30 µL** with a final concentration of thiol groups in the 10–100 µM range. **Note**: If it is an antibody with an average 4 free thiol groups per antibody, you can dilute to 1-2 mg/mL.	
2. Prepare Reagent solution: Briefly spin the tube containing **Reagent** (orange label) and **Solution A** (green label) before opening the tubes. Pipette 800 µL of **Solution A** into the **Reagent tube**. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap.	
4. Prepare sample solution: Mix 30 µL of sample with 70 µL of **Reagent solution** at RT for 15 minutes. **Note:** Aliquot and store the rest of the **Reagent solution** at -20°C for later usage.	
5. Prepare Blank solution: Briefly spin the tube containing **Blank** (yellow label) before opening it. Pipette 1100 µL of **Solution A** into the **Blank tube.** Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Pipette 100 µL for the assay. **Note:** Aliquot and store the rest of the **Blank solution** at -20°C for later usage.	
6. UV reading: Zero the spectrophotometer with blank solution and measure the UV absorbance of the sample solution at 412 nm.	As (sample): _____ Ab (blank): ____0_____
7. Calculate the concentration of thiol $uM = 235.5713 \times As$	μM = _____
8. Calculate the number of thiols per biopolymer (n) based on the following formula: $n = \frac{uM\ (Maleimide)}{uM\ (Biopolymer)}$	n = _____

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. Information about the chemicals and reagents used in the kit are provided as necessary.

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