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Thiol Assay Kit with Purification

Product Number: **CM90005**

Product Description

CellMosaic's Thiol Assay Kit with Purification is designed to assay the free thiol groups of a biopolymer after reducing or labeling with small molecule thiol-modification reagents. CellMosaic routinely uses this kit for its internal bioconjugation-related research. The product can be used together with antibody or other biopolymer labeling and conjugation kits to optimize loading. Examples of these kits are antibody MMAE conjugation kits with VC-PAB linker (Cat#: CM11409 and CM11409x3), antibody Mc-MMAF conjugation kits (CM11422 and CM11422x3), antibody MMAF conjugation kits with VC-PAB linker (CM11425 and CM11425x3), and F(ab')₂ MMAE conjugation kits (CM11416 and CM11416x3). Please follow the protocols for using the thiol assay kit along with these conjugation kits.

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 (ϵ) of 14,150 M⁻¹cm⁻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 thiol group content will be calculated based on the amount of TNB generated.

Application of the Product

- Purification of a small amount of biopolymer after a reducing or thiol labeling reaction.
- Assay the free thiol groups.

Key Feature of the Product

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

Kit Components

Each package is sufficient for 10 purification and 5 assays (100 μ L volume per assay).

Name	Part #	Quantity
Reagent (orange label)	CM13006	1 unit
Blank (yellow label)	CM13007	1 unit
Buffer A (blue label)	CM02018	12 mL
Solution A (green label)	CM01003	2 mL
Desalting Spin Column	CM03SG50	10
0.5 mL Centrifuge Tubes	N/A	10
1.5 mL Collection Tubes	N/A	10

Storage/Stability

Recommended storage of the kit is at 2-8°C. For reagent and blank dissolved in Buffer A, they can be aliquoted and stored at -20 °C up to 1 year.

Equipment (not provided)

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

General Protocol for Thiol Assay with Purification

1. Sample preparation:

Dilute the reaction mixture of reduced or thiol labeled biopolymer in **Buffer A** (blue label) to a total volume of **30 μL** with a final concentration of thiol groups in the 10–50 μM range.

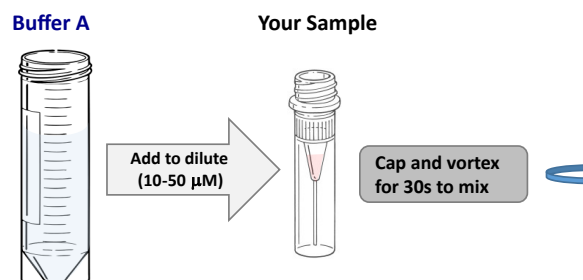
Cap and vortex for 30 s and then spin to ensure no liquid is in the cap.

Note: If it is an antibody with an average 4 free thiol groups per antibody, you can dilute to 2-3 mg/mL.

ADC kit sample preparation:

CM11409, CM11409x3
CM11422, CM11422x3
CM11425, CM11425x3
CM11416, CM11416x3

Pipette 6 μL of reaction mixture from Step C5 following the ADC user manual and mix with 24 μL of **Buffer A**.



2. Control preparation:

Dilute the reducing reagent or thiol-modification reagent that was used in the reaction using **Buffer A** to a total volume of **30 μL** .

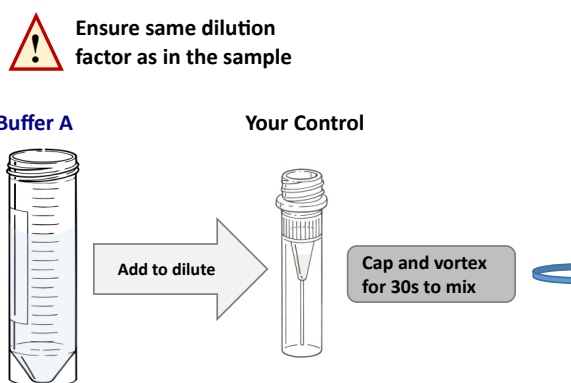
Cap and vortex for 30 s and then spin to ensure no liquid is in the cap.

Note: The final concentration of reducing reagent or thiol-modification reagent should be the same as the starting concentration in the sample preparation.

ADC kits control preparation:

CM11409, CM11409x3
CM11422, CM11422x3
CM11425, CM11425x3
CM11416, CM11416x3

Pipette 7.5 μL of **Reagent A solution** from **step C2** and mix with 300 μL of **Reducing buffer** following the ADC user manual. Next, pipette 6 μL of this diluted **Reagent A solution** and mix with 24 μL of **Buffer A**.

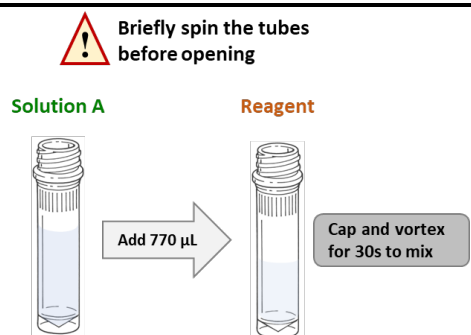


3. Reagent solution preparation:

Briefly spin the tubes containing **Solution A** (green label) and **Reagent** (orange label) before opening the tubes.

Pipette 770 μL of **Solution A** into the **Reagent tube**.

Cap and vortex the solution for 30 s.



4. Purification of sample and control:

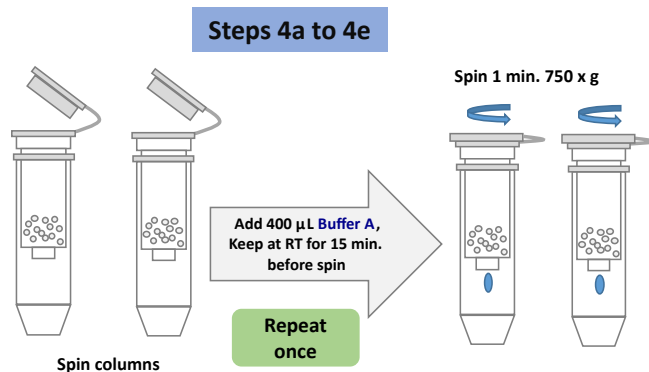
a. Take out two clean desalting spin columns, remove the bottom red cap. Spin for 1 min at 750 x g before opening the top cap.

b. Apply 400 μL of **Buffer A** to the top-center of the resin of each column, let it stay at room temperature (RT) for 15 min to swell the resin.

c. Spin for 1 min at 750 x g.

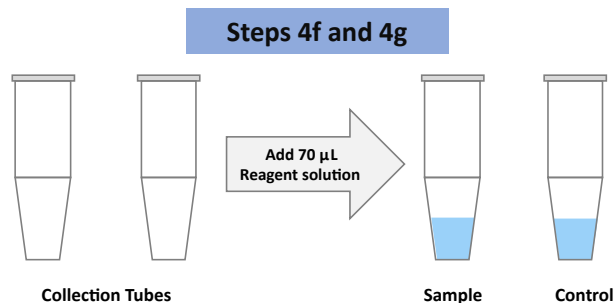
d. Discard the flow through.

e. Repeat steps b through d once.



f. Take out two clean 1.5 mL collection tubes and label one tube as sample and another as control.

g. Add 70 μL of **Reagent solution** to each tube.



h. Insert the spin columns into the collection tubes.

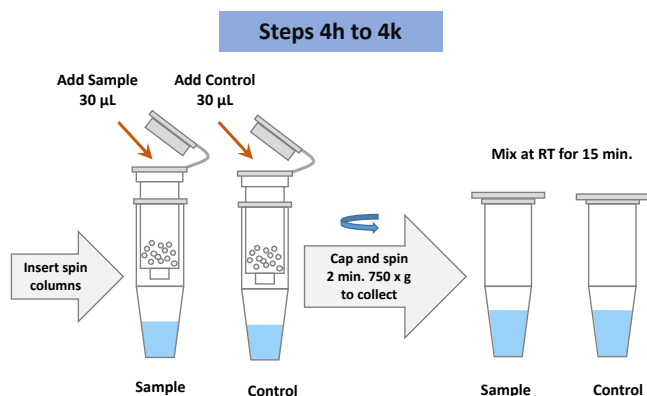
i. Slowly apply 30 μL of sample solution to the top-center of the resin of the spin column that is inserted into the sample tube without disturbing the resin bed. Next, apply 30 μL of control solution to the spin column that was inserted into the control tube.

j. Cap and spin for 2 min at 750 x g.

k. Remove the top filter and cap the collection tubes.

l. Mix the solution of the collection tubes at RT for 4-5 min.

Note: Aliquot and store the rest of the **Reagent solution** at -20°C for later use.



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**.

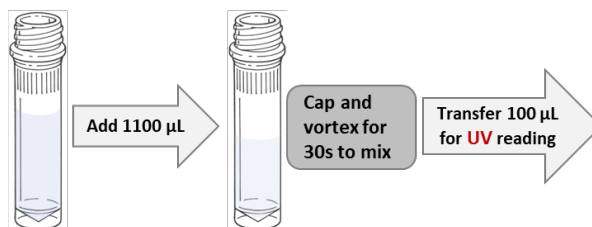
Cap and vortex the solution for 30 s, and then centrifuge to ensure no liquid is in the cap.

Pipette 100 μL for the UV reading.

Note: Aliquot and store the rest of the **Blank solution** at -20°C for later use.

Solution A

Blank



6. UV readings:

- **Zero** the spectrophotometer with **Blank** solution from **step 5** and Buffer A as reference at 412 nm

Ab (blank): 0

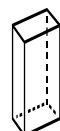
- Measure the UV absorbance of the **Control** from **step 4k** with Buffer A as reference at 412 nm.

Ac (control):

- Measure the UV absorbance of the **Sample** from **step 4k** with Buffer A as reference at 412 nm.

As (sample):

Reference Cell



Buffer A (CM02018)

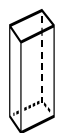


Blank Solution: **Step 5**

Sample Cell

Zero Spectrophotometer

Reference Cell



Buffer A (CM02018)

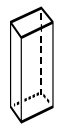


Control Solution: **Step 4K**

Sample Cell

Measure Ac

Reference Cell



Buffer A (CM02018)



Sample Solution: **Step 4K**

Sample Cell

Measure As

7. Calculate the concentration of thiol after dilution in Step 1

$\mu\text{M} =$

$$\mu\text{M} = 235.5713 \times (As - Ac)$$

Cuvette pathlength: 1 cm

8. Calculate the number of thiols per biopolymer (n)

based on the following formula:

$$n = \frac{\mu\text{M (Thiol)}}{\mu\text{M (Biopolymer)}}$$

n = _____

Where μM (Biopolymer) is the concentration of biopolymer after dilution in Step 1.

ADC kit antibody concentration after dilution in Step 1: 13

μM

CM11409, CM11409x3

CM11422, CM11422x3

CM11425, CM11425x3

CM11416, CM11416x3

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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