Amplite™ Rapid Colorimetric Protein Thiol Quantitation Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 5529 (2 Assays)	Multiple storage conditions Component A: -20°C. Do not freeze C, D, E	Absorbance readers

Introduction

It has been widely accepted that protein thiols are very important to protein structure, protein function and biological system redox environment. For example, albumin is the most abundant protein in plasma and the free thiol present in the albumin protein are considered as major plasma antioxidants in the body. The change of thiol status in albumin is related to a lot of diseases and disorders, such as kidney disease and Parkinson's disease. Although there are a few reagents or assay kits available for quantitating the total thiol content in biological systems, a key challenge is to have a rapid and accurate method to quantify the amount of free thiol group in a specific protein.

Amplite™ Rapid Colorimetric Thiol Quantitation Kit provides an accurate method to quantify free thiol group using our proprietary thiol sensor, Thiol Blue™, which has the maximum absorbance at ~680 nm. Thiol Blue™ reacts with the protein samples that contain free thiol groups. The resulted thiol adduct is run through a single spin column to remove the excess Thiol Blue™ sensor, and the absorption spectrum of the purified product is measured. The amount of thiol to protein ratio is calculated from the absorbance ratio of 680 nm and 280 nm. This Amplite™ Rapid Colorimetric Thiol Quantitation Kit can be performed in a traditional cuvette, NanoDrop™ Spectrophotometer or a convenient 96-well absorbance plate reader with a UV-transparent plate.

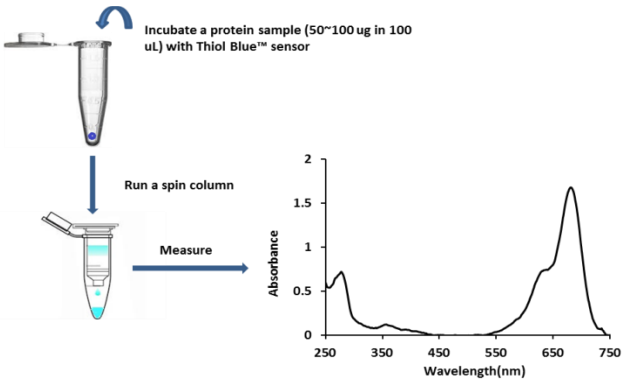


Figure 1. The Amplite™ Rapid Colorimetric Thiol Quantitation Assay Principle

Kit Components

Components	**Amount**	**Storage**
Component A: Thiol Blue™ sensor	2 vials (One vial is for 50 ~100 µg protein)	-20 °C
Component B: Assay Buffer	1 bottle (15 mL)	4 °C
Component C: Spin Column	2 columns	Do not freeze
Component D: Washing Tube (2 mL)	2 tubes	Do not freeze
Component E: Collecting Tube (1.5 mL)	2 tubes	Do not freeze

Assay Protocol for Spectrophotometer

Upon receipt, store Thiol Blue™ (Component A) at -20 °C, keep from light and moisture. When stored properly, the kit components should be stable for six months.

Note 1: Do not freeze Spin Column (Component C).

Note 2: Warm all the components before run the required assays. 50 to 100 μg protein sample is needed for determining the amount of thiol amount.

1. Prepare Sample Solution:

1.1 Use 50 to 100 μg protein sample.

1.2 Adjust the volume to 100 μL with Assay Buffer (Component B).

***Note**: The protein sample should be in pH=6.0 buffer and without DTT or other reagent containing free thiols.*

2. Run Thiol Assay:

2.1 Add the protein sample (from Step 1.2) to one vial of Thiol Blue™ (Component A).

2.2 Mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

2.3 Keep the reaction mixture at room temperature and rotate or shake for 30 - 60 minutes.

3. Prepare Spin Column for Sample Purification:

3.1 Invert the Spin Column (Component C) several times to resuspend the settled gel and remove any bubbles.

3.2 Snap off the tip and place the column in the Washing Tube (2 mL, Component D). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube (not provided).

3.3 Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

3.4 Apply 1 mL Assay Buffer (Component B) to the column, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.

3.5 Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the reaction buffer. Discard the buffer.

Note 1: 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.

Note 2. 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 the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the equation (1) to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.

$$RCF(g) = (1.12 \times 10^{-5}) \times (RPM)^2 \times \mathrm{r} \qquad (1)$$

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

4. Purify Reaction Product:

4.1 Place the column (from Step 3.5) in a clean Collecting Tube (1.5 mL, Component E). Carefully load the sample (100 μL) directly to the center of the column.

4.2 After loading the sample, add 10 μL Assay Buffer (Component B) to the top and centrifuge the column for 5 min at 1,000 xg, and collect the solution into the collecting tube.

5. Run Absorption Spectra with 0.2mL or 0.5 mL Quartz Cuvette

5.1 Dilute the reaction product (from Step 4.2) by 5-folds with Assay Buffer (Component B) depending on the cuvette size used and the absorbance reading.

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

5.2 Measure the absorption spectrum from 250 to 750 nm, or only read the absorbance number at 280 nm and 680 nm.

Data Analysis

For illustrating purpose we use a BSA as an example to calculate the number of thiol groups on BSA with NanoDrop™ Spectrophotometer.

Sample: BSA, 10 mg/mL in pH=6.0 Buffer

Procedures:

1. Use 10 µL (100 µg) of BSA, and then add 90 µL Assay Buffer (Component B) to have total volume of 100µL.
2. Add above 100µL solution to Thiol Blue™ vial (Component A), mixed well.
3. Rotate for 60 min at room temperature.
4. Purify with Spin Column (Component C), and collect the product.
5. Take 2~3 µL of the product and measure the absorbance spectra.

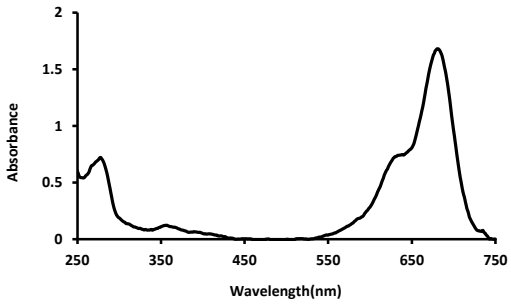
Constants needed:

BSA extinction coefficient at 280 nm: 43824 M^{-1} cm^{-1}

Thiol Blue™ extinction coefficient at maximum absorption (680 ± 3nm): 250,000 M^{-1} cm^{-1}

Correction Factor of Thiol Blue™ at 280nm (CF_{280nm}): 0.101

Results:



OD readings obtained with the above BSA sample: A_{280nm} = 0.699, A_{680nm} = 1.678

Thiol Calculation:

$$\frac{Moles\ of\ Thiol}{\text{Moles of protein}} = \frac{[A_{680nm}]/\varepsilon_{\text{Thiol Blue™}}}{(A_{280nm} - CF_{280nm} \times [A_{680nm}])/\varepsilon_{\text{protein at 280nm}}} \qquad (2)$$

Calculate thiol amount with Equation (2):

Thiol/BSA = (1.678/250000)/(0.699-1.678×0.101)/43824 = 0.56

Warning: This kit is only sold to our authorized distributors and end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.