Version 2 Last updated 13 February 2019

ab219272
Total Protein Thiol Quantitation Assay Kit (Colorimetric)

For the quantitative detection of Total Protein Thiols in plasma.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

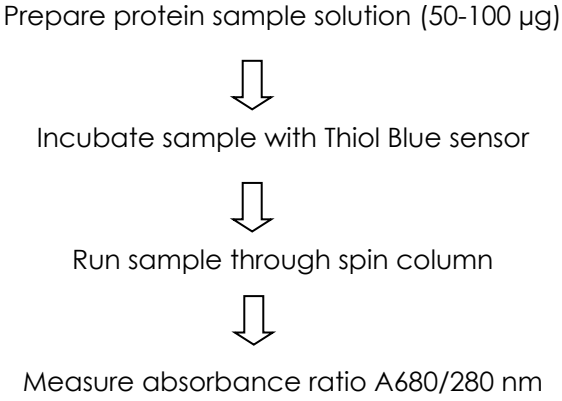
1. Overview

Total Protein Thiol Quantitation Assay Kit (Colorimetric) (ab219272) provides an accurate method to quantify free thiol groups in proteins. The assay uses our proprietary thiol sensor, Thiol Blue, which has the maximum absorbance at OD 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.

The assay can be performed in a traditional cuvette, Spectrophotometer or a convenient 96-well absorbance plate reader with a UV-transparent plate.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C, except Thiol Blue that should be stored at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Thiol Blue	2 x 1 vial	-20°C
Assay Buffer	15 mL	4°C
Spin Column	2 units	4°C
2 mL Washing Tube	2 units	RT
1.5 mL Collecting Tube	2 units	RT

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Spectrophotometer capable of measuring absorbance at A280nm and A680nm (i.e., NanoDrop spectrophotomer)
- 0.2 mL or 0.5 mL Quartz cuvettes
- Centrifuge with swing buckets capable of reaching 1,000 x g
- Optional: 12 x 75 mm test tubes

If performing assay in a microplate reader:

- Microplate reader capable of measuring absorbance at A280nm and A680nm
- 96-well UV transparent plate

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Thiol Blue (2 vials):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C away from light and moisture.

9.2 Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.3 Spin column (2 columns):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. Do not freeze.

9.4 2 mL Washing tube (2 tubes):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. Do not freeze.

9.5 1.5 mL Collecting tube (2 tubes):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. Do not freeze.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents as directed in the previous sections.
- Protein sample should be in pH 6.0 and without DTT or reagent containing free thiols or cell debris.
- The protocol described here is for 0.2 mL / 0.5 mL Quartz cuvettes.

Δ Note: DTT or other reagents containing free thiols will interfere with the assay. Ensure your sample does not contain interfering substances.

10.1 Prepare Sample Solution:

10.1.1 Use 50-100 µg protein sample.
10.1.2 Adjust the volume to 100 µL with Assay Buffer.

10.2 Run Thiol Assay:

10.2.1 Add the protein sample (from Step 10.1) to one vial of Thiol Blue (Step 9.1).
10.2.2 Mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
10.2.3 Keep the reaction mixture at room temperature and rotate or shake for 30-60 minutes.

10.3 Prepare Spin Column for Sample Purification:

10.3.1 Invert the Spin Column (Step 9.3) several times to resuspend the settled gel and remove any bubbles.
10.3.2 Snap off the tip and place the column in the 2 mL Washing Tube (Step 9.4).

Δ Note: Spin Column 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.

10.3.3 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.

10.3.4 Discard the drained buffer, and then place the column back into the Washing Tube. If column is placed into a 12 x 75 mm test tube instead, centrifuge immediately.
10.3.5 Centrifuge tube for 1 minute in a swinging bucket centrifuge at 1,000 x *g* to remove the packing buffer. Discard the buffer.
10.3.6 Apply 1 mL Assay Buffer to the column, let the buffer drain out by gravity, or centrifuge the column for 1 minute to remove the buffer. Discard the buffer from the collection tube.
10.3.7 Repeat Step 10.3.6 again 3-4 times.
10.3.8 Centrifuge column for 2 minutes in a swinging bucket centrifuge at 1,000 x *g* to remove the reaction buffer. Discard the buffer.

Δ Note: Swinging bucket centrifuges capable of generating a minimum force of 1,000 x *g* are suitable for 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 following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x*g*.

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

RCF = relative centrifugal force

RPM = speed of the rotor

r = radius (cm) measured from the center of the rotor to the middle of the spin column

10.4 Purify Reaction Product:

10.4.1 Place the column (from Step 10.3.8) in a clean 1.5 mL Collecting Tube.
10.4.2 Carefully load the sample (100 µL volume) directly to the center of the column.
10.4.3 After loading the sample, add 10 µL Assay Buffer to the top section and centrifuge the column for 5 minutes at 1,000 x*g*.
10.4.4 Collect the flow-through solution into the collecting tube.

10.5 Measurement in Quartz cuvette (0.2 mL or 0.5 mL):

10.5.1 Dilute the reaction product (from Step 10.4.4) 5-fold with Assay Buffer.

Δ Note: the amount of Assay Buffer to add will depend on the cuvette size used and the absorbance reading.

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

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

11. Calculations

– Before proceeding with the calculation of thiols in the sample, you will need to know the following constants:

Protein extinction coefficient at $A_{280\ nm}$ (ε protein)

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

Correction Factor of Thiol Blue at $A_{280\ nm}$ (CF_{280nm}) = 0.101

– Amount of Thiol amount in the protein sample can be calculated as:

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

For illustrating purposes, we will use BSA as an example to calculate the number of thiol groups present on BSA using a NanoDrop spectrophotometer.

Sample (BSA): 10 mg/mL BSA in pH 6.0 buffer

Following assay procedure described in Section 10:

– Use 10 µL (100 µg) of BSA and then add 90 µL Assay Buffer for a total volume of 100µL.
– Add 100µL solution to Thiol Blue vial to the above and mix well.
– Rotate for 60 minutes at RT.
– Purify with Spin Column, and collect the product.
– Take 2-3 µL of the product and measure the absorbance spectra (figure 1).

Calculations:

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

Calculate thiol amount with Equation:

$$\frac{Thiol}{BSA} = \frac{[1.678] / 250{,}000}{(0.699 - 1.678 \times 0.101)/43824} = 0.56$$

12. Typical Data

Typical standard curve – data provided **for demonstration purposes only.**

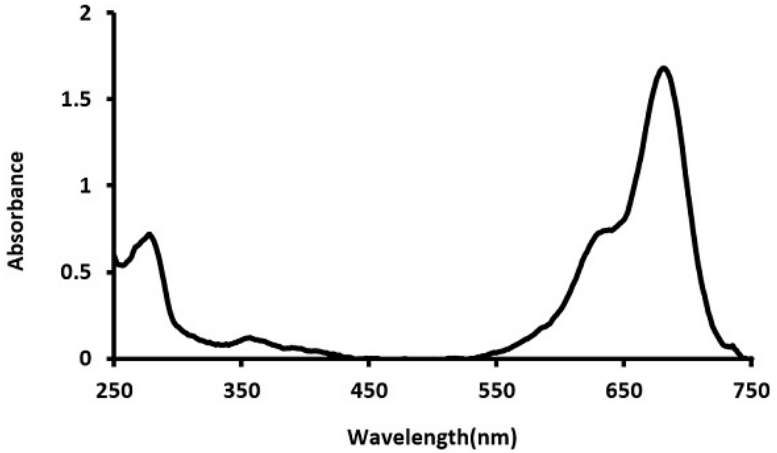


Figure 1. OD readings obtained with BSA sample as described in section 11: A280nm = 0.699, A680nm = 1.678

13. Notes

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