

Thiol and Disulfide Assay Kit

KB-03-007

200 tests (96 well plate)

BOCKit

A brand of  **BioQuoChem**

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All chemicals should be handled with care

➤ This kit is for R&D use only

Introduction

Biologic systems contain redox elements, which function in cell signaling, macromolecular trafficking and physiologic regulation. Oxidative stress includes disruption of this redox circuitry through altered functions of enzymes, receptors, transporters, transcription factors, and structural elements, in addition to the macromolecular damage, both resulting from an imbalance between pro-oxidants and antioxidants performances.

Since many proteins contain redox-sensitive free thiols, the identification and quantification of their different redox states gives us an idea of the oxidative stress level of the sample.

The present assay is based on the classic colorimetric one, first described by Ellman in 1958, and aimed at the detection of reduced thiols, but modified in order to allow the detection of those oxidated to disulfides as well.

Materials

BQCKit Thiol and Disulfide Assay kit *KB03007-200 tests* contains:

Product	Quantity	Storage
Thiol and Disulfide Reagent A*	1 vial	4°C
Thiol and Disulfide Reagent B	2 vials (powder)	RT
Thiol and Disulfide Reagent C*	1 bottle	4°C
Thiol and Disulfide Reagent D*	2 vials (powder)	4°C
Thiol and Disulfide Standard*	2 vials (powder)	4°C

* These reagents are stable during 10 days at Room temperature and are shipped in these conditions. Once received is recommended to keep them at 4°C.

Assay Principle

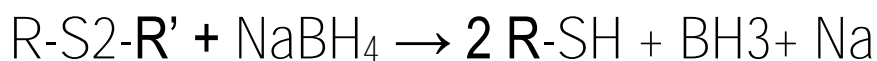
Bioquochem Thiol and Disulfide Assay Kit is recommended for estimations of oxidative stress levels in biological samples such as plasma.

The assay described here measures the formation of 2-nitro-5-thiobenzoate (TNB), which is proportional to the amount of reduced thiols in the sample that are oxidized by the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) through a non-specific reaction. The generated TNB ionizes to a dianion at alkaline pH and thus develops an intense yellow color with an absorbance maximum at 412 nm (Scheme 1).

On the other hand, the assay also allows the measurement of disulfides in the sample, thanks to NaBH_4 , which reduces these to thiols (Scheme 2).



Scheme 1. *Reaction of DTNB with thiol groups*



Scheme 2. *Reduction of disulfides by NaBH_4*

Reagents Preparation

Thiol and Disulfide Reagent B:

Add exactly 2.5 mL of ultrapure water- methanol (50%) to the provided vial. This reagent is not stable: prepare daily and discard after use. The kit includes two vials for the 192 assays. Use one vial for 100 assays.

Thiol and Disulfide Reagent D*:

Add exactly 2.5 mL of methanol to the provided vial. This reagent is not stable: prepare daily and discard after use. The kit includes two vials for the 192 assays. Use one vial for 100 assays.

Thiols and Disulfide Standard:

Add exactly 2 mL of purified H₂O to the provided vial for a final concentration of 10 mM and dilute 1:10 for a final concentration of 1 mM. Then prepare different dilutions as shown below in Table 1.

Sample Preparation

Tissue homogenate



Rinse tissue with PBS (pH 7.4).



Homogenize in 5-10 mL of cold TRIS buffer/g tissue.



Centrifuge at 10,000 x g for 15 min at 4°C.



Collect the supernatant to assay or freeze.

Cell lysate



Centrifuge sample at 1,000-2,000 x g for 10 min at 4°C. Do not use proteolytic enzymes.



Homogenize/sonicate cell pellet with 1-2 mL of cold buffer.



Centrifuge at 10,000 x g for 15 min at 4°C.



Collect the supernatant to assay or freeze.

Plasma



Centrifuge blood simple (with anticoagulant) at 700-1,000 x g for 10 min at 4°C.



Collect the supernatant to assay or freeze.

Assay Protocol

Standard preparation

Oxidative stress levels are expressed as free thiols values. These are related to GSH concentration. Prepare calibration curve in 1 mL tubes.

Table 1. *Reagent volumes needed to carry out the standard curve*

Sample	Standard [μL]	Diluent Purified H_2O [μL]	Free Thiols (mM)
S1 (Blank)	0	100	0
S2	20	80	0.2
S3	40	60	0.4
S4	60	40	0.6
S5	70	30	0.7
S6	80	20	0.8
S7	90	10	0.9
S8	100	0	1

Assay Protocol

Performing the Assay

It is possible to calculate both the native (naturally reduced) and total (chemically reduced) free thiols in each sample.

For the native free thiols:

1. Add 20 μL of sample/standard.
2. Add 20 μL of Thiol and Disulfide Reagent A and wait 10 min.
3. Add 220 μL of Thiol and Disulfide Reagent C and 20 μL of Thiol and Disulfide Reagent D. Wait 10 min.
4. Read the absorbance at 412 nm.

For the total free thiols:

1. Add 20 μL of sample.
2. Add 20 μL of Thiol and Disulfide Reagent B and wait 10 min.
3. Add 220 μL of Thiol and Disulfide Reagent C and 20 μL of Thiol and Disulfide Reagent D. Wait 10 min.
4. Read the absorbance at 412 nm.

Data Analysis

1. Zeroed the absorbance values:

$$\Delta A_{412 \text{ nm}} = A_{412 \text{ nm sample/standard}} - A_{412 \text{ nm blank}}$$

2. Plot the zeroed absorbance ($\Delta A_{412 \text{ nm}}$) of standards as a function of their final concentrations (Table 1). See Figure 2 for a typical standard curve.
3. Calculate the free thiols value of the samples using the equation obtained from the linear regression of the standard curve substituted $\Delta A_{412 \text{ nm}}$ values for each sample.
4. The reduced thiols concentration in the sample is then the free thiols value calculation for the native free thiols assay.
5. The disulfides concentration in the sample results from the difference between the free thiols value calculations for the total free thiols assay and the native free thiols assay.

$$\text{Free Thiols (mM)} = (\Delta A_{412 \text{ nm}} - \text{intercept}) / \text{slope}$$

Data Analysis

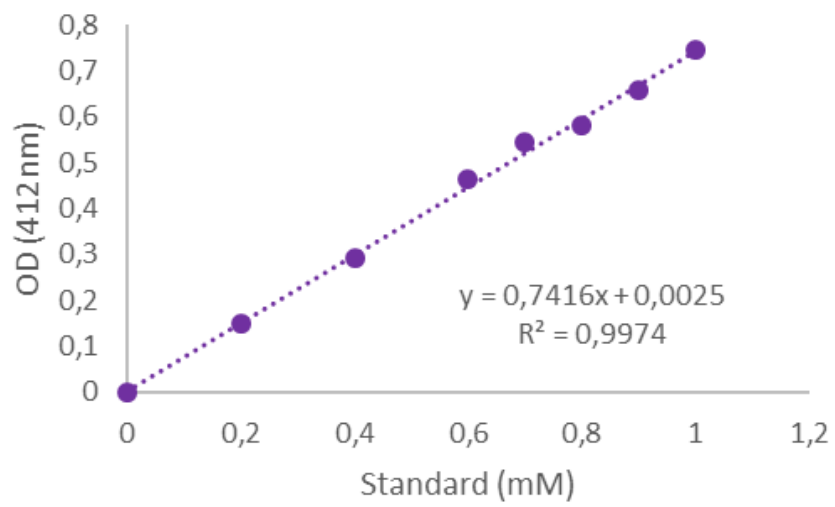


Figure 2. *Typical standard curve for Thiol assay*

Warranties and Limitation of Liability

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Buyer's exclusive remedy and Bioquochem's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery