Thiol & Disulfide Quantification Assay

(Cat. # BAQ080)

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 oxidized to disulfides as well.

G-B iosciences' 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.

R-SH + DTNB → R-TNB + TNB (Yellow) (λMax = 412 nm)

The assay also allows the measurement of disulfides in the sample. Sodium borohydride ($NaBH_4$) is used to reduce disulfides to thiols.

R-S2-R' + $NaBH_4$ → 2 R-SH + BH_3+ Na

ITEM(S) SUPPLIED

Product	Quantity	Storage
Reagent A	1 vial	4ºC
Reagent B	1 vial	RT
Reagent C	2 bottles	4ºC
Reagent D	2 vials	4ºC
Reagent E	1 bottle	4ºC
Standard (GSH)	2 vials	4ºC

STORAGE CONDITIONS

This kit is shipped at ambient temperature. Store all the reagents as indicated on the labels. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure 450 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

PREPARATION BEFORE USE

TDA Reagent B

Add exactly 2 mL of reagent E and 2 ml of H2O ultrapure to the reagent B. This reagent is not stable: prepare daily and discard after use.

TDA Reagent D

Add exactly 2.5 mL of reagent E to the provided vial. This reagent is not stable: prepare daily and discard after use. The kit includes two vials for the 200 assays and four vials for the 400 assays. Use one vial for 100 assays.

Thiols and Disulfide Standard

Add 2 mL of purified H_2O 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.

Standard preparation

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

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

SAMPLE PREPARATION

Tissue Homogenate

1. Rinse tissue with PBS
2. Homogenize in 5-10ml cold Tris buffer for every gram of tissue
3. Centrifuge at 10,000xg for 15 minutes at 4°C
4. Collect the supernatant to assay or freeze at -80°C

Cell Lysate

1. Collect the cells by centrifugation at 1,000-2,000xg for 10 minutes at 4°C. Do not use proteolytic enzymes.
2. Homogenize of sonicate the cell pellet with 1-2ml cold buffer
3. Centrifuge at 10,000xg for 15 minutes at 4°C
4. Collect the supernatant to assay or freeze at -80°C

Plasma

1. Centrifuge blood with an anticoagulant at 700-1,000xg for 10 minutes at 4°C
2. Collect the supernatant to assay or freeze at -80°C

PROTOCOL

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

For the native free thiols:

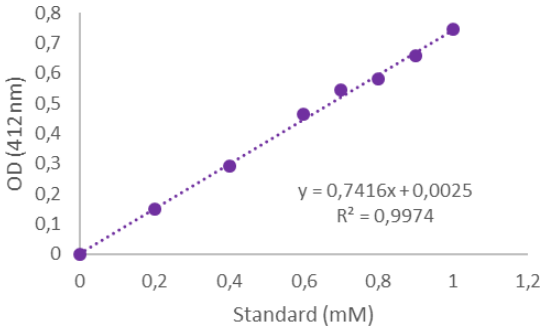
1. Add 20 µL of sample/standard.
2. Add 20 µL of TDA Reagent A and wait 10 min.
3. Add 220 µL of TDA Reagent C and 20 µL of TDA Reagent D. Wait 10 min.
4. Read the absorbance at 412 nm.

For the total free thiols:

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

DATA ANALYSIS

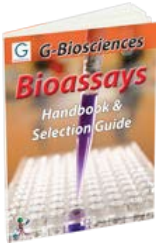
1. Zero the absorbance values:
   ΔA412 nm = A412 nm sample/standard – A412 nm blank
2. Plot the zeroed absorbance (ΔA412 nm) of standards as a function of their final concentrations.



3. Calculate the free thiols value of the samples using the equation obtained from the linear regression of the standard curve substituted ΔA412 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.
   Free Thiols (mM) = (ΔA412 nm – intercept) / slope

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

www.GBiosciences.com