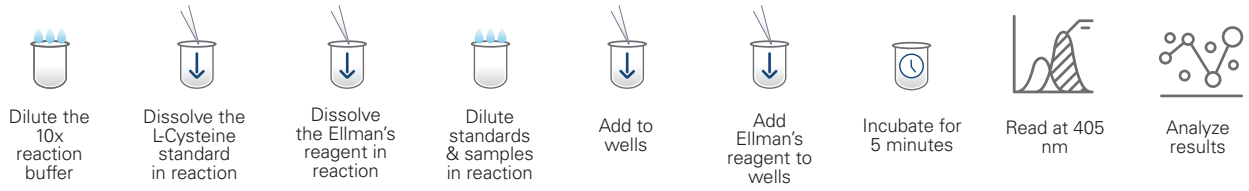
DTNB (ELLMAN'S) THIOL OXIDATIVE STRESS



DESCRIPTION

The DTNB-Thiols assay kit measures sulfhydryl groups with the thiol reagent 5,5-dithiobis(2-nitrobenzoic acid), (DTNB), which forms the 5-thionitrobenzoic acid and a mixed disulfide. Under conditions of oxidative stress, free sulfhydryls decrease and disulfides increase. Determination of the free thiol concentration in biological samples reflects the ability to detoxify lipid and other peroxides. Ethos Biosciences DTNB-Thiols assay can be used with a spectrum of biological samples including body fluids, tissue, and cell specimens.

To complete the assay, aliquots of standard or sample are reacted with rehydrated DTNB-Ellman's Reagent in microtiter wells. Absorbance is read at 405 nm using a reference filter of 630 nm. Concentration in samples is determined from the standard curve.

KIT CONTENTS

- DTNB Reaction Buffer (RB): 10 x Tris buffer that must be diluted before use
- L-Cysteine Standard: 12 mg of L-Cysteine that is dissolved in 10 mL of RB for the assay
- Ellman's Reagent: 12 mg of DTNB that is dissolved in 30 mL of RB for use
- Microtiter Plate: 96-well plate to complete the assay

MATERIALS & EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Deionized water
- 100 mL graduated cylinder
- Disposable 5, 10 and 25 mL pipets
- Pipetting Aid
- Dilution Tubes: i.e. 12 x 100 mm disposable polystyrene tubes
- Suitable Tube Support
- Micro-pipettors/tips to deliver 50, 500 and 1000 uL
- Multi-channel Pipettor/tips capable of delivering 200 uL
- Pipetting reservoir
- Microplate Reader

INITIAL PROCEDURES

1. Dilute the 10X DTNB Reaction Buffer by adding 100 mL of deionized water to the bottle
2. Install the cap securely and gently shake the bottle
3. Secure a 10 mL pipet
4. Transfer 10 mL of RB to the L-Cysteine Standard Bottle
5. Install the cap securely and gently shake the bottle to dissolve the material. The concentration of L-Cysteine will then be 10 mM.
6. Secure a 25 mL pipet.
7. Transfer 30 mL of RB to the Ellman's Reagent Bottle
8. Install the cap securely, and shake to fully dissolve the material

Dilute the Standard:

1. Set-up the tube support with 8 tubes labeled 0-7
2. Using a 5 mL pipet, add 2 mL of the Reaction Buffer to the tube marked "0"
3. With a micropipette; transfer 500 uL of 10 mM L-Cysteine to the tube marked "0"
4. Mix by vortexing. This tube now contains 2 mM L-Cysteine
5. Using a micropipette, transfer 1.0 mL volumes of Reaction Buffer to tubes 1-7
6. With a fresh tip; transfer 1 mL from Tube 0 to tube 1 and mix by aspirating/expelling the fluid 5x
7. Using the same tip, transfer 1 mL from tube 1 to tube 2, and mix as before
8. Continue this serial dilution of standard through to the last tube...in order.

Dilute the Samples:

1. Samples should be diluted in RB
2. Proper dilution must be determined by the user
3. The RB has a pH of 8.5, and diluting the sample in it may cause turbidity
4. These samples may be clarified by centrifugation

ASSAY PROCEDURE

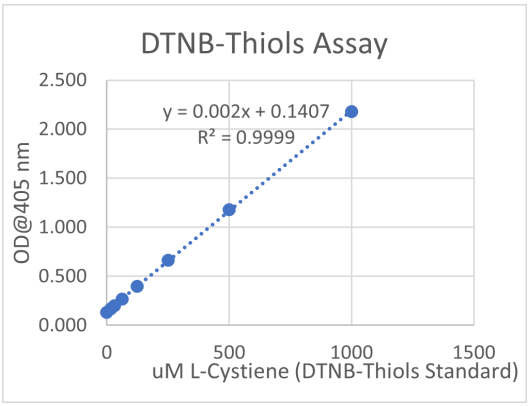
1. Prepare an assay map that shows placement of controls, standard dilutions and samples; Columns 1 and 2 are reserved for the controls and standard dilutions, and the balance of the plate for samples
2. Secure the microtiter plate
3. Using a micropipette; add 50 uL of RB to wells A1 and A2 (A1,2). These wells serve as negative controls, and can be used to "Blank" the plate reader
4. Using the same tip, pre-wet the tip in fluid of tube 7, and transfer 50 uL of diluted standard to wells H1,2
5. Using the same tip, pre-wet the tip in the fluid of tube 6; transfer 50 uL to wells G1,2
6. Continue this procedure; Tube 5 to wells F1,2, Tube 4 to wells E1,2 and so forth
7. The standard curve is present in wells B1,2-H1,2. It includes serial 2-fold dilutions from 1 mM to 0.0156 mM
8. Add diluted samples to the plate, but a fresh tip must be used for every diluted sample
9. Secure the Ellman's Reagent, and pour into a pipetting reservoir
10. Adjust the multi-channel pipettor to deliver 200 uL
11. Equip with fresh tips
12. Pre-wet the tips in Ellman's Reagent, and transfer 200 uL volumes to all of the wells of the plate
13. Color development is nearly immediate, but incubate for 5 minutes at room temperature
14. Determine Optical Density (Absorbance) with a microplate reader at 405 nm with reference filter at 630 nm
15. A1 or A2 are used to "Blank" the machine
16. Calculate the amount of DTNB-Thiols

DATA ANALYSIS

1. Determine the least squares regression line using absorbance versus DTNB-Thiols concentration for each standard.
2. Determine the concentration of diluted samples by substituting the respective absorbances appropriately.
3. Multiply these values by the dilution factor to obtain concentration in undiluted samples.

TYPICAL DATA

As the OD values of the standard curve may vary according to the conditions and timing of the actual assay performance, the operator should establish a standard curve for each test. A typical standard curve is provided below for reference only.



STABILITY OF REAGENTS

Reconstituted Ellman's Reagent and DTNB Thiols Standard are not stable and should be discarded after 1 day.

Revision 4.5 June 2022

PRODUCT INFORMATION

Cat. #	Description
1021	DTNB Thiols Oxidative Stress Assay

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