DTNB-Thiols Assay Kit

Cat. No. Kit-0311 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	DTNB-Thiols Assay Kit is used for the quantitiation of Sulfhydryls (DTNB-Thiols).
Description	The DTNB-Thiols assay kit measures sulfhydryl groups with the thiol reagent 5-5dithiobis[2nitrobenzoic acid] (DTNB), which forms the 5-thionitrobenzoic acid and a mixed disulfide. Under conditioins 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. Exocell's 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.
Stability	Reconstituted Ellman's Reagent and DTNB Thiols are not stable and should be discarded after 1 day.
Storage	Store this kit at 4°C.
Size	96 tests
Kit Components	96 well microtiter plate (1) DTNB (Ellman’s Reagent) Ellman’s Reagent Diluent

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	DTNB Thiols (L-Cysteine Standard 10.0 mg vial) DTNB Thiols Assay Diluent
Materials Required but Not Supplied	1. Eppendorf Tubes 2. Eppendorf Centrifuge for clarification of samples 3. Pipettes capable of delivering 50 ul, 100 ul, and 200 ul of sample 4. Serological Pipettes (1 ml and 5 ml) 5. Tube to hold 5 ml 6. Microplate Reader
Preparation	1. Dissolve Ellman's Reagent in 20 ml of Ellman's Reagent Diluent by adding 1.0 ml to vial, mix thoroughly, and transfer back to Diluent bottle. 2. Dissolve DTNB Thiols (L-Cysteine Standard) in .58 ml of DTNB Thiols Assay Diluent. Make a 1:50 Standard Stock by adding 100 ul of DTNB (L-Cysteine Standard) to 4.9 ml of DTNB Thiols Assay Diluent. 3. The DTNB Thiols standard curve is prepared by adding 200 ul DTNB Thiols Assay Diluent into 7 standard eppendorf tubes labeled #1 to #7. Add 200 ul of DTNB Thiols L- Cysteine Stock solution to tube #1, mix thoroughly and remove 200ul to tube # 2, mix thoroughly and remove 200ul to tube # 3, and so on until tube #7. 4. In the assay the 7 standards will contain 1000, 500, 250, 125, 62.5, 31.25, and 15.6 uM, respectively. 5. Prepare samples by diluting in DTNB Thiols Assay Diluent, if necessary, and clarifying by centrifugation.
Assay Protocol	1. In microtiter plate, add 50 ul of DTNB Thiols Assay Diluent to wells A1 and A2. Add 50 ul of standard from tube #1 to wells B1 and B2, continue adding standard #2 to wells C1 and C2 and so on until you end up adding standard #7 to wells H1 and H2. 2. Add 50 ul of clarified sample #1 to A3 and A4 and continue adding samples, in duplicate, to fill up the plate. 3. Add 150 ul of Ellman's Reagent to each well.

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	4. Plate can be read immediately but incubate no longer then 10 minutes before reading. 5. Read in a microplate reader at 405 nm with reference filter at 630 nm. 6. Measure absorbance with A1 or A2 being used as blank. 7. Calculate amount of DTNB-Thiols.
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.

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