G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Ellman's Reagent

5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB)

(Cat. # BC87)

INTRODUCTION

Ellman's reagent, 5, 5'-dithio-*bis*-(2-nitrobenzoic acid), also known as DTNB, is a versatile water-soluble compound for quantitating free sulfhydryl groups in solution. DTNB reacts with a free sulfhydryl group to yield a mixed disulfide and 2-nitro-5-thiobenzoic acid (NTB), a measurable yellow-colored product with molar extinction coefficient of 14.15 mM^{-1} cm^{-1}at 412 nm. DTNB is very useful as a sulfhydryl assay reagent because of its specificity for -SH groups at neutral pH, high molar extinction coefficient and short reaction time.

PROPERTIES

- **Chemical Name:** 5, 5'-Dithio-*bis*-(2-nitrobenzoic acid)
- **Molecular Formula:** $C_{14}H_8N_2O_8S_2$
- **Molecular Weight:** 396.3
- **CAS#:** 69-78-3

ITEM(S) SUPPLIED (Cat. #: BC87)

Description	Size
Ellman's Reagent	5g

STORAGE CONDITION

Shipped at ambient temperature. Store at room temperature upon receiving. Stable for up to 2 years.

PROTOCOL

1. Make 10mM DTNB stock solution by dissolving 40mg DTNB in 10ml DMSO. The stock solution can be stored at 4°C for 3 months. Dilute the stock solution 100 fold with 0.1M Tris-HCl pH 7.5 to make 0.1mM DTNB working solution.
2. Aliquot 950µl of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50µl test sample and mix by brief vortexing. Set a blank by adding 50µl of 0.1M Tris-HCl pH 7.5 to 950µl of 0.1mM DTNB work solution.
   NOTE: *The test sample may need to be diluted before applied to the assay and the dilution factor should be recorded. The 50µl test sample applied to the assay reaction should have a sulfhydryl concentration less than 0.5mM. Concentrations exceeding 0.5mM free sulfhydryl will result in high absorbance values and less accurate estimation of the concentration based on the extinction coefficient.*
3. Incubate 2 minutes at room temperature.
4. Measure the absorbance of the test sample with a spectrophotometer against blank at 412nm.

CALCULATION

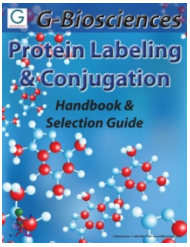
Calculate the concentration of free sulfhydryls in the sample from the molar extinction coefficient of NTB (14.15 mM^{-1} cm^{-1}) as follow:

mM free sulfhydryls = Absorbance / (path length x 14.15) x 20 x dilution factor

NOTE: *Path length is the cuvette path length in centimeters (cm)*
20 is the dilution factor of 50µl sample to 1ml assay volume

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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

Last saved: 8/14/2012 CMH

www.GBiosciences.com