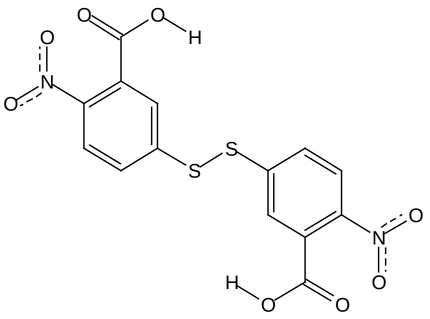
ProductInformation

5,5'-DITHIO-BIS(2-NITROBENZOIC ACID)

Product Number **D8130**

CAS #: 69-78-3
Synonyms: DTNB; Ellman's Reagent; 3,3'-dithio-bis(6-nitrobenzoic acid), 3-Carboxy-4-nitophenyl disulfide; 2,2'-Dinitro-5,5'-dithiobenzoic acid

Product Description



Appearance: Yellow powder
Molecular formula: $C_{14}H_8N_2O_8S_2$
Molecular weight: 396.3
Melting point: 237-238°C, with decomposition[1]
Extinction coefficient at 412 nm:[2]
14140 at pH 8 in phosphate buffer
14290 in 8 M urea
13880 in 6 M guanidine HCl

Frequently called Ellman's Reagent or DTNB, this was originally developed for use as a sulfhydryl reagent. D8130 is tested for suitability for use as a reagent in sulfhydryl group determination.[2] This aromatic disulfide reacts with aliphatic thiol groups to form a mixed disulfide of the protein and one mole of 2-nitro-5-thiobenzoate per mole of protein sulfhydryl group. DTNB has little if any absorbance, but when it reacts with -SH groups on proteins under mild alkaline conditions (pH 7-8), the 2-nitro-5-thiobenzoate anion (TNB^{2-}) gives an intense yellow color at 412 nm.[3]

The optimal molar extinction coefficient value for TNB^{2-} has been somewhat controversial. The original work by Ellman[4] cited a value of 13,600 M^{-1} cm^{-1}. Since this initial reference, values ranging from 11,400 to 14,150 M^{-1} cm^{-1} have been reported for TNB^{2-}. In a detailed study, the following comments were made:

The molar absorption coefficient of purified TNB^{2-} at 412 nm is 14,150 M^{-1} cm^{-1} and the absorption maximum is at 409.5 nm in dilute salt solutions (0.1 M NaOH or 0.1 M phosphate buffer with 1 mM EDTA, pH 7.27). In 6 M guanidinium chloride the spectrum of TNB^{2-} is shifted slightly (lambda max. = 421 nm) so that molar absorption coefficient at 412 nm is 13,700 M^{-1} cm^{-1}. Similar shifts and changes in molar absorptivity occur in 8 M urea and in 3 M NaOH. The spectrum of TNB $^{2-}$ is apparently sensitive to the presence of 2% (w/v) sodium dodecyl sulfate. At pH 7.27, 99.8% of TNB is in the form of the intensely colored dianion (TNB^{2-}), while the rate of decomposition due to hydroxide ion is minimal.[5,6]

In a novel application, DTNB was used as a cleavable cross-linking agent. A modified insulin was treated with DTNB, then was connected to glucose oxidase in the presence of the carbodiimide (E1769). When glucose was added to an aqueous solution containing this hybrid enzyme, the modified insulin was released (together with the modified glucose oxidase enzyme). Insulin release was specific to the glucose.[7]

An alternative method for determination of sulfhydryl group number involves the use of 4-vinylpyridine (V3877). The DTNB assay has a number of problems, including pH-induced disulfide disruption[8], reoxidation of the R-SH groups and thermochromic effects. Ellman's reagent is sensitive to various buffer ions, so the extinction coefficient used to calculate the number of sulfhydryl groups must be matched to the reaction conditions; if the sulfhydryl groups are in disulfide bonds, they must be reduced by sodium borohydride under anaerobic conditions prior to reaction with DTNB. Ming et al. discusses advantages and disadvantages of both assay methods.[9]

Applications and additional references:
Determination of free sulfhydryl and disulfide groups in alpha-amylase, papain, and lysozyme[10,11,12]
Disulfide determination in peptides[13]
Hydrogen sulfide determination[14]
HPLC determination of alkylthiol using DTNB; EDTA to reduce metal ion interference[14,15]
Molar absorptivity studies[2,6,10]
Sulfhydryl measurements in blood, urine, tissue, and purified proteins[1,16]
Studies with calf thymuse histone H3[17,18]

Preparation Instructions

DTNB is soluble in ethanol at 8 mg/mL, giving a clear yellow to yellow-green solutions. However, approximately 5 mM DTNB can be made in about a 100 mM sodium or potassium phosphate buffer solution, pH 7.2 containing 0.1 mM EDTA. (This should be stable for greater than 2 months if stored in the dark between 0-5°C, possibly up to 6 months.)

For use in one Sigma enzyme assay, a 1.7 mM stock solution of D8130 is prepared just before use in deionized water, titrated into solution by the addition of a minimal volume of 0.1 M NaOH. A very pale yellow solution is obtained.

The stability of DTNB decreases with increased hydroxide concentration. One study reported for room temperature solutions in water, 0.02% decomposition per hour at pH 7.0, but at pH 8.0, 0.2% decomposition per hour.[5] Another study reported room temperature studies in 0.1 M phosphate buffer:

(pH 7.0)	No loss in 7 weeks
(pH 8.0)	Loss of 5% in 48 hours
(pH 9.3)	Loss of 9% in 4 hours
(pH 12)	Complete decomposition within 15 minutes.[19]

Storage/Stability

DTNB is stable stored at room temperature. After 3.5 years, samples were still suitable for thiol determination and did not show any significant change in purity by TLC.[1]

References

1. Ellman, G.L., *Arch. Biochem. Biophys.*, 82, 70 (1959).
2. Gething, M.J. and Davidson, B.E., *Eur. J. Biochem.,* 30, 352 (1972).
3. Habeeb, A.F.S.A., *Methods in Enzymology*, 25, 457 (1972).
4. Ellman, G.L., *Arch. Biochem. Biophys.,* 74, 443 (1958).
5. Riddles, P.W. et al., *Anal. Biochem.,* 94, 75 (1979).
6. Riddles, P.W. et al., *Methods in Enzymology,* 91, 49 (1983).
7. Ito, Y. et al., *Bioconjugate Chem.,* 5, 84-87 (1994).
8. Man, M. et al., *Anal. Biochem.,* 57, 429
9. Ming, D. et al., *Biotechniques*, 18, 808 (1995).
10. Robyt, J.F. et al., *Arch. Biochem. Biophys.,* 147, 262 (1971).
11. Brockelhurst, K. et al, *Biochem. J.,* 811-816 (1972).
12. McKelvy, J.F. et al., *Arch. Biochem. Biophys.,* 132, 99-110 (1969).
13. Anderson, W.L. et al., *Anal. Biochem.,* 67, 493 (1975).
14. Nashef, A.S. et al., *Anal. Biochem.,* 79, 394-405 (1977).
15. Kuwata, K. et al., *Anal. Chem.,* 54, 1082 (1982).
16. Harrap, K.R., *Biochem. Pharmacol.,* 16, 725-731 (1967).
17. Bode, J., *Anal. Biochem.,* 94, 465-469 (1979).
18. Palau, J., *Biochim. Biophys. Acta,* 536, 323-327 (1978).
19. Danehy, J.P, Elia, V.J. and Lavelle, C.J., *J. Org. Chem.,* 36, 1003 (1971).

KMR/MAM 01/02