

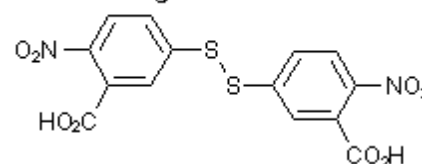
Quantitation of sulfhydryls

DTNB, Ellman's reagent

Product Description

Catalog number: UP01566I, 1g
Name: **DTNB**
5,5'-Dithio-bis(2-nitrobenzoic acid)
Formula: C₁₄H₈N₂O₈S₂ C₁₄H₈N₂O₈S₂, CAS: [69-78-3],
Physical data: **M.W.= 396.36**
 Appearance: yellow crystals or powder ; Purity: 99.0%
Storage: Store cold. Keep dry.
 Warm to room temperature before opening

Ellman Reagent



Catalog number: UP04901V
Name: **acetyl-Cysteine**
Formula: C₅H₉NO₃S, CAS: [616-91-1], **M.W.= 136.2**, (x)
Storage: Room temperature

Directions for use

Protocol 1. Ellman's Test: Quantitative Determination of Peptides by Sulfhydryl (-SH) Groups

(Ellman 1959, Bulaj 1998)

This is the standard "Ellman's Test" for the determination of free thiols. (Ellman 1959). It works well for small peptides and proteins synthesized using standard solid phase synthetic methods. Peptides from these syntheses are usually in their reduced form, and are usually stable to oxidation in acidic solutions. Free thiol can be determined in solutions collected from chromatographic separations or from reconstituted lyophilized samples. This protocol has been used for peptides (3 to 26mer) with a single Cys residue present and lacking tryptophan. (Bulaj 1998, Horn). The technique should be feasible for multiple Cys residues.

- 1) Prepare the DTNB stock solution and the Tris dilution buffer:
 DTNB solution: 50 mM sodium acetate (NaAc), 2 mM DTNB in H₂O . Keep refrigerated. Tris solution: 1 M Tris / pH 8.0. Keep refrigerated.
 Prepare eventually a Standard SH (acetyl Cysteine) calibration curve., starting 10µM
- 2) Prepare the DTNB working reagent: (per sample) add 50 µL of the DTNB solution, 100 µL Tris solution, and water 840 µL (final volume will be 1000µl with 10µl sample)
- 3) Mix solution carefully using pipette. Place cuvette into UV spectrophotometer and take a background scan using the solution as background.
- 4) Add 10µl sample solution (or 10µl sample buffer or water for the blank test, or 10µl of standards) to 990µl DTNB reagent.
- 5) Mix well and incubate 5min at room temperature (or 37°C)
- 6) Measure Optical Absorbance at 412 nm
- 7) Calculate SH content:
 method1: Divide OD_{412nm} by 13600 M⁻¹ cm⁻¹ (the extinction coefficient of the reagent) to get the molarity in the assay, multiply by the total volume/sample volume ratio (1000/10=100 for the standard protocol with 10µl sample) of the solution.

For any question,
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SH concentration(sample) = (Tot. vol./sample vol.) x OD_{412nm} / 13600 = 100 x OD_{412nm} / 13600
method2: Plot a calibration curve of ODs as function of standard (acetylCysteine) concentrations, and estimate SH concentration from sample ODs.

Protocol 2. Determination of total sulfhydryl groups, protein-bound sulfhydryl groups, and free sulfhydryl groups in biological samples using DTNB (Ellman's reagent). Sensitivity 50 μ M to 1000 μ M.
([Sedlak 1968](#))

Total SH determination

- 1- Prepare the dilution buffer (30mM Tris HCl, 3mM EDTA pH8.2). Store at +4°C.
Dissolve in 800 ml distilled H₂O. Adjust pH with 3 N HCl to 8.2. Add distilled H₂O to make 1000 ml.
 - 2- Prepare DTNB working solution. Store at +4°C.
Dissolve 29.7mg of DTNB in 25 ml Methanol.
 - 3-Prepare a set of SH standard (acetylCystein) with sample dilution buffer (or distilled water).
Dissolve acetylCystein at 1000 μ M, then serial dilutions down to 31 μ M. To be use immediately
- 3-Add the following components to test tubes:
- 20 μ l Sample/Standard
 - 75 μ l dilution buffer
 - 25 μ l DTNB reagent
 - 400 μ l Methanol

Spin down samples at 3000 x g for 5 min at room temperature. Using a multichannel pipettor, transfer 3 x 90 μ l supernatant into a flat-bottom microplate. Measure extinction at 412 nm.

Free SH / bound SH

Free SH (cysteine, DTT...) present in proteous samples should be separated from protein bound sulfhydryls. As conventionnal desalting methods (dessalting, gelfiltration) may affect SH content (by air oxydation), Uptima recommends to remove, by a TCA precipitations method, proteins from buffer prior to SH quantitation.

50 μ l Sample/Standard + 2 x 25 μ l TCA

Spin down for 15 min at 1500 rpm and room temperature and keep the supernatant for later use.

Set up the following reaction in a flat-bottom microplate:

- 200 μ l Tris· HCl pH 8,9
- 20 μ l DTNB
- 50 μ l Supernatant from the TCA precipitation

Measure at 412 nm.

Calculate the difference of the Total-SH and the Free SH.

Literature

- Sedlak J, Lindsay RH (1968) Anal. Biochem. 25:192-205.
- Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70-77. (Original determination)
- Bulaj, G.; Kortemme, T.; Goldenberg, D. P. (1998) Biochemistry 37, 8965-8972. (Recent usage)
- Van Horn, J. D.; Bulaj, G.; Burrows, C. J. (2001) Unpublished results.

Related documents and products

UP09111 acetylCystein, UltraPure

For any information, please ask :
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Additional information

Melting point: 240 - 245 C; Isoelectric point: pI = 3.5

Stable. Incompatible with strong bases, strong oxidizing agents.

Toxicology: Skin, eye and respiratory irritant. May be harmful - toxicology not fully investigated.

Toxicity data : IPR-MUS LD50 2080 mg kg-1

Risk phrases : R36 R37 R38.

Transport information: Non-hazardous for air, sea and road freight.

Personal protection: Safety glasses, adequate ventilation.

Safety phrases : S26 S36.