Protocol

Ellman's Test Protocol

Quantitative Determination of Thiols

Introduction

Thiols play a very important role in the maintenance of living organisms. One important function of thiols is the formation of disulfide bonds through an oxidation reaction that results in critical posttranslational modification of proteins. Normally, the cell attempts to maintain a thiol-disulfide homeostasis, which includes the presence of free thiols that can be quantified through the use of the chromogenic reagent 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). DTNB has an oxidizing disulfide bond that when in the presence of free thiols is reduced forming a mixed disulfide and releasing one molecule of 5-thio-2-nitrobenzoic acid (TNB). Thus, free thiol concentration can be determined by observing absorption at 412 nm and measuring TNB. In this protocol we describe a procedure to measure free thiols in solutions containing proteins synthesized using a standard solid phase synthetic method. Peptides from these syntheses are usually in a reduced form and are stable to oxidation in acidic solutions.

Materials

- DNTB (GoldBio Catalog # D-250)
- Molecular grade biology H_2O
- Tris (GoldBio Catalog # T-400)
- UV spectrophotometer
- 10 µL of biological sample
- Sodium acetate

Method

1. Prepare DTNB stock solution with a final concentration of 50mM sodium acetate and 2mM DTNB in molecular biology grade water. Keep refrigerated.

2. Prepare a Tris solution with a final concentration of 1M Tris and adjust the pH to 8.0.

3. Set a standard SH (acetyl cysteine) calibration curve starting at 10µM.

4. Prepare the DTNB working reagent by mixing 50 µl of the DTNB solution, 100 µl of Tris solution and 840 µl of molecular grade biology H_2O. **DO NOT ADD SAMPLE YET.** The final volume will be 1000 µl when 10 µl of sample is added.

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5. Mix the solution carefully and place cuvette into UV spectrophotometer. Take a background scan using the solution as the background.

6. Add 10 µl of sample solution to 990 µl of DTNB reagent.

7. Mix well and incubate at room temperature for 5 minutes.

8. Measure the optical absorbance at 412 nm.

9. You can repeat this at higher sample volumes with lower water volumes to develop data points for a curve.

Calculations

To calculate the molarity of (-SH) groups in the assay, find the absorbance average and divide according to this formula:

$$Molarity = \frac{Absorbance\ Average}{13600 M^{-1} cm^{-1}}$$

Associated Products

- DNTB (GoldBio Catalog # D-250)

References

Bulaj, G., Kortemme, T., and Goldenberg, D. P. (1998). Ionization−Reactivity Relationships for Cysteine Thiols in Polypeptides†. *Biochemistry,* 37(25), 8965-8972. Doi:10.1021/bi973101r.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics,* 82(1), 70-77. Doi:10.1016/0003-9861(59)90090-6.