Enzymatic Assay of THIOREDOXIN REDUCTASE (EC 1.6.4.5)

PRINCIPLE:

$$\text{Thioredoxin-}S_2 + \text{DTNB} \xrightarrow[\text{NADPH}]{\text{Thioredoxin Reductase}} \text{TNB} + \text{Thioredoxin - } (SH)_2$$

Abbreviations used:
DTNB = 5,5'-Dithio-bis(2-Nitrobenzoic Acid)
TNB = 5-Thio-2-Nitrobenzoic Acid
NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 25°C, pH = 7.0, A_{412nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 500 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

B. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

C. 7 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Solution (NADPH)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Prod. No. N-6505.)

D. 0.5% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 5 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-4503.)

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REAGENTS: (continued)

E. 0.1% (w/v) Thioredoxin Solution (Thioredoxin)
(Prepare 1 ml in deionized water using Thioredoxin, Sigma Prod. No. T3303.)

F. 100% Ethanol
(Use 200 Proof USP Ethyl Alcohol, available from Equistar Chemical Company.)

G. 100 mM 5'5-Dithio-bis(2-Nitrobenzoic Acid Solution) (DTNB)
(Prepare 1 ml in Reagent F using 5,5'-Dithio-bis(2-Nitrobenzoic Acid), Sigma Prod. No. D-8130.

H. Thioredoxin Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 3 units/ml of Thioredoxin Reductase in Reagent D.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes.

	Test	Blank
Reagent A (Buffer)	0.14	0.14
Reagent B (EDTA)	0.07	0.07
Reagent C (NADPH)	0.02	0.02
Reagent D (BSA)	0.02	0.07
Reagent E (Thioredoxin)	0.10	0.10
Deionized water	0.265	0.265
Enzyme Solution	0.05	------

Mix by inversion and equilibrate to 25°C. Monitor the A_{412nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (DTNB)	0.035	0.035

Immediately mix by inversion and record the increase in A_{412nm} for approximately 2 minutes. Obtain the ΔA_{412nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATION:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{412nm/min}\ \text{Test} - \Delta A_{412nm/min}\ \text{Blank})(0.7)(df)}{(1)(0.05)}$$

0.7 = Volume (in milliliter) of assay
df = Dilution factor
1 = Change in absorbance at 412 nm that is equivalent to one unit
0.05 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will cause an increase in absorbance of 1.0 at 412 nm (when measured in a coupled assay with E. coli thioredoxin and DTNB) per minute per ml at pH 7.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 0.70 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 10 mM ethylenediaminetetraacetic acid, 0.2 mM β-nicotinamide adenine dinucleotide phosphate, reduced form, 0.05% (w/v) bovine serum albumin, 0.014% (w/v) thioredoxin, 5 mM 5,5'-dithio-bis(2-nitrobenzoic acid), 0.15 unit thioredoxin reductase.

REFERENCES:

Thelander, L. (1967) *Journal of Biological Chemistry*, 242, 852-859

Moore, E.C., and Reichard, P., and Thelander, L. (1964) *Journal of Biological Chemistry*, 239, 3445-3452

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.