Enzymatic Assay of THIOREDOXIN REDUCTASE  
(EC 1.6.4.5)

PRINCIPLE:

\[
\text{Thioredoxin Reductase} \\
\text{Thioredoxin-S}_2 + \text{DTNB} \rightarrow \text{TNB} + \text{Thioredoxin - (SH)}_2 \\
\text{NADPH}
\]

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

CONDITIONS:  
T = 25°C, pH = 7.0, \( A_{412} \), 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 \( \beta \)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Solution (NADPH)  
(Prepare 1 ml in deionized water using \( \beta \)-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.

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Reagent B (EDTA)</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Reagent C (NADPH)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Reagent D (BSA)</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Reagent E (Thioredoxin)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized water</td>
<td>0.265</td>
<td>0.265</td>
</tr>
<tr>
<td>Enzyme Solution</td>
<td>0.05</td>
<td>------</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (DTNB)</td>
<td>0.035</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in \( A_{412\text{nm}} \) for approximately 2 minutes. Obtain the \( \Delta A_{412\text{nm}}/\text{minute} \) using the maximum linear rate for both the Test and Blank.
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**CALCULATION:**

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

- \(0.7\) = 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:**


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