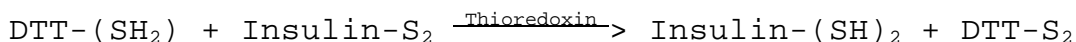


Suitability Assay of THIOREDOXIN

PRINCIPLE:



Abbreviations used:

DTT-(SH₂) = DL-Dithiothreitol

Insulin-S₂ = Insulin (native disulfide bridge)

Insulin-(SH)₂ = Insulin (reduced disulfides)

DTT-S₂ = DL-Dithiothreitol (disulfide bridge)

CONDITIONS: T = 25°C, pH 7.0, A_{650nm}, Light path = 1 cm

METHOD: Turbidimetric Rate Determination

REAGENTS:

- A. 100 mM Sodium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.0 at 25°C with 1 M NaOH.)
- B. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- C. 100 mM DL-Dithiothreitol Solution (DTT)
(Prepare 1 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632.)
- D. 50 mM Tris HCl Buffer, pH 7.5 at 25°C
(Prepare 10 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 25°C with 1 M HCl.)
- E. 1% (w/v) Insulin Solution (Insulin)
(Prepare by dissolving 10 mg of Insulin, Sigma Prod. No. I-3505, in 1 ml of Reagent D. This will produce a milky solution. Adjust the pH to below 3 at 25°C with 5 N HCl. The solution should begin to clear. Titrate again with 5 N NaOH. The solution should become turbid at pH 7 and then clear at pH 7.5. The final pH should be 7.5 at 25°C.)

Suitability Assay of THIOREDOXIN

REAGENTS: (continued)

- F. Thioredoxin Solution (Thioredoxin)
(Immediately before use prepare a solution containing
0.025 - 0.1 mg/ml of Thioredoxin (protein) in cold
deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters)
the following reagents into a suitable container:

Reagent A (Buffer)	7.56
Reagent B (EDTA)	0.24
Reagent E (Insulin)	1.20

Mix by swirling.

Pipette (in milliliters) the following reagents into
suitable containers:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.75	0.75
Reagent F (Thioredoxin)		0.08

Deionized Water	0.16	0.24

Mix by swirling and then add:

Reagent C (DTT)	0.01	0.01
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Immediately mix by swirling and incubate at 25°C for
20 minutes.¹ Then begin to record the increase in $A_{650\text{nm}}$
every 5 minutes for a total of 15 minutes. Obtain the
 $\Delta A_{650\text{nm}}/\text{minute}$ using the maximum linear rate for both the
Test and Blank.²

CALCULATIONS:

$$\text{S.A.} = \frac{(\Delta A_{650\text{nm}}/\text{min Test} - \Delta A_{650\text{nm}}/\text{min Blank})(1)(\text{df})}{(\text{mg})}$$

S.A. = Specific Activity

df = Dilution factor

1 = Total volume (in milliliter) of assay

mg = mg Thioredoxin

Suitability Assay of THIOREDOXIN

SPECIFICATION:

Specific Activity (using insulin): $\Delta A_{650nm} = 1-3$ per mg protein per minute in the presence of 1 mM EDTA.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 5 mM Tris, 63 mM sodium phosphate, 0.1% (w/v) insulin, 1 mM DL-dithiothreitol, 2 mM ethylenediaminetetraacetic acid, and 0.002 - 0.008 mg thioredoxin.

REFERENCE:

Holmgren, A. (1979) *Journal of Biological Chemistry* **254**, 9627-9632

NOTES:

1. The turbidity of the reaction mixture begins to increase after approximately 20 minutes.
2. If the third A_{650nm} reading (at 10 minutes) exceeds 0.8, dilute the thioredoxin solution (Reagent F) and run the assay again because rates above this value are not linear.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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