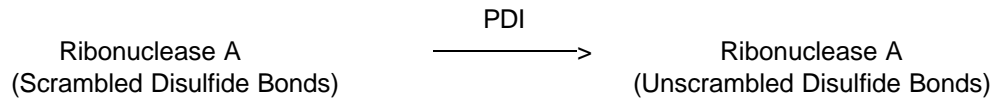


**Enzymatic Assay of PROTEIN DISULFIDE-ISOMERASE
(EC 5.3.4.1)**

PRINCIPLE:



Abbreviation used:
PDI = Protein Disulfide-Isomerase

CONDITIONS: T = 30°C, pH 7.5, $A_{260\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Sodium Phosphate Buffer with 5.0 mM Ethylenediaminetetraacetic Acid, pH 7.5 at 30°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, and Ethylenediaminetetraacetic Acid Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.5 at 30°C with 1 M NaOH.)
- B. 50 mM Tris HCl Buffer with 25 mM Potassium Chloride and 5 mM Magnesium Chloride, pH 7.5 at 30°C (TKM)
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, Potassium Chloride, Sigma Prod. No. P-4504, and Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20. Adjust to pH 7.5 at 30°C with 1 M NaOH.)
- C. 10 mM Acetic Acid Solution (HOAC)
(Prepare 25 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283.)
- D. 100 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632.)
- E. 0.0083% (w/v) Ribonucleic Acid Solution (RNA)
(Prepare 5 ml in Reagent B using Ribonucleic Acid, Sigma Prod. No. R-6625.)

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REAGENTS: (continued)

- F. Scrambled Ribonuclease A Enzyme Solution (SRNase A)
(Immediately before use, prepare a solution containing 0.05% (w/v) of Ribonuclease A, with Scrambled Disulfide Bonds, Sigma Prod. No. R-2638, in cold Reagent C. Keep at 4°C. This solution is stable for several weeks at 4°C.)
- G. Protein Disulfide-Isomerase Enzyme Solution (PDI)
(Immediately before use, prepare a solution containing 0.02 mg/ml of Protein Disulfide-Isomerase in cold Reagent A. Keep at 4°C.)

PROCEDURE:

Step 1: PDI Assay

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.94	0.95
Reagent D (DTT)	0.01	0.01
Reagent G (PDI)	0.01	-----

Mix by inversion and incubate at 30°C for 5 minutes. Then add:

Reagent F (SRNase A)	0.05	0.05
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Immediately start to record time. Mix by inversion and remove 0.010 ml aliquots at 1, 5, and 9 minutes time points (Reaction Mix T₁, T₅, and T₉). Proceed with the RNase assay (Step 2).

Step 2: RNase Assay

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

	<u>Test1</u>	<u>Test2</u>	<u>Test3</u>	<u>Blank</u>
Reagent E (RNA)	3.00	3.00	3.00	3.00

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PROCEDURE: (continued)

Incubate at 25°C for 5 minutes. Then add:

PDI Reaction Mix T ₁	0.01	----	----	----
PDI Reaction Mix T ₅	----	0.01	----	----
PDI Reaction Mix T ₉	----	----	0.01	----
Blank Mixture	----	----	----	0.01

Mix by inversion and record the increase in A_{260nm} for approximately 5 minutes. Obtain the r A_{260nm}/minute using the maximum linear rate for both the Tests and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{260\text{nm}}/\text{min Test}^1 - \text{r } A_{260\text{nm}}/\text{min Blank})(\text{df})(1.01)(100)}{(\text{T})(1)(0.01)}$$

100 = Dilution of enzyme resulting from using 0.01 ml of the PDI reaction mixture

df = Dilution factor

1.01 = Volume (in milliliters) of assay

T = Time (in minutes) of assay

1 = Change in A_{260nm} which is equal to 1 unit

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

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

UNIT DEFINITION:

One unit is the amount of enzyme which reactivates one unit of ribonuclease² from scrambled ribonuclease (R-2638) per minute at pH 7.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 1.01 ml reaction mix, the final concentrations are 47 mM sodium phosphate, 4.7 mM ethylenediaminetetraacetic acid, 1 mM DL-dithiothreitol, 0.0025% (w/v) scrambled ribonuclease A, 0.5 mM acetic acid, and 0.2 µg protein disulfide-isomerase.

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REFERENCES:

Hillson, D.A., Lambert, N., and Freedman, R.B. (1984) *Methods in Enzymology* **107B**, 281-294

NOTES:

1. Average the values for the time points at 5 and 9 minutes. The 1 minute time point is not reliable enough to be included in the average.
2. One unit of ribonuclease is defined as the amount of enzyme that will release 1.0 A_{260nm} absorbance unit per minute at pH 7.5 at 25°C.
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.