

## ENZYMATIC ASSAY of PROTEIN DISULFIDE ISOMERASE using Insulin as substrate

Sigma's previous assay procedure used scrambled RNase as substrate. The use of insulin as substrate enables a lower background and a more reliable measure of the enzyme activity.

### Unit definition:

One unit will cause a change of A<sub>650nm</sub> of 0.01 per minute at 25°C at pH 7.5 of a 1 mg/ml solution of insulin in presence of DTT.

### Materials:

#### Solutions:

10 mg/ml insulin (I-5500) in 50 mM Tris-HCl buffer pH 7.5 (may be stored in frozen portions of 1.2 ml)

100 mM DTT (D-0632)

100 mM Sodium EDTA pH 7.0

100 mM Sodium Phosphate Buffer pH 7.0

Reaction cocktail (for 6 assays), to be prepared freshly:

7.56 ml	Na-Phosphate buffer solution #4
0.24 ml	Na-EDTA solution #3
1.2 ml	Insulin solution #1

### Equipment and Supplies:

Spectrophotometer (preferently thermostated at 25°C)

Test tubes

Suitable heating block or bath thermostated at 25°C

### Method

Determine the protein concentration of the PDI solution (Lowry)

Fill 0.75 ml of the cocktail into a 1 ml test tube and add approx. 15 µg protein of PDI sample and up to 0.25 ml of sodium phosphate buffer (#4) to complete to 1ml reaction volume.

Add 10 µl of DTT solution (#2), mix and incubate in a 25°C bath.

Determine the turbidity by reading A<sub>650</sub> every 5 minutes up to OD 0.8.

Note that the turbidity starts to appear after approximately 30 minutes, and the rate of its appearance is linear with time.

A control should be run without PDI (cocktail + buffer + DTT)

**Calculation:**

Subtract the reading of the control (without PDI). Turbidity starts to build up in this control after 30-35 minutes.

Calculate the absorbance per minute in the linear range. At least 4 readings should be determined.

Specific activity:

$$\text{S.A. (in units/mgP/min): } (A_{650}/\text{min}) / (0.01 \times \text{mg protein in reaction mixture})$$

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