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 A650nm 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 (preferentially 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 A650 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:

Substract 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 reading should be determined.
Specific activity:

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\text{S.A. (in units/mgP/min): } \frac{(A650/\text{min})}{(0.01 \times \text{mg protein in reaction mixture})}
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This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.