Enzymatic Assay of RHODANESE
(EC 2.8.1.1)

PRINCIPLE:

Thiosulfate + Cyanide $\xrightarrow{\text{Rhodanese}}$ Sulfite + Thiocyanate

CONDITIONS: T = 25°C, pH = 8.6, A_{460nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 200 mM Potassium Phosphate Buffer, pH 8.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.6 at 25°C with 1 M KOH.)

B. 125 mM Sodium Thiosulfate Solution
(Prepare 10 ml in deionized water using Sodium Thiosulfate, Pentahydrate, Sigma Prod. No. S-8503.)

C. 250 mM Potassium Cyanide Solution (Sodium Cyanide)
(Prepare 10 ml in Reagent A using Potassium Cyanide ACS Reagent Grade, Aldrich Prod. No. 20781-0)

D. 14% (w/v) Nitric Acid Solution
(Prepare 50 ml in deionized water using concentrated Nitric Acid, Aldrich Stock No. 25811-3.)

E. 410 mM Ferric Nitrate Solution (Ferric Nitrate)
(Prepare 25 ml in Reagent D using Ferric Nitrate, Nonahydrate, Sigma Prod. No. F-3002.)

F. 37% (w/w) Formaldehyde Solution (Formaldhyde)
(Use Formaldehyde, 37% Solution Formalin, Sigma Prod. No. F-1635.)

G. 12.5 mM Sodium Thiosulfate Solution with 0.025% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepare 25 ml in deionized water using Sodium Thiosulfate, Pentahydrate, Sigma Prod. No. S-8503 and Albumin, Bovine, Prod. No. A-4503 or equivalent.)

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REAGENTS: (continued)

H. Rhodanese Enzyme Solution
(Immediately before use, prepare a solution containing 2.0 - 3.0 units/ml Rhodanese in Reagent G.)

PROCEDURE:

Pipette (in milliliters) the following reagents into 4 dram vials:[1]

	Test	Blank
Reagent A (Buffer)	0.30	0.30
Reagent B (Sodium Thiosulfate)	0.50	0.50
Reagent C (Sodium Cyanide)	0.25	0.25

Mix by swirling and equilibrate to 25°C. Then add:

Reagent H (Enzyme Solution)	0.20	------

Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

Reagent F (Formaldehyde)	0.25	0.25
Reagent H (Enzyme Solution)	------	0.20

Mix by swirling and then add:

Reagent E (Ferric Nitrate)	1.25	1.25
Deionized Water	12.50	12.50

Mix by swirling and transfer the solutions to suitable cuvettes and record the A_{460nm} for both the Test and Blank using a suitably thermostatted spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{460nm}\ \text{Test} - A_{460nm}\ \text{Blank})(15.25)(df)}{(5)(3.17)(0.2)}$$

5 = Conversion factor for 5 minutes to 1 minute
3.17 = Millimolar extinction coefficient of Thiocyanate at 460 nm
df = Dilution factor
15.25 = Total volume of assay
0.2 = Volume (in milliliters) of enzyme used

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

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of cyanide to thiocyanate per minute at pH 8.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.25 ml reaction mix, the final concentrations are 88 mM potassium phosphate, 52 mM sodium thiosulfate, 50 mM sodium cyanide, 0.004% (w/v) BSA, and 0.4 - 0.6 unit rhodanese.

NOTES:

1. This assay should be run in a fume hood with a mechanical exhaust system since small quantities of HCN may be liberated.

2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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