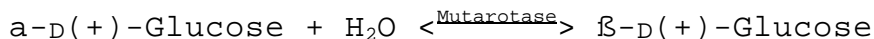


**Enzymatic Assay of MUTAROTASE  
(EC 5.1.3.3)**

**PRINCIPLE:**



**CONDITIONS:** T = 25°C, pH = 7.4, 589 nm

**METHOD:** Continuous Polarimetric

**REAGENTS:**

- A. 5 mM Ethylenediaminetetraacetic Acid Solution, pH 7.4 at 25°C (EDTA)  
(Prepare 500 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Stock No. ED4S. Adjust to pH 7.4 at 25°C with 1 M NaOH.)
- B.  $\alpha\text{-D}(+)\text{-Glucose}$  Tube (Glucose Tube)  
(Weigh 100 mg into suitable tubes using  $\text{D}(+)\text{-Glucose}$ , Anhydrous, Prod. No. G-8270. NOTE: This product contains 6 - 7% of the ( $\beta\text{-}$ ) anomer.)
- C. Mutarotase Enzyme Solution  
(Immediately before use, prepare a solution containing 125 - 250 units/ml of Mutarotase in cold Reagent A.)

**PROCEDURE:**

Spontaneous Optical Rotation Determination

Using a suitable polarimeter, zero a 10 cm cell at 589 nm (Na Lamp) at 25°C.

At zero time add 10.00 ml of Reagent A (EDTA) to Reagent B (Glucose Tube) and dissolve rapidly by agitation. Transfer to the polarimeter cell and begin to record the rotation at 1.0 minute intervals for the first 10 minutes (earliest reading should be taken 3.5 minutes after the addition of Reagent A), at 5 minute intervals for the second 10 minutes and at 15 minute intervals until the rotation is constant.<sup>1</sup>

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**PROCEDURE: (continued)**

Test Optical Rotation Determination

Using a suitable polarimeter, zero a 10 cm cell at 589 nm (Na Lamp) at 25°C.

Add 9.90 ml of Reagent A (EDTA) to Reagent B (Glucose Tube) and dissolve rapidly by agitation. At zero time, add 0.10 ml of Reagent C (Enzyme Solution). Mix by swirling and transfer to the polarimeter cell as rapidly as possible. Begin to record the rotation at 30 second intervals for 10 minutes.

**CALCULATIONS:**

Graphs:

1 - Spontaneous Rotation Plot

Using the spontaneous rotation data, plot the rotation vs. time in minutes on semi-log paper. Extrapolate to zero time to obtain the initial rotation. The initial rotation corresponds to an  $\alpha$ -D(+)-Glucose concentration of 555  $\mu$ moles per reaction mixture.

2 - Blank Rotation Plot

Using the spontaneous rotation data, plot the rotation, vs time in 1.0 minute intervals. Determine the change in rotation for 5.0 minutes.

3 - Standard Curve

Using the spontaneous rotation data, plot the rotation, vs  $\mu$ moles  $\alpha$ -D(+)-glucose. This plot consists of two points; the rotation at 555  $\mu$ moles (Initial Rotation) and the rotation at 195  $\mu$ moles (Final Rotation).

4 - Test Plot

Using the test rotation data, plot the rotation, vs time in minutes and determine the change in rotation for 5 minutes.

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**CALCULATIONS:**

Obtain the following values:

A = Initial rotation from Graph #1

B = r Rotation per 5.0 minutes from Graph #2

C = Blank rotation per 5.0 minutes,  $C = A - B$

D = Conversion of C to  $\mu$ moles  $\alpha$ -D-(+)-Glucose from graph #3

E = Concentration of  $\alpha$ -D-(+)-Glucose per reaction mix at Initial Rotation,  $E = 555 \mu$ mole

F = Spontaneous or blank rate in  $\mu$ moles  $\alpha$ -D-(+)-Glucose/min,

$$F = \frac{E - D}{5.0 \text{ minutes}}$$

G = Test Rotation 5.0 minutes from Graph #4

H = Test Rotation after 5.0 minutes,  $H = A - G$

I = Conversion of H to  $\mu$ moles  $\alpha$ -D-(+)-Glucose from Graph #3

J = Test rate in  $\mu$ moles  $\alpha$ -D-(+)-Glucose/min,

$$J = \frac{E - I}{5.0 \text{ minutes}} - F$$

$$\text{Units/mg enzyme} = \frac{J}{(5) (\text{mg enzyme/RM})}$$

RM = Reaction Mix

5 = Time of assay (in minutes) as per unit definition

**UNIT DEFINITION:**

One unit will increase the spontaneous mutarotation of  $\alpha$ -D-glucose to  $\beta$ -D-glucose by 1.0  $\mu$ mole per minute at pH 7.4 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 10.00 ml reaction mix, the final concentrations are

5 mM EDTA, 1% (w/v)  $\alpha$ -D-(+)-glucose and 12.5 - 25 units mutarotase.

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**NOTES:**

1. Average time for equilibration is 3.5 - 4 hours and will be at +53°.
2. All product 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.**