Enzymatic Assay of GLYCOLATE OXIDASE[1] (EC 1.1.3.15)

PRINCIPLE:

Glycolate + O_2 $\xrightarrow{\text{Glycolate Oxidase}}$ Glyoxylate

Glyoxylate + Phenylhydrazine ⟶ Glyoxylate Phenylhydrazone

CONDITIONS: T = 25°C, pH = 7.8, A_{324nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Phosphate Buffer, pH 7.8 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.8 at 25°C with 1 M KOH.)

B. 40 mM Glycolate Solution
(Prepare 25 ml in Reagent A using Glycolic Acid, Sigma Prod. No. G-1884. Adjust to pH 8.0 at 25°C with 1 M KOH.)

C. 100 mM L-Cysteine HCl Solution
(Prepare 25 ml in Reagent A using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 6.0 at 25°C with 1 M KOH. **PREPARE FRESH**.)

D. 100 mM Phenylhydrazine HCl Solution
(Prepare 25 ml in Reagent A using Phenylhydrazine Hydrochloride, Sigma Prod. No. P-6926. Adjust to pH 6.0 with 1 M KOH. **PREPARE FRESH AND KEEP FROM LIGHT**.)

E. 1 mM Flavin Mononucleotide Solution (FMN)
(Prepare 10 ml in deionized water using Flavin Mononucleotide, Sodium Salt, Sigma Prod. No. F-2253. **PREPARE FRESH AND KEEP FROM LIGHT**.)

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REAGENTS: (continued)

F. Glycolate Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 unit/ml of Glycolate Oxidase in cold Reagent A.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable amber bottle (**PREPARE FRESH. SOLUTION IS STABLE FOR ONLY A FEW HOURS**):

Reagent A (Buffer)	22.00
Reagent B (Glycolate)	5.00
Reagent C (L-Cysteine)	1.00
Reagent D (Phenylhydrazine)	1.00

Mix and adjust to pH 7.8 at 25°C with either 1 M HCl or 1 M KOH, if necessary. Oxygenate by bubbling 99.9% pure O_2 through the solution for 5 to 7 minutes (**this must be done prior to each assay**).

Immediately pipette (in milliliters) into suitable quartz cuvettes:

	Test	Blank
Oxygenated Reaction Cocktail	2.90	2.90
Reagent A (Buffer)	------	0.10
Reagent E (FMN)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{324nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{324nm} for approximately 15 minutes. Obtain the ΔA_{324nm}/minute using the maximum linear rate[2] for both the Test and Blank.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{324nm}/\text{min Test} - \text{r } A_{324nm}/\text{min Blank})\ (3.1)\ (df)}{(17)\ (0.1)}$$

3.1 = Total volume (in milliliters) of assay
df = dilution factor
17 = Millimolar extinction coefficient of Glyoxylate Phenylhydrazone at 324 nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will produce 1.0 µmole of glyoxylate from glycolate per minute at pH 7.8 at 25°C, in the presence of phenylhydrazine.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 6.5 mM glycolic acid, 3.22 mM cysteine, 3.22 mM phenylhydrazine, 0.03 mM flavin mononucleotide and 0.02 unit glycolate oxidase.

REFERENCES:

Baker, A.L. and Tolbert, N.E. (1966) *Methods in Enzymology* **IX**, 339-340

NOTES:

1. Not to be used for Sigma Prod. No. G-4136.

2. The maximum linear rate is generally found between 10 - 15 minutes.

3. This assay is based on the cited reference.

NOTES: (continued)

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.