

**Enzymatic Assay of GLYCOLATE OXIDASE
(EC 1.1.3.15)**

PRINCIPLE:

Glycolate + O₂ $\xrightarrow{\text{Glycolate Oxidase}}$ Glyoxylate

Glyoxylate + Phenylhydrazine \longrightarrow Glyoxylate
Phenylhydrazone

CONDITIONS: T = 25°C, pH = 8.3, A_{324nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Phosphate Buffer, pH 8.3 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.3 at 25°C with 1 M KOH.)
- B. 40 mM Glycolic Acid Solution (Glycolic Acid)
(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 (L-Cysteine)
(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 (Phenylhydrazine)
(Prepare 25 ml in Reagent A using Phenylhydrazine Hydrochloride, Sigma Prod. No. P-6926. Adjust to pH 6.0 at 25°C with 1 M KOH. **PREPARE FRESH AND KEEP FROM LIGHT.**)
- E. 1 mM Flavin Mononucleotide Solution (FMN)
(Prepare 5 ml in deionized water using Flavin Mononucleotide, Sodium Salt, Sigma Prod. No. F-2253. **PREPARE FRESH.**)

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REAGENTS: (continued)

- F. 5 mM Flavin Mononucleotide with 0.1% (w/v) Bovine Serum Albumin (Enzyme Dil)
(Prepare 10 ml in deionized water using Flavin Mononucleotide, Sodium Salt, Sigma Prod. No. F-2253, and Albumin, Bovine, Sigma Prod. No. A-4503. **PREPARE FRESH AND KEEP FROM LIGHT.**)
- G. Glycolate Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Glycolate Oxidase in cold Reagent F.)

PROCEDURE:

To prepare a reaction cocktail, pipette (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 (Glycolic Acid)	5.00
Reagent C (L-Cysteine)	1.00
Reagent D (Phenylhydrazine)	1.00

Mix by swirling and equilibrate to 25°C. If necessary, adjust to pH 8.3 with either 1 M KOH or 1 M HCl. Oxygenate by bubbling pure O₂ through the solution for 5 to 7 minutes (**this must be done prior to each assay**).

Immediately pipette (in milliliters) into suitable quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Oxygenated Reaction Cocktail	2.9	2.9
Reagent E (FMN)	0.1	0.1
Reagent F (Enzyme Dil)	-----	0.1

Immediately mix by inversion. Monitor the A_{324nm} until constant, using a suitably thermostatted spectrophotometer.
Then add:

Reagent G (Enzyme Solution)	0.1	-----
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Immediately mix by inversion and record the increase in A_{324nm} for approximately 15 minutes. Obtain the r A_{324nm}/min

using the maximum linear rate¹ for both the Test and Blank.

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CALCULATIONS:

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

3.1 = Total volume (in milliliters) of assay
df = Dilution factor
17 = Millimolar extinction coefficient of Glyoxylate
Phenylhydrazine at 324 nm
0.1 = Volume (in milliliter) 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 8.3 at 25°C, in the presence of phenylhydrazine.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 94 mM potassium phosphate, 6.5 mM glycolic acid, 3.2 mM cysteine, 3.2 mM phenylhydrazine, 0.2 mM flavin mononucleotide, 0.003% (w/v) bovine serum albumin and 0.01 - 0.02 unit glycolate oxidase.

REFERENCES:

Baker, A.L. and Tolbert, N.E. (1966) *Methods in Enzymology*, IX, 338-342

NOTES:

1. The maximum linear rate is generally found between 10 - 15 minutes.
2. This assay is based on the cited reference.

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NOTES: (continued)

3. 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.