Enzymatic Assay of MANDELONITRILE LYASE
(EC 4.1.2.10)

PRINCIPLE:

Mandelonitrile $\xrightarrow{ML}$ Benzaldehyde + HCN

Abbreviations used:
ML = Mandelonitrile Lyase
HCN = Hydrogen Cyanide

CONDITIONS: T = 25°C, pH = 5.4, A_{275nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer
(Prepare 100 ml in deionized water using Sodium Acetate Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.4 at 25°C with 1 M HCl.)

B. 95% (v/v) Ethanol
(Prepare 10 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

C. 168 mM $_{DL}$-Mandelonitrile Solution (Substrate)
(Prepare 10 ml in cold 95% Ethanol using $_{DL}$-Mandelonitrile, Sigma Prod. No. M-7010. **PREPARE FRESH**.)

D. 0.1% (w/v) Bovine Serum Albumin Solution (Enzyme Diluent)
(Prepare 10 ml in cold deionized water using Albumin, Bovine, Sigma Prod. No. A-6003.)

E. Mandelonitrile Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Mandelonitrile Lyase in cold Reagent D.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	2.85	2.85
Reagent C (Substrate)	0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the A_{275nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------
Reagent D (Enzyme Diluent)	------	0.10

Immediately mix by inversion and record the increase in A_{275nm} for approximately 5 minutes. Obtain the $r\ A_{275}$/minute using the maximum linear rate for both Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r\ A_{275nm}/\text{min Test} - r\ A_{275nm}/\text{min Blank})(3)(df)}{(1.2)(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
1.2 = Millimolar extinction coefficient of Benzaldehyde at 275 nm
0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will form 1.0 µmole of benzaldehyde and HCN from mandelonitrile per minute at pH 5.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 95 mM sodium acetate, 2.8 mM DL-mandelonitrile, 0.003% (w/v) bovine serum albumin, 1.6% (v/v) ethanol, and 0.1 - 0.2 unit of mandelonitrile lyase.

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REFERENCES:

Phillips, J.P., Feuer, H. and Thyagarajan, B.S. (1967) *Organic Spectral Data*, Volume IX, 64

Jorns, M.S. (1979) *Journal of Biological Chemistry* **254**, 12145-12152

NOTES:

1. This assay is based on the cited references.
2. 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.