

**Enzymatic Assay of L-ARGININE DECARBOXYLASE
(EC 4.1.1.19)**

PRINCIPLE:

L-Arg + Pyridoxyl 5-Phosphate L-Arginine Decarboxylase > Agmatine + CO₂

Abbreviations:

L-Arg = L-Arginine

CONDITIONS: T = 37°C, pH 5.2

METHOD: Manometric Assay using Warburg Flasks

REAGENTS:

- A. 500 mM Sodium Acetate Buffer, pH 5.2 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Prod. No. S-8625. Adjust pH to 5.2 with 1 M NaOH.)
- B. 100 mM L-Arginine Hydrochloride Solution
(Prepare 50 ml in Reagent A using L-Arginine, Hydrochloride, Prod. No. A-5131.)
- C. 10 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 25 ml in Reagent A using Pyridoxal 5-Phosphate, Prod. No. P-9255.)
- D. L-Arginine Decarboxylase Solution
(Immediately before use, prepare a solution containing 3 - 5 units/ml of L-Arginine Decarboxylase in Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into Warburg flasks.

Main Chamber	Thermo Barometer Substrate <u>Flask</u>	Enzyme <u>Blank</u>	<u>Test¹</u>	<u>Blank</u>
Reagent A (Buffer)	2.8	0.30	0.30	2.80
Reagent B (Ornithine HCl)	-----	2.50	2.50	-----
 Side Arm				
Reagent D (Enzyme Solution)		-----	-----	0.10
			0.10	
Reagent C (PRP)	-----	0.10	0.10	0.10
Reagent A (Buffer)	0.20	0.10	-----	-----

Be sure to confirm the stability of the pressure with the flask sealed off, before proceeding with the assay. This is to insure temperature equilibrium and the absence of leaks in the flask.

The enzyme activity is determined by calculation of the rate of production of CO₂ at 37°C.¹ The reaction rate should be linear for about 20 minutes.

CALCULATIONS:

$$\frac{\text{Units}}{\text{ml Arginine Decarboxylase}} = \frac{(C) (k) (\text{Dilution Factor})}{1000 \left(22.4 \frac{L}{\text{mole}} \right) (\text{ml Arginine Decarboxylase})}$$

- C = mm of CO₂ gas evolved/minute.²
- k = Warburg flask constant in μL/mm.³
- 22.4 L = Volume gas occupies under STP conditions.
- 1000 = Conversion factor for L to ml

UNIT DEFINITIONS:

One unit will release 1.0 μmole of CO₂ from L-arginine per minute at pH 5.2 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.0 ml reaction mix, the final concentrations are 500 mM sodium acetate, 83 mM L-arginine, 0.33 mM pyridoxal

5-phosphate, and 0.3 - 0.5 units L-arginine decarboxylase.

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REFERENCES:

Blethen, S.L., Boeker, E.A., and Snell, E.E. (1968)
Journal of Biological Chemistry **243**, 1671-1677

NOTES:

1. The tests are done at least in triplicate since it is common for the flasks to have small leaks.
2. The mm of CO₂ gas evolved (C) is corrected for any temperature and barometric changes (ThermoBarometer) during the experiment and also for the Substrate Blank and Enzyme Blank

mm CO₂ corrected = mm CO₂ measured Test - mm CO₂ measured of
[Thermobarometer + Substrate Blank + Enzyme Blank]

Values of the corrected mm CO₂ produced are plotted versus time. The best straight line is drawn, not necessarily through the origin. The slope, C = mm CO₂/time, is obtained.

3. The flask constant, k, is calculated according to the equation:

$$k = \frac{[(Vg) \left(\frac{273}{T}\right) + V_1 a]}{P}$$

where

P = Standard pressure as mm of manometer fluid.

Vg = Gas volume in flask and manometer.

V₁ = Volume of liquid in flask.

T = Absolute temperature.

a = Solubility of gas; for CO₂ at 37°C, a = 0.57

4. This assay is based on the cited reference.
5. 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.