Enzymatic Assay of L-ARGININE DECARBOXYLASE  
(EC 4.1.1.19)

**PRINCIPLE:**

\[
\text{L-Arg} + \text{Pyridoxyl 5-Phosphate} \xrightarrow{\text{L-Arginine Decarboxylase}} \text{Agmatine} + \text{CO}_2
\]

Abbreviations:

L-Arg = L-Arginine

**CONDITIONS:**  \( T = 37^\circ \text{C}, \text{pH} 5.2 \)

**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. 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.)

**C.** 10 mM Pyridoxal 5-Phosphate Solution (PRP)  
(Prepare 25 ml in Reagent A using Pyridoxal 5-Phosphate.)

**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.

<table>
<thead>
<tr>
<th>Main Chamber</th>
<th>Thermo Barometer</th>
<th>Enzyme Substrate Flask</th>
<th>Blank</th>
<th>Test¹</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.8</td>
<td>0.30</td>
<td>0.30</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td>Reagent B (Ornithine HCl)</td>
<td>------</td>
<td>2.50</td>
<td>2.50</td>
<td>------</td>
<td></td>
</tr>
</tbody>
</table>

Side Arm

| Reagent D (Enzyme Solution) | ------ | ------ | 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:**

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

\[C = \text{mm of CO}_2 \text{ gas evolved/minute}.²\]
\[k = \text{Warburg flask constant in } \mu\text{L/mm}.³\]
\[22.4 L = \text{Volume gas occupies under STP conditions.}\]
\[1000 = \text{Conversion factor for L to ml}\]

**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:

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

\[ \text{mm CO}_2 \text{ corrected} = \text{mm CO}_2 \text{ measured Test} - \text{mm CO}_2 \text{ measured of} \]
\[ \text{[Thermobarometer + Substrate Blank + Enzyme Blank]} \]

Values of the corrected mm CO₂ produced are plotted 
versus time. The best straight line is drawn, not 
necessarily through the origin. The slope, 
\( C = \text{mm CO}_2/\text{time} \), is obtained.

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

\[
k = \frac{\left( \frac{273}{T} \right)^2 + V_1a}{P}
\]

where

\( P \) = Standard pressure as mm of manometer fluid. 
\( V_g \) = Gas volume in flask and manometer. 
\( V_1 \) = 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.