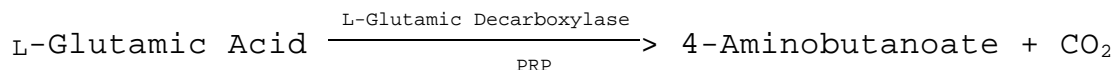


**Enzymatic Assay of L-GLUTAMIC DECARBOXYLASE
(EC 4.1.1.15)**

PRINCIPLE:



Abbreviation used:

PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, pH = 5.0

METHOD: Manometric Assay using Warburg Flasks

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 100 mM L-Glutamic Acid Solution (L-Glu)
(Prepare 50 ml in Reagent A using L-Glutamic Acid, Monosodium Salt, Sigma Prod. No. G-1626. Adjust to pH 5.0 at 37°C with either 1 M NaOH or 1 M HCl.)
- C. 10 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 25 ml in Reagent A using Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255.)
- D. L-Glutamic Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing 3 - 5 units/ml of L-Glutamic Decarboxylase in cold 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)	2.80	2.80	0.30	0.30
Reagent B (L-Glu)	-----	-----	2.50	2.50
 Side Arm				
Reagent A (Buffer)	0.20	-----	-----	0.10
Reagent C (PRP)	-----	0.10	0.10	0.10
Reagent D (Enz Solution)	-----	0.10	0.10	-----

Be sure to confirm the stability of the pressure with the flask sealed off and before proceeding with the assay. This is to ensure 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. The reaction rate should be linear for about 20 minutes.

CALCULATIONS:

$$\frac{\text{Units}}{\text{ml L-Glutamic Decarboxylase}} = \frac{(C)(K)(df)}{(22.4 \text{ l/mole})(\text{ml L-Glutamic Decarboxylase})}$$

- C = mm of CO₂ gas evolved/minute²
- K = Warburg flask constant³ in µl/mm
- df = Dilution factor
- 22.4 l = Volume gas occupies under STP conditions

UNIT DEFINITION:

One unit will release 1.0 µmole of CO₂ from L-glutamic acid per minute at pH 5.0 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 100 mM sodium acetate, 83 mM L-glutamic acid, 0.33 mM pyridoxal 5-phosphate, and 0.3 - 0.5 unit L-glutamic acid decarboxylase.

REFERENCE:

Fonda, M.L. (1985) *Methods in Enzymology* **113**, 11-16

Umbreit, W.W., Burris R.H., and Stauffer, J.F. (1951) *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co., Minneapolis, MN

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 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:

$$K = \frac{[(V_g) \left(\frac{273}{T}\right) + V_f a]}{P_o}$$

P_o = Standard pressure as mm of manometer fluid
V_g = Gas volume (in milliliters) in flask and manometer
V_f = Volume of Liquid in flask
T = Absolute temperature
a = Solubility of gas; (for CO₂ at 37°C, a = 0.57)

The flask constant, K, must be calculated for each Warburg flask used, as described in Umbreit, W.W.,

Burris, R.H. and Stauffer, J.F. (1951)

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NOTES:

4. This assay is based on the cited references.
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.