N BIO - BICARBONATE

(PEPC method)

Kit Name	Kit Size	Cat No
N BIO-Bicarbonate	1 x 10 ml	MBIC04005M
Bicarbonate monovials	10 x 1 ml	VBIC10001M

INTRODUCTION

The measurement of Carbon Dioxide is useful in the assessment of acid base balance disturbances. Elevated CO2 is observed in metabolic alkalosis and compensated respiratory acidosis. Low CO2 is observed in compensated respiratory alkalosis and metabolic acidosis. Differentiation between the metabolic and respiratory conditions is only possible through additional laboratory determinations.

METHOD PRINCIPLE

Early methods for the determination of carbon dioxide were based on either volumetric or manometric determination of the CO2 released from a sample by acid treatment. These methods used the instruments of Van Slyke until they were replaced by the Natelson microgasometer,3 which still uses manometric determination of total Co2. Methods have been developed for Auto Analyzers4 but these suffer from baseline drift5 and require equipment which many laboratories do not have. Enzymatic methods for CO2 have been introduced by Wilson, Menson and Norris8 using phosphoenolpyruvate carboxylase. The present procedure is a enzymatic assay utilizing Phosphoenolpyruvate Carboxylase (PEPC) and a NADH analog.

$$\text{PEP} + \text{HCO3-} \xrightarrow{\text{PEPC} + \text{Mg}^{++}} \text{Oxaloacetate} + \text{H2PO4-}$$

$$\text{Oxaloacetate} + \text{reduced cofactor} + \text{H+} \xrightarrow{\text{MDH}} \text{Malate} + \text{cofactor}$$

Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEPC), to form oxaloacetate. The cofactor then in the presence of malate dehydrogenase (MDH) is oxidized by the oxaloacetate.

KIT CONTENTS

Reagent Name	MBIC04005M	VBIC01001M
R1-Bicarbonate Reagent	4 x 5 ml	10 x 1 ml
R2-Bicarbonate Calibrator	1 vial	1 vial

Please refer the calibrator value mentioned on the via label.

WORKING REAGENT PREPARATION AND STABILITY

The reagents are stable at 2-8°C till expiry date printed on the package when stored at 2-8°C.

CONCENTRATIONS IN THE TEST

PEP	7.0 mmol/L
Mg ++	8.0 mmol/L
PEPC	≥ 500 U/ml
MDH	≥ 600 U/ml
NADH	0.45 mmol/L

WARNINGS AND NOTES

-Product for in vitro diagnostic use only.

1. A sample with concentration over 50mmol/L should be diluted by 0.9% NaCl and re-assayed.
2. Do not pipette by mouth to avoid CO2 exhalation.
3. Venous blood is recommended, and collect it air-tightly.

GENUINE BIOSYSTEM

4. Sample should be stored at 2~8°C and analyze within 1 hour.
5. Sodium azide is included, avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with water. In case of contact with eyes or if ingested, seek immediate medical attention.
6. Discard the kit when it is muddy or the absorbency of reagent blank < 1.6.

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 405 nm
- Thermostat at 37°C
- General laboratory equipment

SPECIMEN

Fresh Serum, heparinized plasma.

EDTA, citrate and oxalate should not be used as anticoagulants, as they will affect results. Samples should be kept tightly closed, as CO_2 will diffuse from the sample causing erroneous values.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analyzers. Applications for them are available on request.

MANUAL PROCEDURE

Wavelength	405 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Calibrator (C)	Test (T)
R1 Bicarbonate Reagent	1000 µl	1000 µl	1000 µl
Bring up the temperature of determination. Then add,			
Distilled Water	10 µl		
R2 - Bicarbonate calibrator		10 µl	
Sample			10 µl

Mix well, incubate for 8 minutes at 37°C and read the absorbance of calibrator, sample against reagent blank.

CALCULATION

Bicarbonate concentration = Abs.(T) / Abs(S) x calibrator concentration

REFERENCE VALUES

22.0 to 29.0 mmol/l

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

Linearity: 50mmol/L

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Roger L.Forrester,et al. Clin Chem , 1976, 22: 243~245
2.Methods of Enzymatic Analysis(Bergmeyer,ed.) 1985, VII:572~577
3.National Clinical Laboratory Operator Program, Ye Ying-wu, etal,1997,186 ~ 187

SYSTEM PARAMETERS

Method	End Point
Wavelength	405 nm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	8 mins
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Standard vial
Units	mmol/l
Factor	----
Reaction Slope	Decreasing
Linearity	50 mmol/l

IVD CE

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