BICARBONATE

(PEPC)

ENZOPAK

Last update 09-2020

Ref. CC1-HCO.036, 20x1.1 ml

INTENDED USE

This reagent is intended for the *in-vitro* quantitative determination of total CO_2 in human serum or plasma.

INTRODUCTION

Approximately 90% of carbon dioxide present in serum or plasma is in the form of bicarbonate, the measurement of bicarbonate, usually in conjunction with tests such as glucose, urea, sodium, potassium and chloride is useful in the assessment of disturbances of acid base balance resulting from metabolic or respiratory causes.

PRINCIPLE

This reagent is based upon phosphoenolpyruvate carboxylase (PEPC) utilizing bicarbonate present in the sample to produce oxaloacetate and phosphate. Malate dehydrogenase (MDH) then catalyzes the reduction of oxaloacetate to malate and the oxidation of NADH to NAD^+. The resulting decrease in absorbance can be measured at 380nm and is proportional to the amount of bicarbonate present in the sample.

HCO_3^- + Phosphoenolpyruvate $\xrightarrow{PEPC}$ Oxalocetate + H_2PO_4

Oxaloacetate + NADH $\xrightarrow{MDH}$ Malate + NAD^+

PRESENTATION

All reagent to be stored at 2-8^{0}C	No. of Bottles/Pouches 20 x 1.1 ml.
• 1 Bicarbonate (Powder)	20
• 2 Bicarbonate (Buffer)	1
• Bicarbonate Std (25 mmol/L)	1

PRECAUTION

Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper and lead plumbing. Flush with plenty of water when disposing.

PREPARATION OF WORKING REAGENT

Reconstitute the contents of each vial of 1 Bicarbonate with the volume of 2 Bicarbonate buffer stated on the vial label. In order to minimize contamination of the reagent with CO_2

1. It is recommended that buffer bottle should be closed immediately after use. Buffer with a pH < 6.5 strongly indicative of CO_2 contamination and should not be used for reconstitution. Keep the buffer bottle in boiling water for few minutes before reconstitution.
2. Buffer which has been stored for prolonged periods should not be used for reconstitution.
3. Avoid shaking the reagent as this will increase contamination of the product with atmospheric CO_2.
4. Do not mouth pipette.

REAGENT STORAGE AND STABILITY

Prior to reconstitution when stored at 2-8^{0}C the reagent is stable until the expiration date stated on the label. After reconstitution the reagent is stable for at least 15 days at 2-8^{0}C. Discard the turbid reagent or that which has an absorbance less than 0.8 at 380 nm (1 cm) when measured against distilled water.

SPECIMEN COLLECTION AND HANDLING

Serum or heparinized plasma free of hemolysis is suitable specimens for use with this reagent. The whole blood should be collected and handled anaerobically to minimize exposure to air. Serum bicarbonate is stable for one hour when stored under anaerobic conditions in an ice bath.

REACTION PARAMETERS

- Type of reaction : End point.
- Temperature : 37^{0}C
- Wavelength : 380 nm (375-380 nm) / 340 nm
- Reaction time : 5 min
- Reagent volume : 1.0 ml
- Sample volume : 0.01 ml
- Cuvette path length : 1.0 cm
- Auto Zero : Distilled water

TEST PROCEDURE: FOR 380 nm

Pipette into test tube	Blank	Std.	Test
Working Rgt. (ml)	1.0	1.0	1.0
Distilled Water(ml)	0.01	-	-
Standard (ml)	-	0.01	-
Sample (ml)	-	-	0.01

Stopper the tube, mix well and allow to stand for 5 min. at 37^0 C and Read the absorbance of Blank, standard and test against distilled water at 380 nm (375-380 nm).

TEST PROCEDURE: FOR 340 nm:

Pipette into test tube	Blank	Std.	Test
Working Rgt. (ml)	1.0	1.0	1.0
Distilled Water(ml)	0.01	-	-
Standard (ml)	-	0.01	-
Sample (ml)	-	-	0.01

Stopper the tube, Mix well and allow to stand for 5 minutes at 37^{0}C.

Distilled Water (ml)	3.0 ml	3.0 ml	3.0 ml

Mix well and read the absorbance of blank, standard and test against distilled water at 340 nm immediately.

TEST RESULTS

A1 = Absorbance of Blank - Absorbance of Sample

A2 = Absorbance of Blank - Absorbance of Standard

$$\text{Bicarbonate (mmol/L)} = \frac{A1}{A2} \times \text{Standard Value (mmol/L)}$$

Example

Absorbance of reagent blank = 1.3

Final Absorbance of standard = 0.94

Final Absorbance of sample = 1.0

Standard value = 25 mmol/L

A1= 1.3 – 1.0 =0.30

A2 = 1.3 – 0.94 = 0.36

$$\text{Bicarbonate (mmol/L)} = \frac{0.30 \times 25}{0.36} = 20.8 \text{ mmol/L}$$

LIMITATIONS FOR INTERFERENCE

1. Bicarbonate levels are elevated or depressed due to a variety of diseases and conditions. Other tests may be necessary for differential diagnosis.
2. Keep exposure of the reagent to air to a minimum and avoid extraneous carbonate contamination.
3. For bichromatic analysers a blank wavelength of 500 nm may be used which will reduce interference from these substances.

NORMAL VALUES

23.0-29.0 mmol/L
23.0-29.0 mEq/L

LINEARITY

The Bicarbonate reagent is linear upto 50 mmol/L (50 mEq/L) at 380nm and 40 mmol/L (40 mEq/L) at 340nm.

SENSITIVITY

The sensitivity of the assay is such that a change in absorbance of 0.001 AU equals 0.08 mmol/L (0.08 mEq/L).

REFERENCES

1. Zilva JF, Pannall PR. "Hydrogen ion Homeostasis: Blood Gas level" in Clinical Chemistry in Diagnosis and Treatment. LLoyd-Luke London 1979. Chapter iv:78-113.
2. Henry RJ. Clinical Chemistry: Principles and Technics. Harper and Row New York 1974.
3. Tietz NW. Fundamentals of Clinical Chemistry, WB Saunders Co. Philadelpha 1976; 15:885.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third edition 1990; 3:57-9

Regd. Office.3/7, B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com Ph: +91·265·2281631
Email: mail@reckondiagnostics.com
An ISO 13485:2016 Certified Company

RECKON DIAGNOSTICS P. LTD.
THE TEST OF CONFIDENCE - SINCE 1989