BICARBONATE

ENZOPAK

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INTENDED USE:

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

CLINICAL SIGNIFICANCE:

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^+

WARNING:

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.

PRESENTATION:

	No. of Bottles/Pouches
All reagent to be stored at 2-8^{0}C	12 x 1.1 ml.
• 1 Bicarbonate (Powder)	12
• 2 Bicarbonate (Buffer)	1
• Bicarbonate Std (25 mmol/L)	1

WORKING REAGENT PREPARATION:

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 (1cm) when measured against distilled water.

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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

PROCEDURE: FOR 380 nm

(a) Label one test tube or cuvette for a reagent blank. Add 1.0 ml. of completely dissolved reagent to each tube or cuvette and bring to reaction temperature.
(b) Add 0.01 ml of H_2O to the reagent blank and 0.01 ml of standard, controls and unknown sample to the appropriately labelled tube. Mix and incubate for 5 minutes.
(c) Zero spectrophotometer at 380 nm with distilled or deionised H_2O.
(d) Read and record absorbance of the reagent blank.
(e) Read and record the absorbance of the Standard, controls and each unknown sample.

PROCEDURE:

PIPETTE INTO TEST TUBES	STANDARD	TEST
• WORKING REAGENT (ml)	1.0 ml	1.0 ml.
• STANDARD (ml)	0.01 ml	–
• SAMPLE (ml)	–	0.01 ml.

Mix well and allow to stand for 5 min. at 37^0 C and Read the absorbance of standard and test at 380 nm (375-380 nm).

CALCULATION:

$$\text{Bicarbonate} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 25 \text{ (mmol/Lit)}$$

PROCEDURE: FOR 340 nm:

1. Label one test tube or cuvette for a reagent blank, standard, control and unknown sample.
2. Add 1.0 ml of completely dissolved reagent to each tube or cuvette and bring to reaction temperature.
3. Add 0.01 ml of water to the reagent blank and 0.01 ml of standard, control and unknown sample to the appropriately labeled tube. Stopper the tube mix and incubate for 5 minutes at 37^{0}C
4. Add 3.0 ml Distilled water to each tube.
5. Zero spectrophotometer at 340 nm with distilled or deionised water.
6. Read and record absorbance of the reagent blank, standard, Control and each unknown sample immediately.

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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

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

Distilled Water (ml)	3.0 ml	3.0 ml	3.0 ml

Read the absorbance of blank, standard and test at 340 nm immediately.

CALCULATION:

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

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

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).

LIMITATIONS:

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.

REFERENCES:

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