

# BICARBONATE

## ENZYMATIC METHOD

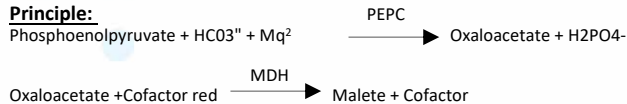
**Summary:**

Measurement of bicarbonate is used in the diagnosis of the acid-base-balance in the blood. Elevated and decreased values indicate disorders associated with disturbances of the metabolic and respiratory systems.

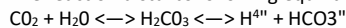
**Method:**

Enzymatic test using phosphoenolpyruvate carboxylase (PEPC) and a stable NADH analog.

**Principle:**



The reaction disturbs following equilibrium.



This results in a conversion of CO<sub>2</sub> to bicarbonate (HCO<sub>3</sub><sup>-</sup>) which then is included in the reaction. Therefore, the total CO<sub>2</sub> concentration is measured. The decrease of reduced cofactor concentration is measured at 405 or 415 nm and is proportional to the concentration of total carbon dioxide in the sample.

**Composition:**

- Buffer pH 7.5 12.5 mmol/L
- Phosphoenolpyruvate (PEP) > 400 U/L
- Phosphoenolpyruvate carboxylase (PEPC) > 4100
- Malate dehydrogenase (MDH) 0.6 mmol/L
- NADH analog
- Activators, stabilizers, surfactant, preservative
- Standard: 30 mmol/L

**Preparation and stability:**

The reagent is stable up to expiry date, if stored at 2 - 8 °C and contamination is avoided.  
Do not freeze the reagent!  
Ready for use.  
Onboard Stability: 28 days.

**Specimen:**

Serum or heparin plasma  
Serum or plasma should be separated from cells immediately and stored at 2 - 8 °C. Exposure of samples to air should be avoided. Samples should be stored tightly sealed to prevent loss of carbon dioxide and assayed as soon as possible after collection.

Stability :	1 day at	20 - 25 °C
	7 days at	4 - 8 °C
	2 weeks at	-20 °C

Discard contaminated specimens!

**Limitations - interference:**

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated bilirubin up to 50 mg/dL, free bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 1400 mg/dL triglycerides.

<b>Manual procedure:</b>	
Wavelength:	405 nm – 415nm
Temperature:	+37°C
Cuvette:	1 cm
Zero adjustment:	against reagent blank
	Sample/ Calibrator
Sample/Calibrator	5 µl
R1	500 µl
Mix, incubate for 2 minutes. And read absorbance A <sub>1</sub> and after exactly 10 min. read A <sub>2</sub> against reagent blank.	
<b>Calculation:</b>	
Bicarbonate mmol/L = [(A <sub>2</sub> - A <sub>1</sub> ) <sub>sample</sub> / (A <sub>2</sub> - A <sub>1</sub> ) <sub>standard</sub> ] x std.conc (mmol/L)	

**Measuring/reportable range:**

4-50 mmol/L  
At higher concentrations, dilute the sample with 0.9% NaCl (e.g. 1 + 1). Multiply the result by the appropriate factor (e.g. 2).

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**Expected values:**

Adults: 22-29 mmol/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the BICARBONATE results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

**Imprecision:**

Reproducibility was determined using human samples and controls in an internal protocol (n = 20). The following results were obtained:

Repeatability (within run):		
Mean concentration (mmol/L)	CV	SD (mmol/L)
17.6	0.80 %	0.14
19.9	0.80 %	0.16
30.1	0.80 %	0.28
Reproducibility (run to run):		
Mean concentration (mmol/L)	CV	SD (mmol/L)
16.8	3.16 %	0.53
20.3	2.40 %	0.49
30.0	2.26 %	0.68

**Method comparison:**

A comparison of BIOANALYTIC Bicarbonate (y) with a commercially available assay (x) using 107 samples gave following results: y = 0.989 x + 0.354 mmol/L; r = 0.998.

**Quality Control:**

Human Control Serum:

- BIOANALYTIC BICARBONATE CON L1 4 x 1 ml
- BIOANALYTIC BICARBONATE CON L2 4 x 1 ml

**Calibration:**

Standardization: This BICARBONATE method was calibrated against an international standard defined for BICARBONATE.

S1: BIOANALYTIC BICARBONATE CAL. SET

**Literature:**

- 1 Müller-Plathe O. Acid base balance and blood gases. In: Thomas L, editor. Clinical laboratory diagnostics. 1<sup>st</sup> ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 318-329.
- 2 Norris KA, Atkinson AR, Smith WG. Colorimetric enzymatic determination of serum total carbon dioxide as applied to the Vickers multichannel 300 discrete analyser. Clin Chem 1975; 21: 1093-1101.
- 3 US patent #5,801,006
- Güder WG, Zawta B et al. The Quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: GfT Verlag; 2001; p. 18-9.

**Order information (Cat No.) :**

CR530	B24045	B28045	B32045	B35045	B80045
B21045	B25045	B30045	B33045	B36045	
B22045	B27045	B31045	B34045	B37045	

**Manufacturer**

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti  
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### SYMBOLS

<b>IVD</b>	for in vitro diagnostic use only
<b>LOT</b>	lot of manufacturing
<b>REF</b>	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

