BICARBONATE

Kinetic method

5 x 25 ml **CL07-125S**

INTENDED USE
Quantitative determination of total carbon dioxide in serum and plasma.

CLINICAL MEANING
Carbon dioxide (CO_2) contained in plasma is made 90% by bicarbonate (HCO_3^-). The measurement of total CO_2 as part of an electrolytic profile (Na^+, K^+, Cl^-) is useful mainly to evaluate HCO_3^- concentration in presence of acid-basis disorders due to metabolic or respiratory dysfunctions.

PRINCIPLE
The enzymatic method used requires carbonic acid measurement as shown in the following scheme:

$$HCO_3^- + \text{phosphoenolpyruvate} \xrightarrow{\text{PEPC+ } Mg^{2+}} \text{oxaloacetate} + H_2PO4^-$$

$$\text{oxaloacetate} + \text{cofactor reduced} \xrightarrow{\text{MDH}} \text{malate} + \text{co-factor}$$

PEPC is specific for bicarbonate ion (HCO_3^-) and its action interferes with the subsequent equilibrium inducing the conversion of CO_2 in $HCO3^-$:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO3^-$$

So total carbon dioxide concentration in the sample is measured. The decrease of reduced co-factor concentration, measured spectrophotometrically at 405 or 415 nm, is proportional to total carbon dioxide concentration in the sample.

SAMPLE
Non hemolized serum or plasma treated with heparin as anticoagulant. The sample must be divided immediately from the clot / cells and analyzed as soon as possible after collection.
STABILITY: 1 day at 20-25°C, 7 days at 2-8°C; 2 weeks at -20°C.
NOTE: store the sample in an air-tight container to avoid loss of carbon dioxide.

REAGENTS
Only for in Vitro diagnostics. Liquid monoreagent ready to use.

Package components:	**CL07-125S**
REAGENT Buffer pH 7.5, Phosphoenolpyruvate 12.5 mmol/L; Phosphoenolpyruvate carboxylase (PEPC) > 400 U/L; malate dehydrogenase (mdh) > 4100 U/L; NADH 0.6 mmol/L Activators, surfactants, stabilizers, preservatives.	**5 x 25 ml**
STANDARD Values found on label	**1 x 4 ml**

STABILITY: stored at 2-8°C and kept away from the sun, reagents are stable up to the expiration date on the label.

NECESSARY ITEMS – NOT PROVIDED
Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

MANUAL ASSAY PROCEDURE

Wavelength:	405 or 415 nm
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37°C
Reaction time:	5 minutes
Linearity:	up to 50 mmol/L
Reagent/Sample Ratio:	100/1

Let reagents and samples reach room temperature before use.

Pipette in cuvette or test tubes labeled as it follows:

	Std	Sample
Reagent	1000 µl	1000 µl
Standard	10 µl	
Sample		10 µl

Mix accurately and incubate at 37°C. Read absorbance A1 exactly after 2 minutes, and absorbance A2 exactly after additional 5 minutes against blank reagent.
$\Delta A = (A2 - A1)$ sample or standard

CALCULATION
Carbon dioxyde [mmol/L] = (ΔAc / ΔAst) x standard conc. [mmol/L]

REFERENCE VALUES
22-29 mmol/L (22-29 meq/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference range if necessary.

QUALITY CONTROL – CALIBRATION
Each laboratory should implement a quality control program. In each analysis control serums should be used, both with normal and higher levels. The results must fall within the declared range.
To calibrate use the aqueous carbon dioxide standard found in the kit.

PERFORMANCES CHARACTERISTICS
CO_2 from air or the breath of the analyst is a major interference in this assay. Reagent handling, specimen collection and all storage instructions must be strictly followed to minimize this interference.

Sensitivity: the sensitivity of the method is about 2.0 mEq/L.

Linearity: up to 50 mmol/L (50 mEq/L).
For higher values, dilute samples with CO_2 free distilled water and multiply the result by the dilution factor.

Precision:

Within run (n=10)	Average (mmol/L)	SD (mmol/L)	CV %
Sample 1	10,3	0,5	1,8
Sample 2	27	0,8	1,9

Between run (n=20)	Average (mmol/L)	SD (mmol/L)	CV %
Sample 1	10,3	0,7	3,1
Sample 2	27	0,92	5,4

Interferences:

Bilirubin conjugated up to 60 mg/dl, free bilirubin up to 40 mg/dl, hemoglobin up to 500 mg/dl, lipemia up to 1400 mg/dl triglycerides. No interference was observed by ascorbic acid up to 30 mg/dl,

Correlation against a reference method

Correlation of FAR (Y) method with another kit available on the market (X) testing 85 samples gave the following result:

Y = 1.04 X – 0.028 r = 0.997

DISPOSAL
The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS
Contact with the skin and ingestion should be avoided. Use the normal precautions expected with chemicals utilization.

REFERENCES

1. Norris KA et al Clin. Chem. (1975) 21:1093.
2. Forrester RL et al. Clin. Chem.(1976) 22: p.243-245.

MANUFACTURER

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KEY SYMBOLS