

Cat. No. 12751 R 2 x 10 ml

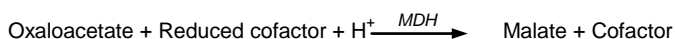
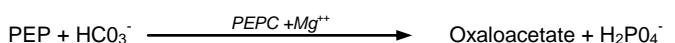
For 20 tests

Bicarbonates

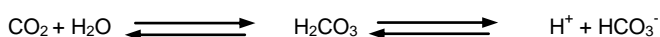
Enzymatic UV-method

Liquid Reagent

Test Principle



PEPC catalyses the first reaction which produces oxaloacetate. In the presence of MDH, the reduced cofactor is oxidized by oxaloacetate. The decrease in concentration of the reduced cofactor is monitored between 405 and 415 nm and is proportional to the total carbon dioxide concentration in the sample. PEPC is specific for the bicarbonate ion (HCO_3^-) and its action disturbs the following equilibrium which results in conversion of the CO_2 to HCO_3^-



Concentrations in the test

Buffer pH = 7.6 at 25 °C		
PEP (phosphoenolpyruvate)	12.5	mmo/L
PEPC (phosphoenolpyruvate carboxylase)	≥ 400	U/L
MDH (Malate dehydrogenase)	≥ 4100	U/L
NADH (nicotinamide adeninedinucleotide)	0.6	mmo/L
Activator, stabilizer and preservative.		

Standard: The Concentration as indicated on vial.

Stability for reagent and calibrator

HCO₃⁻ Reagent R: liquid, ready to use.

The reagent is stable up to expiry date given on label when stored at 2 - 8 °C.

Stability after open the bottle 2 month when stored at 2 - 8 °C.

Note: The reagent should be clear, turbidity indicates deterioration.

Specimen collection and handling

1. Fresh, non-hemolyzed serum collected under anaerobic conditions is the recommended specimen.
2. The specimen should be promptly separated from the clot and stored tightly sealed to prevent the loss of carbon dioxide.
3. Oxalate, citrate, and EDTA should not be used as they cause shifts of electrolytes and water between plasma and cells.
4. The sample may be stored in ice water under anaerobic conditions for up to one hour.

Calibrator

Bicarbonate Calibrator Cat. No. 16051

Quality control

Bicarbonate Control N Cat. No. 15301

Bicarbonate Control P Cat. No. 15311

Procedure

Wavelength	Hg 410 (405 - 415 nm)
Spectrophotometer	405 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against distilled water
Reaction	end point

Assay

	Blank	Standard	Sample
Blank	10 µl	---	---
Standard	---	10 µl	---
Sample	---	---	10 µl
Reagent	1000 µl	1000 µl	1000 µl

Mix well, Incubate for 5 min. at 37°C and read absorbance (A).

Calculation

$$\Delta A_{\text{Sample}} = A_{\text{Blank}} - A_{\text{Sample}}$$

$$\Delta A_{\text{Standard}} = A_{\text{Blank}} - A_{\text{Standard}}$$

$$\text{Conc. HCO}_3^- \text{ (mmo/L)} = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Calibrator}}} \times \text{Conc. Standard (mmo/L)}$$

Notes:

1. When samples are pigmented (icteric or hemolyzed) or opalescent (hyperlipemic) a sample blank should be included:
 - Place 1 ml distilled water in a test tube.
 - Add 0.01 ml (10 µl) sample.
 - Zero spectrophotometer with distilled water.
 - Read and record absorbance of serum blank.
 - Subtract sample blank absorbance from test absorbance.
 - Calculate as usual.

Linearity

Up to 50 mmol/L. Samples exceeding 50 mmol/L must be diluted (1+1) with sodium chloride solution 0.9 %, reassayed and the result multiplied by 2.

Interference

1. CO₂ from air or the breath of the analyst is a major interference in this assay. Reagent preparation, specimen collection, and all storage instructions must be strictly followed to minimize this interference.
2. A number of conditions and substances have been reported to affect serum carbon dioxide levels.

Precautions

1. Reagent contains sodium azide at 0.1%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.
2. Don't ingest.
3. Don't pipet by mouth to avoid CO₂ contamination from the expired air.

Reference range

Men / Women	22 – 29 mmol/L
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References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA., pp 884-887(1982).
2. Norris, K.A., et al, Clin. Chem. 21:1093(1975).
3. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.