DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Liquid Regents – ready to use

CARBON DIOXIDE
(CO_2, BICARBONATE)

PEP-C

Single Reagent

Diagnostic reagent for quantitative in vitro determination of bicarbonate (CO_2) in human serum or plasma on photometric systems

Ref.No.	Kit Size	Configuration
N00045B	**1 x 10 L**	Single Reagent
N03122B	**1 x 1 L**	Single Reagent
N00043/20	**5 x 50 ml**	Single Reagent
N88911	**5 x 50 ml**	Single Reagent
N0414917	**9 x 65 ml**	Single Reagent
NA0820	**5 x 20 ml**	Single Reagent
NT1020	**5 x 20 ml**	Single Reagent
NK0712	**5 x 50 ml**	Single Reagent
NB0920	**2 x 100 ml**	Single Reagent

Additionally offered:

D06520SV	1 x 3 mL	CO_2 Standard
D16525	3 x 3 mL	CO_2 Control
D16525SV	1 x 3 mL	CO_2 Control

TEST PARAMETERS

Method:	Colorimetric, endpoint, decreasing reaction, enzymatic, PEP-C
Wavelength:	405 nm, 415 nm
Temperature:	37 °C
Sample:	Serum or heparin plasma,
Linearity:	up to 50 mmol/L
Sensitivity:	The lower limit of detection is 1 mmol/L

SUMMARY [1]

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.

TEST PRINCIPLE

$$PEP + HCO_3^- \xrightarrow{PEP\text{-}C+Mg^{2+}} Oxaloacetate + H_2PO_4^-$$

$$Oxalacetate + NADH + H^+ \xrightarrow{MDH} Malate + NAD^+$$

The reaction disturbs following equilibrium:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

This results in conversion of the CO_2 to bicarbonate HCO_3^- which then is included in the reaction. Therefore, the total CO_2 concentration is measured.

The decrease in absorbance resulting from the oxidation of NADH to NAD^+ is measured at 405 or 415 nm and is proportional to the concentration of total carbon dioxide (CO_2) in the sample.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Buffer, pH 7.5		
Phosphoenolpyruvate (PEP)	12.5	mmol/L
Phosphoenolpyruvate carboxylase (PEP-C)	> 400	U/L
Malate dehydrogenase (MDH)	> 4100	U/L
NADH analog	0.6	mmol/L

REAGENT PREPARATION

The reagent is ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light. Close immediately after use Avoid contamination. Do not freeze the reagent.
Storage:	at 2 – 8 °C
Stability:	up to the expiration date indicated on labels

SAMPLE STABILITY AND STORAGE

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 [4]:	at 20 – 25 °C	1 day
	at 4 - 8 °C	7 days
	at -20 °C	2 weeks

Freeze only once!
Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution 9 g/L
General laboratory equipment

STANDARD

(not included in the kit – has to be ordered separately)

Concentration	30 mmol/L
Storage:	2 – 25 °C
Stability:	In original container up to the expiration date Once opened, the standard is stable for 3 months, if recapped immediately after use.

CLOSE IMMEDIATELY AFTER USE!
Protect from light.
Always use fresh aliquots for calibration!

MANUAL TEST PROCEDURE

Pipette into test tubes	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Sample	-	-	10 µL
Standard	-	10 µL	-
Distilled water	10 µL	-	-
Mix, incubate at 37 °C and read absorbance A1 after exactly 2 minutes against reagent blank. Incubate for exactly further 8 min. at 37 °C and read absorbance A2 against reagent blank. Calculate: ΔA = (A2 - A1) sample or standard			

CALCULATION

$$CO_2\ [mmol/L] = \frac{\Delta A\ Sample}{\Delta A\ Standard} \times Conc.\ of\ Std\ [mmol/L]$$

UNIT CONVERSION

mmol/L = mEq/L

REFERENCE RANGES [1] *

Adults: 22 – 29 mmol/L (mEq/L)

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

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine CO_2 concentrations within a measuring range from 4 to 50 mmol/L. Samples with CO_2 concentrations higher than 50 mmol/L should be diluted 1+1 with NaCl solution (9 g/L) and the results multiplied by 2.

PRECISION

Intra-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	17.6	0.14	0.80
Sample 2	19.9	0.16	0.80
Sample 3	30.1	0.28	0.93

Inter-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	16.8	0.53	3.16
Sample 2	20.3	0.49	2.40
Sample 3	30.0	0.68	2.26

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SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 1 mmol/L

INTERFERING SUBSTANCES
no interference up to:

Ascorbic acid	30 mg/dl
Bilirubin, conjugated	50 mg/dl
Bilirubin, free	40 mg/dl
Hemoglobin	500 mg/dl
Triglycerides	1400 mg/dl

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON
A comparison of Dialab Carbon Dioxide (y) with a commercially available assay (x) using 107 samples gave following results:
y = 0.989 x + 0.354 mmol/L; r = 0.998

CALIBRATION

The assay requires the use of a carbon dioxide standard or calibrator.
We recommend the Dialab **CO_2 Standard**.
This method has been standardized against a primary standard on basis of sodium carbonate.

QUALITY CONTROL

All controls with carbon dioxide values determined by this method can be used.
We recommend the Dialab **CO_2 Control**.
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special applications for automated analysers can be made on request.

WASTE MANAGEMENT

Please refer to local legal requirements.

WARNINGS AND PRECAUTIONS

1. Reagent contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

REFERENCES

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6. Bakker AJ, Mücke M Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.