BICARBONATE FL

BR F060 CH	6 x 10 ml
BR F245 CH	12 x 20 ml
BR F400 CH	4 x 100 ml

INTENDED USE

Reagent for quantitative in vitro determination of bicarbonate in biological fluids.

SUMMARY OF TEST

Bicarbonate is the second largest fraction of the anions in plasma. Total carbon dioxide (CO_2) content of plasma consists of carbon dioxide dissolved in an aqueous solution (dCO_2), CO_2 loosely bound to amine groups in proteins (carbamino compounds), HCO_3^- and small amounts of carbonate (CO_3^{2-}) ions, and carbonic acid (H_2CO_3).

PRINCIPLE OF THE METHOD

Bicarbonate reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEP-C), to form oxaloacetate and phosphate.

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

The oxaloacetate is then converted to malate by the action of malate dehydrogenase (MDH) and reduced nicotinamide adenine dinucleotide analog (NADH-analog).

$$Oxaloacetate + NADH\text{-}analog + H^+ \xrightarrow{MDH} Malate + NAD^+\text{-}analog$$

The decrease in absorbance at 405 or 415 nm resulting from the oxidation of NADH analog is proportional to the amount of CO_2 in the sample.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

CO2 R1 **F060: 6 x 10 ml (liquid) blue cap**
F245: 12 x 20 ml (liquid) blue cap
F400: 4 x 100 ml (liquid) blue cap

Composition: Buffer 0.1 M, PEP 10 mM, PEC-C > 100 U/l, MDH > 1000 U/l, NAD-analog 0.5 mM, stabilizer and preservative.

Standard: bicarbonate solution 30 mmol/l - 5 ml

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Use single reagent ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: preferably within 60 days at 2-8°C keep away from direct light sources.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, heparin plasma.
Specimens should be protected from direct exposure to light. Samples stored at 2-8°C in the dark and in closed vials are stable up to 3 days and 1 month at -20°C.

TEST PROCEDURE

Wavelenght: 415 nm (allowed 400 ÷ 415 nm)
Lightpath: 1 cm
Temperature: 37°C

dispense:	blank	standard	sample
reagent	1 ml	1 ml	1 ml
incubate at 37°C for 5 minutes			
water	10 µl	-	-
standard	-	10 µl	-
sample	-	-	10 µl

Mix, incubate 1 minute at 37°C, then record absorbance against reagent blank as A_1. After exactly 5 minutes, record again absorbance as A_2.

RESULTS CALCULATION

serum/plasma sample:

$$\text{Bicarbonate (mmol/l)} = \frac{A_2\text{-}A_1 \text{ (sample)}}{A_2\text{-}A_1 \text{ (standard)}} \times \text{standard value}$$

EXPECTED VALUES

Newborn:	13 - 22 mmol/l - mEq/l
Infant, child:	20 - 28 mmol/l - mEq/l
Adults:	22 - 29 mmol/l - mEq/l
Adults > 60yr:	23 - 31 mmol/l - mEq/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose a suitable human based control sera has to be used.
Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 50 mmol/l.
If the limit value is exceeded, it is suggested to dilute sample 1+4 with distilled water and to repeat the test, multiplying the result by 5.

Sensitivity/limit of detection (LOD)
the limit of detection is 0.7 mmol/l.

Interferences
no interference was observed by the presence of:

lipids	≤ 1700 mg/dl
bilirubin	≤ 70 mg/dl
hemoglobin	≤ 1000 mg/dl

Precision

intra-assay (n=10)	mean (mmol/l)	SD (mmol/l)	CV%
sample 1	13.78	0.12	0.87
sample 2	35.72	0.27	0.76

inter-assay (n=20)	mean (mmol/l)	SD (mmol/l)	CV%
sample 1	14.43	0.86	5.97
sample 2	35.59	1.09	3.05

Methods comparison
a comparison between Chema and a commercially available product gave the following results:

Bicarbonate Chema = x
Bicarbonate competitor = y
n = 86

$y = 1.037x - 0.191$ mmol/l $r^2 = 0.992$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

HU Bergmeyer - Methods of enzymatic analysis, third edition, Vol. VII (1987) p. 572.
Norris K.A., Atkinson A.R. and Smith W.G. - Clin. Chem. 1093-1101, 21/8 (1975)
Forrest R.L., Wataji L.J., Silverman D.A. and Pierre K.J. - Clin. Chem. 243-245, 22/2 (1976)
Peled N. - Clin. Chem. 199-200, 27/1 (1981)

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE