BECKMAN COULTER

AU/DxC AU US

Instructions For Use

CO2 BICARBONATE

REF OSR6137 4 x 25 mL
OSR6237 4 x 50 mL
OSR6637 4 x 173 mL

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Bicarbonate in human serum and plasma on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Bicarbonate measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance. The determination of bicarbonate (HCO_3^-) is used in conjunction with other clinical and laboratory information for the evaluation of acid-base status. An elevation of the Bicarbonate level may be observed in compensated respiratory acidosis and metabolic alkalosis. Low Bicarbonate levels may be observed in compensated respiratory alkalosis and metabolic acidosis.[1] Additional laboratory determinations will permit differentiation between metabolic and respiratory conditions.

METHODOLOGY

The bicarbonate reagent utilizes the enzymatic method developed by Forrester et al.[2] In this procedure bicarbonate (HCO_3^-) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MD) catalyzes the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH). This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured bichromatically at 380/410 nm proportional to the Bicarbonate content of the sample.

$$PEP + HCO_3^- \xrightarrow[\text{Magnesium}]{\text{PEPC}} \text{Oxaloacetate} + H_2PO_4$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{\text{MD}} \text{Malate} + NAD^+$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Once separated from cells, Bicarbonate in serum and plasma is stable for 1 day at 20 - 25°C, 7 days at 4 - 8°C and 2 weeks at -20°C when protected from exposure to air.[3]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum and plasma samples (sodium heparin and lithium heparin) free from hemolysis, are the recommended specimens. Obtain blood specimen aseptically by venipuncture or skin puncture. Separate serum or plasma from cells promptly to minimize hemolysis.

Oxalate, citrate and EDTA have been reported to interfere with assay results. If plasma is used, the recommended anticoagulant is heparin.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Bicarbonate Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

MD (microbial)	2,000 U/L
PEPC (microbial)	2209.0 U/L
Magnesium	2.8 mmol/L
PEP	3.46 mmol/L
NADH	1.6 mmol/L
Sodium azide as a preservative	0.095% (w/w)

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Bicarbonate Calibrator (Cat # ODC0019) or Chemistry Calibrator (Cat # DR0070)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR6637 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Bicarbonate reagents are ready to use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 to 8°C.
2. Opened reagent is stable for 7 days when stored in the refrigerated compartment of the analyzer.

INDICATIONS OF DETERIORATION

Absorbance measurements exceeding specified limits may be an indication of reagent deterioration.

Visible signs of microbial growth, turbidity or any change in color in the Bicarbonate reagent may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration is daily. Calibration of this bicarbonate procedure is accomplished by use of Bicarbonate Calibrator (Cat # ODC0019) or Chemistry Calibrator (Cat # DR0070).

Chemistry Calibrator (Cat # DR0070) is traceable to NERL Carbon Dioxide Standards (Cat # 2340-C and 2340-E).

Bicarbonate Calibrator and Chemistry Calibrator are traceable to NIST SRM 351.

Recalibration of this test is required when any one of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.
3. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Absorption of atmospheric CO_2 by the reagent on board the analyzer can impair calibration stability. This effect will vary depending upon the rate of use. Consequently each laboratory should establish a calibration frequency appropriate to their usage pattern.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mEq/L, for conversion to SI units (mmol/L) the result is multiplied by 1.0.

REPORTING RESULTS

EXPECTED RESULTS

Adult[4,5]	21 - 31 mEq/L

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS (mg/dL)

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[6] show that the following substances interfere with this Bicarbonate procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbic acid:	No significant interference up to 20 mg/dL Ascorbic Acid
Hemolysis:	No significant interference up to 500 mg/dL Hemoglobin
Icterus:	No significant interference up to 40 mg/dL unconjugated Bilirubin
	No significant interference up to 20 mg/dL Conjugated Bilirubin
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

High lactate dehydrogenase (LDH) concentrations in patient samples may cause falsely increased bicarbonate results - if markedly elevated LDH levels are suspected please assess results in conjunction with patient's LDH values.

Drugs and other substances may affect Bicarbonate determinations. The information presented is based on results from studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[7]

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LIMITATIONS

Visible signs of a crystalline precipitate which settles out upon storage may be evident in the reagent bottle. Reagent with crystalline precipitate may be used without affecting results. However, if any trends or sudden shifts in control values are detected, which are not corrected by recalibration then an alternative vial of reagent should be used.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Bicarbonate procedure is linear from 2.0 to 45.0 mEq/L[8]. Result exceeding linearity range and marked with F-flag should be reported as greater than 45.0 mEq/L.

SENSITIVITY

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined in accordance with the CLSI EP17-A2 guideline[9]. The LoB is calculated from n ≥ 60 measurements of an analyte-free sample, and corresponds to the concentration below which analyte-free samples are found with 95% confidence. The Limit of Detection (LoD) corresponds to the sample concentration above the LoB which is detectable with 95% confidence whereas Limit of Quantitation (LoQ) corresponds to the sample concentration that can be quantitatively determined with imprecision 20% CV.

LoB = 1.20 mEq/L

LoD = 1.95 mEq/L

LoQ = 1.95 mEq/L

METHODS COMPARISON

Reference[10,11]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	0.964
Intercept	-0.26

Correlation Coeff. (r)	0.9938
No. of Samples (n)	110
Range (mEq/L)	2.70 - 41.24

PRECISION

Reference[12,10]

Estimates of precision, based on CLSI recommendations[12], are consistent with typical performance. The within run precision is less than 3% CV or SD less than 1 mEq/L and total precision is less than 7% CV or SD less than 1.5 mEq/L. Assays of 3 serum pools were performed and this data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, mEq/L	**SD**	**CV%**	**SD**	**CV%**
12.3	0.30	2.5	0.92	7.5
31.0	0.35	1.1	1.23	4.0
40.3	0.34	0.8	1.47	3.6

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
CO21U	Bicarbonate OSR6x37 DR0070 Serum
CO22U	Bicarbonate OSR6x37 ODC0019 Serum

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

† Beckman Coulter System Calibrator: Cat. No. DR0070 or Bicarbonate Calibrator Cat. No.: ODC0019

* Values set for working in mEq/L (mmol/L)

* Values set for working in mEq/L

‡ Depends on usage pattern in the laboratory.

REVISION HISTORY

Update to Interference Section.

Preceding version revision history

Updated Specimen Section

Updated REPORTING RESULTS section
Updated PROCEDURAL NOTES section

Updated References section

REFERENCES

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11. CLSI/NCCLS, Method Comparison and Bias Estimation Using Patient Samples, Approved Guideline, 2nd Edition, EP09-A2, 2010.
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