RANBUT

D-3-Hydroxybutyrate
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of D-3-Hydroxybutyrate in serum and plasma. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.			
RB 1007	R1a.	Buffer	1 x 105 ml
10 x 10 ml	R1b.	Enzyme/Coenzyme	10 x 10 ml
	CAL.	Standard	1 x 5.5 ml
GTIN:	05055273205008		
RB 1008	R1a..	Buffer	10 x 50 ml
10 x 50 ml	R1b.	Enzyme/Coenzyme	10 x 50 ml
	CAL.	Standard	1 x 5.5 ml
GTIN:	05055273205015		

UV METHOD

A kinetic enzymatic method to measure the level of D-3-hydroxybutyrate in serum or plasma. The method is based on the oxidation of D-3-hydroxybutyrate to acetoacetate by the enzyme 3-Hydroxybutyrate dehydrogenase. Concomitant with this oxidation the cofactor NAD^+ is reduced to NADH and the associated change of absorbance can be directly correlated with the D-3-hydroxybutyrate concentration.

PRINCIPLE[1]

$$\text{D-hydroxybutyrate} + NAD^+ \xrightarrow[\text{dehydrogenase}]{\text{3-hydroxybutyrate}} \text{acetoacetate} + H^+ + NADH$$

SAMPLE

Serum, heparinized plasma or EDTA plasma.

REAGENT COMPOSITION

Contents		Initial Concentration of Solutions
R1a.	**Buffer**	
	Tris Buffer	100 mmol/l, pH 8.5
	EDTA	2 mmol/l
	Oxalic acid	20 mmol/l
R1b.	**Enzyme/Coenzyme**	
	NAD^+	2.5 mmol/l
	3-HBDH	0.12 U/ml
CAL.	**Standard**	
	D-3-hydroxybutyrate	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

R1a and CAL contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Please dispose of all biological and chemical materials according to local guidelines.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Buffer
Contents stable as supplied up to the expiry date if stored at +2 to +8°C.

R1b. Enzyme/Coenzyme
Cat. No. RB 1007 10 x 10 ml
Reconstitute the contents of one vial R1b with **10 ml** of Buffer R1a. Stable for 24 hours at +15 to +25°C or 7 days at +2 to +8°C.
Cat. No. RB 1008 10 x 50 ml
Reconstitute the contents of one vial R1b with a portion of Buffer R1a and then transfer the entire contents to bottle R1a, rinsing several times. Stable for 24 hours at +15 to +25°C or 7 days at +2 to +8°C.

CAL. Standard
Contents ready for use. Stable up to the expiry date if stored at +2 to +8°C.

MATERIALS PROVIDED

Buffer
Enzyme/Coenzyme
Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)

PROCEDURE

Select RBU in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

	Reagent Blank S0	Standard S1	Sample
ddH20	25 µl	-	-
Standard (CAL)	-	25 µl	-
Sample	-	-	25 µl
Reagent 1 R1	1000 µl	1000 µl	1000 µl

Mix and aspirate into the RX **monza**.

RX MONZA CALIBRATION
Recommended with change of reagent lot or as indicated by quality control procedures, using CAL Standard supplied in the kit.

FOR MANUAL USE

Wavelength:	340nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm light path
Temperature:	+37°C
Measurement:	against reagent blank

Pipette into test tubes:

	Micro		Semi Micro	
	Standard or Sample	Reagent Blank	Standard or Sample	Reagent Blank
Standard or sample	75 µl	---	25 µl	---
Distilled Water	---	75 µl	---	25 µl
Reagent	3.00 ml	3.00 ml	1.00 ml	1.00 ml

Mix, incubate for 60 seconds at +37°C and then take the first reading. Read again after 1 and 2 minutes. Determine the mean absorbance change per minute (–A) and use this in the calculation.

MANUAL CALCULATION
D-3-hydroxybutyrate Concentration (mmol/l) =

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{standard conc.}$$

QUALITY CONTROL
Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants, i.e. bacterial growth, may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCES
Hb was tested up to 1 g/dl and was found not to interfere.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [3]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

NORMAL VALUES
Plasma fasting levels = 0.03 - 0.3 mmol/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance data were obtained using a RX **monza** analyzer at +37°C.

LINEARITY
The test is linear between concentrations of 0.100 and 5.75 mmol/l. Samples over this concentration should be diluted 1 + 2 with 0.9% NaCl solution and the result multiplied by 3.

SENSITIVITY
The minimum detectable concentration of D-3-Hydroxybutyrate with an acceptable level of precision was determined as 0.100 mmol/l.

PRECISION

Within run precision

	Level 2	Level 3
Mean (mmol/l)	0.324	1.17
SD	0.012	0.044
CV (%)	3.78	3.76
n	20	20

Total precision

	Level 2	Level 3
Mean (mmol/l)	0.324	1.17
SD	0.017	0.059
CV (%)	5.25	5.06
n	20	20

METHOD COMPARISON
The Randox method (Y) was compared to another commercially available test kit (X). 44 patient samples with values spanning the range 0.39 to 2.8mmol/l were tested. Linear regression analysis of the data resulted in the following equation.

Y = 1.045 X -0.0553
and a correlation coefficient of r = 0.9954

REFERENCES

1. McMurray, C.H., Blanchflower, W.J., Rice, D.A., *Clin Chem*., 1984; **30:** No. 3.
2. Li, P.K., Lee, S.T., Macgillvray, M.H., *et al. Clin. Chem.* 1980; **26:** 1713-1717.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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