

Liquid Reagents – ready to use

β-HYDROXYBUTYRATE

Enzymatic, NBT

2 Reagents

Diagnostic reagent for quantitative in vitro determination of β-Hydroxybutyrate in human serum or plasma on photometric systems

REF

Cont.

D10017	5 x 25 ml	4 x 25 ml	Reagent 1
		1 x 25 ml	Reagent 2

Additionally offered:

D10090SV	1 x 3 ml	β-Hydroxybutyrate Standard	
D98485	5 x 3 ml	Calibrator	Diacal Auto
D98481	12 x 5 ml	Control normal	Diacon N
D98482	12 x 5 ml	Control abnormal	Diacon P

TEST PARAMETERS

Method: Enzymatic, NBT
Increasing Reaction, Endpoint

Wavelength: 546 nm

Temperature: 37°C

Sample: Serum, plasma

Linearity: up to 6.9 mmol/L

Sensitivity: The lower limit of detection is 0.01 mmol/L

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Buffer, pH 8.4	115 mmol/L
β-Hydroxybutyrate dehydrogenase	≥ 3 kU/L
Diaphorase	2.1 kU/L
Reagent 2:	
NAD ⁺	21 mmol/L
Oxalic acid	66 mmol/L
Nitroblue tetrazolium (NBT)	1.7 mmol/L

REAGENT PREPARATION

Substrate Start:

Reagents are ready for use.

NOTE: After a long cool storage, a slightly violet coloured sediment in Reagent 2 can accrue which does not influence the measurement. However, it should be re-dissolved into solution by shaking the bottle gently before further measurement.

Sample Start:

Not possible.

REAGENT STABILITY AND STORAGE

Conditions: protect from light
close immediately after use
do not freeze the reagents!

Storage: at 2 – 8 °C
Stability: up to the expiration date

SAMPLE STABILITY AND STORAGE

serum, plasma: [3] at 20 – 25°C 1 month
at 2 – 8°C 1 month
at -20 °C 1 month

Discard contaminated specimens.

STANDARD

(has to be ordered separately)

Concentration 1,00 mmol/L
Storage: 2 – 8°C
Stability: up to the expiration date
CLOSE IMMEDIATELY AFTER USE!

INTERFERING SUBSTANCES

no interference up to:

ascorbic acid 30 mg/dl
bilirubin 60 mg/dl
haemoglobin 500 mg/dl
triglycerides 2400 mg/dl

The addition of oxalic acid to the reagent eliminates interferences with lactate and lactate dehydrogenase.

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate start

Pipette into test tubes	Blank	Cal.	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample or Std./Cal.	-	25 µl	25 µl
Distilled water	25 µl	-	-
Mix. Incubate 3 – 5 min. at 37°C. Read absorbance A1 against reagent blank, then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix. Incubate exactly 5 min. at 37°C and read absorbance A2 against reagent blank. ΔA = (A2 – A1)			

CALCULATION

With standard or calibrator

$$\beta\text{-Hydroxybutyrate} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std./Cal.}} \times \text{Conc. Std./Cal [mmol/L]}$$

UNIT CONVERSION

mg/dl x 0.096 = mmol/L

REFERENCE RANGE [1]

	[mmol/L]	[mg/dL]
Fasting	0.02 – 0.27	0.21 – 2.81

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

TEST PRINCIPLE

β -Hydroxybutyrate + NAD⁺ $\xrightarrow{\beta\text{-Hydroxybutyrate-dehydrogenase}}$ Acetoacetate + NADH + H⁺

NADH + NTB (oxidised) $\xrightarrow{\text{Diaphorase}}$ NAD⁺ + NTB (reduced)

The absorbance of the blue dye at 546 nm is proportional to the β -hydroxybutyrate concentration in the sample.

PERFORMANCE CHARACTERISTICS

LINEARITY

The test has been developed to determine β -Hydroxybutyrate concentrations within a measuring range from 0.01 mmol/L to 6.9 mmol/L.

If values exceed this range, samples should be diluted 1+1 with NaCl solution (9 g/L) and the results multiplied by 2.

PRECISION (at 37°C)

Intra-assay n = 20	Mean [mmol/L]	SD mmol/L]	CV [%]
Sample 1	0.30	0.004	1.31
Sample 2	0.75	0.008	1.02
Sample 3	1.13	0.006	0.53

Inter-assay n = 20	Mean mmol/L]	SD mmol/L]]	CV [%]
Sample 1	0.26	0.01	3.78
Sample 2	1.02	0.02	2.33
Sample 3	2.14	0.05	2.47

METHOD COMPARISON

A comparison of Dialab β -Hydroxybutyrate (y) with a commercially available test (x) using 120 samples gave following results:

$$y = 1.00 x + 0.003 \text{ mmol/L}; r = 0.999.$$

QUALITY CONTROL

All control sera with β -hydroxybutyrate values determined by this method can be used.

We recommend:

REF

Cont.

D98481 12 x 5 ml **DIACON N** Assayed Control Serum Normal

D98482 12 x 5 ml **DIACON P** Assayed Control Serum Abnormal

CALIBRATION

The assay requires the use of a β -hydroxybutyrate standard or calibrator.

We recommend:

REF

Cont.

D10090SV 1 x 3 ml **β -Hydroxybutyrate Standard**

D98485 5 x 3 ml **DIACAL AUTO** Assayed Multi Calibration Serum

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p 155-60
2. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 785-787.
3. Data on file at Dialab GmbH

2°C 8°C

IVD



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