

EN

## β-HYDROXYBUTYRATE

REF 984325

1	3 x 16 ml Reagent 1
2	3 x 4.5 ml Reagent 2

### INTENDED USE

Reagent for photometric determination of β-Hydroxybutyrate in homogenous liquid samples using automated Thermo Scientific Arena or Gallery analyzer.

### METHOD

Enzymatic test with D-3-Hydroxybutyric-Dehydrogenase (HBDH) and Nitrotriazolium Blue (NTB).  
Method is performed at 37 °C, using 540 nm filter.

### PRINCIPLE OF THE PROCEDURE

β-Hydroxybutyrate + NAD<sup>+</sup> → HBDH → Acetoacetate + NADH + H<sup>+</sup>  
NADH + NTB (oxidized) → Diaphorase → NAD<sup>+</sup> + NTB (reduced)

### REAGENT INFORMATION

Reagent 1 (R1) 3 x 16 ml  
Reagent 2 (R2) 3 x 4.5 ml

**Note:** Labels of reagents vials have two barcodes.

For Arena analyzers, turn the short barcode on the left side to the barcode reader.

For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.

### Concentrations

R1	Buffer	pH 8.4
	HBDH	≥ 2 kU/l
	Diaphorase	≥ 1.7 kU/l
R2	NAD	≥ 4.3 mmol/l
	NTB	≥ 0.34 mmol/l

### Precautions

See the separate Safety Data Sheet for hazardous components.

The reagent R1 contains sodium azide (< 0.1 %) and the reagent R2 contains ProClin 300 as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

### Preparation

The reagents R1 and R2 are ready-to-use.

**Note:** Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

**Warning:** after long storage in a refrigerator, it is possible that a slightly coloured violet precipitate appears. This does not influence the result, but the precipitate should be resolved through gentle swirling of the bottle.

### Storage and Stability

Reagents in unopened vials are stable at 2...8 °C until the expiry date printed on the label. Do not freeze the reagents.

Refer to the Application Notes of your analyzer for the on board stability of reagents.

### SAMPLES

#### Sample Type

Food and other sample material.

#### Sample concentration and Arena/Gallery application

All method related details are in the separate application note.

Arena and Gallery applications have a primary dilution of 1+0, this means that the automated dilution is not in use.

#### Sample preparation

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter. Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml Sample).

#### Carrez clarification:

Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium ferrocyanide, 85 mmol = 3.60 g K<sub>4</sub>[Fe(CN)<sub>6</sub>] × 3 H<sub>2</sub>O/100 ml), 5 ml Carrez-II-solution (zinc sulphate, 250 mmol = 7.20 g ZnSO<sub>4</sub> × 7 H<sub>2</sub>O/100 ml) and 10 ml 0.1 M NaOH. Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

### TEST PROCEDURE

See a separate application for the Arena or Gallery analyzer.

### Materials required but not provided

Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

β-Hydroxybutyrate Std Cat no. 984392 (one level, water based) is not included in the kit.

### Calibration

Water based β-Hydroxybutyrate Std can be used or other. Ordering code for β-Hydroxybutyrate Std is 984392 (3x3 ml). The standard is ready-to-use.

### Quality Control

Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:

β-Hydroxybutyrate Std can be used. If β-Hydroxybutyrate Std is used also for calibration, an additional internal control is recommended to be used.

### CALCULATION OF RESULTS

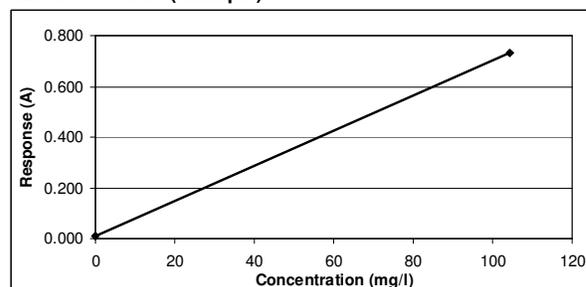
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:

mg/l × 0.009606 = mmol/l

mmol/l × 104.10 = mg/l

### Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (mg/l)
Water	0.009	0
HB Std	0.734	104

Calibration factor of this example is 143.7.

Note that the calibration curve is lot dependent.

#### LIMITATIONS OF THE PROCEDURE

##### Interference

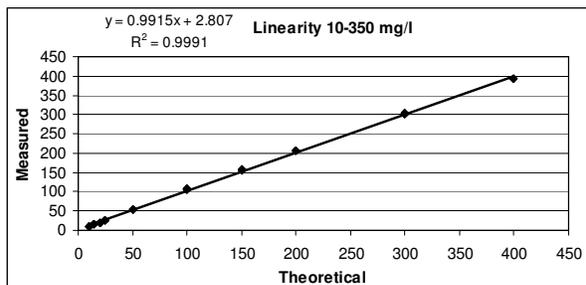
The determination is specific for  $\beta$ -Hydroxybutyrate. L-Hydroxybutyric acid is not measured.

##### MEASURING RANGE

The test has been developed to determine  $\beta$ -Hydroxybutyrate concentrations within a measuring range from 10 to 350 mg/l.

##### PERFORMANCE CHARACTERISTICS

The results obtained in individual laboratories may differ from the performance data given.



##### Determination limit (=Test limit low)

The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 10 mg/l.

##### Precision

###### Arena analyzer

	Mean 52 mg/l		Mean 156 m/l		Mean 249 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.413	0.8	1.176	0.8	1.239	0.5
Between run	0.372	0.7	1.326	0.9	2.131	0.9
Total	0.556	1.1	1.772	1.1	2.465	1.0

A precision study was performed using Arena 20XT for 5 days, with the number of measurements being n = 50.

###### Gallery analyzer

	Mean 20 mg/l		Mean 254 m/l		Mean 356 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.179	0.9	1.191	0.5	2.070	0.6
Between run	0.357	1.8	3.707	1.5	4.199	1.2
Total	0.400	2.0	3.894	1.5	4.682	1.3

A precision study was performed using Gallery for 5 days, with the number of measurements being n = 50.

##### WASTE MANAGEMENT

Please refer to local legal requirements. Empty the cuvette waste bin daily immediately after the analysis.

##### OTHER REMARKS

All results must be verified by laboratory quality control samples.

Manufacturer does not warrant that the product is error-free or will accomplish any particular result. In no event shall the manufacturer be liable for special, incidental, indirect, punitive or consequential damages (including, but not limited to, loss of profits, loss of goodwill, loss of data or loss of use damages) arising out of the use or disposition of the products.

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##### Date of revision (yyyy-mm-dd)

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##### Changes from previous version

New insert.