



GHB Enzymatic Assay

Procedure

KK-GHB-U

Pre-Analytics

Sample required: ~8 µl urine, serum

Sample storage: urine, serum: at 2-8°C for at least 2 weeks; at -20°C for longer storage

Special Equipment

Open clinical chemistry analyzer: optical filter at 340 nm; incubation chamber at 37 °C

Test components

Reagents	KK-GHB	Comments
Incubation buffer	1 x 12 mL	ready to use
Cofactor	1 x lyoph.	add 5.6 mL DM H ₂ O
Enzyme	2 x lyoph.	add 4.2 mL DM H ₂ O, do not vortex
Calibrators (10, 100 mg/L)	2 x lyoph.	add 2 mL DM H ₂ O
Controls low/high (appr. 15/75 mg/L)	2 x lyoph.	add 2 mL DM H ₂ O

Limitations

Positive GHB results should be confirmed by chromatographic methods like ionic chromatography or GC-MS.

Automated Assay Procedure

BÜHLMANN GHB is an enzymatic assay to be performed on clinical chemistry analyzers according to specific protocols provided upon request.

Dissolve lyophilized reagents 15 min prior to starting the assay.

Assay Procedure (Konelab 30)

100 µl Incubation buffer (R1)

+ 8 µl sample (S)

+ 7 µl DM H₂O

+ 50 µl Cofactor (R2)

1.5-2 min Incubation at 37°C

↓ ↻ +85 µl Substrate (R3)

Read immediately at 340 nm (M1)

Incubate for 5-6 min at 37°C

↓ ↻ Read again at 340 nm (M2)

Instrument Calibration

The standard curve is programmed with 2 calibrators using a linear regression mode. Absorbance is read twice using endpoint mode at 340 nm.

Validated Applications

Siemens Viva E (Selectra E)

Siemens Advia 1800

Beckman AU400/640 (offline sample dilution required)

Beckman AU680

Roche Cobas Mira

Roche Cobas 6000

Roche Hitachi 912

Thermo Konelab T-series

Onboard Stability

The enzyme can be kept at up to 15 °C for 2 months.

Interfering Substances

Interfering Substances were evaluated on the Thermo Konelab 30.

Therapeutic drugs and drugs of abuse: Common therapeutic drugs and drugs of abuse that have been tested showed no interference. Refer to the instruction for use to obtain further details.

Ethanol: 1 g/L Ethanol raises the GHB value by 3 mg/L. Up to 3‰ the measured GHB concentration is below 10 mg/L.

Serum Indices: No interference is detected with the following substances up to the listed concentrations: Triglycerides (Intralipid® 275 mg/dL; equivalent to 7.7 mmol/L triglycerides), conjugated bilirubin (360 µmol/L; 30 mg/dL), unconjugated bilirubin (513 µmol/L; 30 mg/dL) or haemoglobin (3.1 mmol/L; 500 mg/dL) on Konelab 30.

GHB Enzymatic Assay

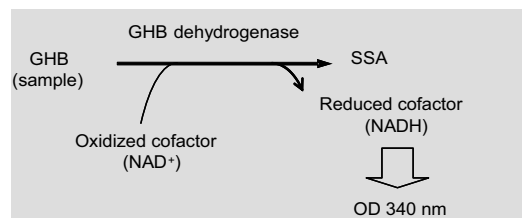
Characteristics KK-GHB-U

Intended Use

Direct determination of Gamma-hydroxybutyric acid (GHB) in urine and serum by enzymatic assay. For Research Use Only in the US. Not for use in diagnostic procedures.

Principle of the Assay

GHB is metabolized by a GHB-specific recombinant dehydrogenase. Oxidized nicotinamide adenine dinucleotide (NAD⁺), a cofactor is transformed to NADH during the reaction. The formation of NADH can be measured at 340 nm and is directly proportional to the amount of GHB present in the sample.



Conversion of GBL to GHB

Being converted to the active metabolite GHB in the body, GBL, a GHB precursor is often consumed as a drug itself. KK-GHB is a screening test for GHB. However, if requested, a conversion can be achieved by a sample pre-treatment at basic pH:

- Add 25 µL 2 N NaOH to 1000 µL urine to obtain a final concentration of 50 mM NaOH and vortex. Afterwards, the samples can directly be analyzed.

Assay Performance Data

Assay performance characteristics have been determined on Konelab 30:

Dynamic Range		5 - 230 mg/L
Analytical Sensitivity:	LoB	<1.0 mg/L
	LoD	1.5 mg/L
Functional Sensitivity :	LoQ	
Urine:		5.0 mg/L
Serum:		<5.0 mg/L
Total Precision serum and urine:		<10 % CV
Repeatability serum and urine:		<5 % CV
Between day precision serum and urine:		<5 % CV
Between run precision serum and urine:		<10 % CV
Linear Range		5 - 230 mg/L
Spiking Recovery	Urine	95 - 107 %
	Serum	106 - 113 %

Enzyme Specificity

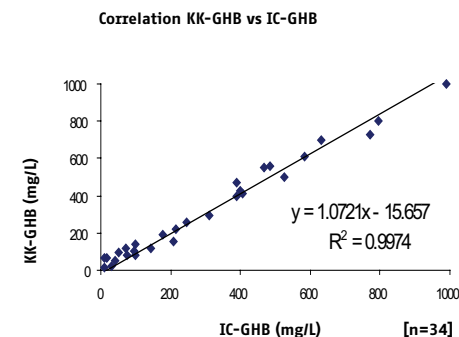
Gamma-Butyrolactone (GBL):	4 %
Other analogues or precursors: (GHV, GVL, 1,4-BD, BHB (R,S,R/S), AHB (R,S,R/S), succinic acid)	<0.1 %

For Research Use Only in the US

GHB Enzymatic Assay is available for Research Use Only in the US for GHB quantification in urine and serum samples. Not for use in diagnostic procedures.

Correlation to Reference Methods

A high correlation to ionic chromatography and LC-MS/MS has been shown.



Ordering code: GHB Enzymatic Assay is available For Research Use Only in the US. KK-GHB-U Not for use in diagnostic procedures.

Canada: GHB Enzymatic Assay is Health Canada Licensed (No. 89980).
Ordering code: KK-GHB.



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