



GHB Enzymatic Assay

Procedure

KK-GHB

A Commitment to Diagnostics

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 CE-certified 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

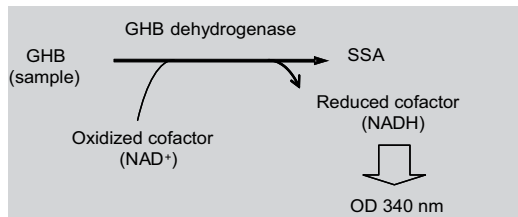
A Commitment to Diagnostics

Intended Use

For in vitro diagnostics. Direct and quantitative determination of Gamma-hydroxybutyric acid (GHB) in urine and serum by enzymatic assay.

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: >0.1 %
GHV, GVL, 1,4-BD, BHB (R,S,R/S),
AHB (R,S,R/S), succinic acid

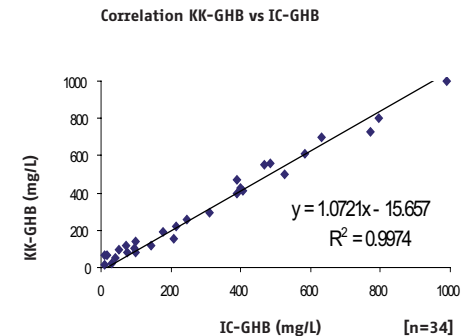
Expected Values

Reference values have been established from individuals, who did not consume GHB.

	Urine	Serum
n	46	65
Median (mg/L)	<1.5	<1.5
97.5 th Percentile (mg/L)	3.1	6.1

Correlation to Reference Methods

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



Ordering code:

KK-GHB

CE-marked product



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