



GHB

Procedure

KK-GHB

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 H ₂ O
Enzyme	2 x lyoph.	add 4.2 ml H ₂ O, do not vortex
Calibrators (10, 100 mg/L)	2 x lyoph.	add 2 ml H ₂ O
Controls low/high (appr. 15/75 mg/L)	2 x lyoph.	add 2 ml 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 H₂O deionized

+ 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

Every 10 days, whenever a fresh vial is used, or when the controls are out of their confidence range. The standard curve is programmed with 2 calibrators using a linear regression mode. Absorbance is read twice using endpoint mode at 340 nm.

CE protocols available

Siemens/Dade Viva E (Selectra E)
Olympus AU 400/640 (offline sample dilution required)
Roche Cobas Mira
Roche Cobas 6000
Roche Hitachi 912
Thermo Konelab T-series

Onboard stability

Important: Keep the Enzyme at 2-6 °C. If the analyzer in use does not fulfil this requirement, reagents should be recapped and stored in the refrigerator below 6°C. On board, a temperature above 6°C may cause a loss of enzyme activity. Thus the OD obtained in the end point measurement might decrease. Controls included in the kit can be used to monitor the assay performance. If the controls are outside the indicated confidence ranges, a recalibration of the standard curve is mandatory.

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

Characteristics

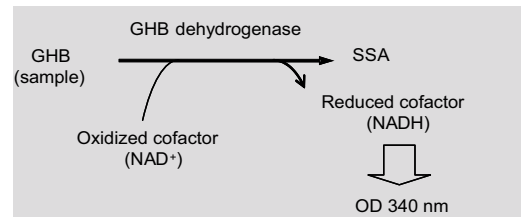
KK-GHB

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 μ l **2 N NaOH** to **1000 μ l 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 - 250 mg/L

Analytical Sensitivity: 1.5 mg/L

Functional Sensitivity : 2.8 - 4.5 mg/L

Urine: 2.8 mg/L

Serum: 4.5 mg/L

Precision: <10 % CV

Intra-Assay Precision

Urine at 10 - 100 mg/L: 0.8 - 4.6 % CV

Serum at 10 - 100 mg/L: 1.0 - 3.8 % CV

Inter-Assay Precision

Urine at 10 - 100 mg/L: 2.4 - 7.4 % CV

Serum at 10 - 100 mg/L: 2.7 - 8.3 % CV

Dilution Linearity

Mean urine (98 - 102 %): 100 %

Mean serum (100 - 110): 105 %

Spiking Recovery

Mean urine (102 - 115 %): 105 %

Mean serum (103 - 112 %): 109 %

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

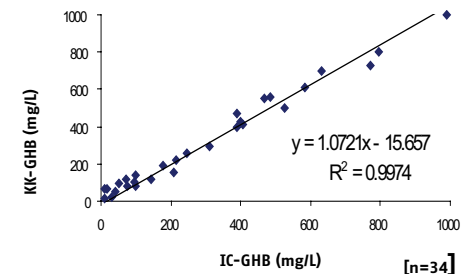
Reference values have been established from individuals, who did not consume GHB.

	Urine	Serum
n	75	50
Median (mg/L)	3.3	0.7
97.5 th Percentile (mg/L)	10.6	4.0

Correlation to Reference Methods

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

Correlation KK-GHB vs IC-GHB



Ordering code:

KK-GHB



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