

Enzymatic Assay on Clinical Chemistry Analyzers

Laborious and costly chromatographic methods so far limit the routine measurement of GHB.

The BÜHLMANN enzymatic GHB assay allows quick, easy and cost efficient quantification of GHB abuse or intoxication:

- Adaptable to most clinical chemistry analyzers
- Direct measurement of urine and serum samples
- Results within 10 min
- High correlation to chromatographic methods

US: Available For Research Use Only for GHB quantification in urine and serum samples. Not for use in diagnostic procedures.





Introduction

Gamma-hydroxybutyric acid (GHB) is an endogenous substance present in the central nervous system (CNS). GHB binds at the specific excitatory GHB receptor and is a weak antagonist for the inhibitory GABA3 receptor.

In the past, GHB was used as an anesthetic, and in treatment of insomnia and clinical depression. Today, the only accepted therapeutic applications of GHB is treatment of alcoholism and narcolepsy.

Gamma-hydroxybutyric acid (GHB) well known as Liquid Ecstasy and Liquid X is voluntarily consumed not only by partygoers: lt is increasingly used to substitute expensive more drugs. Furthermore, GHB is administered on the sly to daze people in order to rape or rob them (k.o. drops).

At low doses. GHB causes euphoria, increases enjoyment of movement and music, libido and sociability. Effects of GHB at higher doses include nausea, dizziness, drowsiness, agitation, visual disturbances, depressed breathing, amnesia, unconsciousness, and death. The duration of GHB effects lasts from 1.5 to 3 hours or even longer, when consumed in large doses or mixed with alcohol.

GHB Dosage

In general, doses between 0.5 and 3 g are consumed, corresponding to approximately 0.5 to 3 ml of liquid at a concentration of 1 g/ml. The exact dosage is difficult and thus consumers are at high risk of GHB intoxication.

Since GHB has been banned by most countries as а drug of abuse, increased consumption of GBL was observed. GBL and other precursors of GHB will be processed very fast to GHB in the organism and show the same effect and symptoms.

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BÜHLMANN GHB enzymatic assay



Intended Use

The BÜHLMANN enzymatic GHB kit is designed for the direct determination of GHB present in human urine and serum samples.

Principle of the Assay

GHB is converted to its metabolite SSA by recombinant GHB-dehydrogenase. During the reaction Nicotinamide adenine dinucleotide (NAD+) is reduced to NADH which can be detected at 340 nm. The increase in absorbance resulting from the reduction of NAD+ into NADH is proportional to the amount of GHB in the sample (see below).

Application on Clinical Chemistry Analyzers

The BÜHLMANN GHB enzymatic assay can be applied on clinical chemistry analyzers allowing a three reagent protocol according to the specific application note.

The sample (urine/serum) can be pipetted directly from the primary tube to the reaction tube without any preparation steps.

Incubation buffer and cofactor are added to the reaction tube and mixed. After a short incubation of 2 minutes the added recombinant enzyme is and mixed. The result is calculated from two point linear regression mode after 5 minutes when the reaction is complete. Calibration is stable for 10 days.

Assay Performance Characteristics

On Konelab 30 the following test characteristics have been established:

Dynamic Range	5 to 250 mg/L
Analytical Sensitivity	1.5 mg/L
Functional Sensitivity	serum 4.5 mg/L
	urine 2.8 mg/L
Total Precision	<10 %CV

Correlation Enzymatic vs. Chromatographic GHB Assay



GHB Ion chromatography [mg/L]

Fig.: 1: Correlation enzymatic vs. chromatographic GHB assay

Evaluation of the assay shows comparable sensitivity and an excellent correlation to well established chromatographic methods.

For Research Use Only in the US

GHB Enzymatic Assay is available for Research Use Only in the US. It can be employed for GHB quantification i n u rine a nd serum samples in a research setting. Not for use in diagnostic procedures.

Ordering Code: KK-GHB-U

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

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

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