α - HBDH DGKC
Multi-Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	*Quantity*	*Reagent*	*Storage*
GL708HD	10 x 15 ml 2 x 15 ml	HBDH DGKC - 1 HBDH DGKC - 2	2 - 8°C
GL718HD	5 x 50 ml 1 x 50 ml	HBDH DGKC - 1 HBDH DGKC - 2	2 - 8°C

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative, kinetic determination of α-Hydroxybutyrate Dehydrogenase (α-HBDH) in serum and plasma, based on the recommendations of DGKC, on automated and semi-automated analysers

SUMMARY AND EXPLANATION: [1]

Utilising alpha-oxobutyrate (instead of pyruvate) as substrate in the LDH reaction results in an increased rate only in the presence of LDH1 and LDH2. Thus the assay helps in the differentiation of nonspecific LDH determinations. Elevated HBDH usually indicates the activity of the cardiac LDH isoenzyme

PRINCIPLE OF THE TEST: [2]

This is an optimised standard method based upon the recommendations of the Deutsche Gesellschaft fur Klin Chemie: The rate of NADH consumption is directly proportional to the α-HBDH activity in the patient sample.

α-oxobutyrate + NADH + H$^+$ $\xrightarrow{\alpha\text{–HBDH}}$ α-hydroxybutyrate + NAD$^+$

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Colourless liquid.
Reagent 2: Clear, pale beige liquid.
Any significant changes from the above could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety Precautions:
Product is not hazardous under EU specification. Contain minute quantity of Sodium Azide. Material Safety Data Sheet is available upon request.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	*Ingredients*	*Concentration in Tests*
Reagent 1	PHOSPHATE Buffer pH 7.5	50 mmol/l
	2-Oxobutyrate	3 mmol/l
	PRESERVATIVES	---
Reagent 2	NADH	0.18 mmol/l
	PRESERVATIVES & STABILISERS	---

REAGENT PREPARATION AND STABILITY:

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label.

Monoreagent procedure: Add 1 volume of Reagent 2 to 5 Volumes of Reagent 1.
Working reagent is stable 4 weeks at 2-8°C.

Bireagent procedure: Liquid reagent 1 and 2 are ready for use.

TYPE OF SPECIMEN:

Use serum free of haemolysis as specimen.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum should be separated from cells as soon as possible after collection.

Stability: up to 4 Days at 2-8°C.

TEST PROCEDURE:

Materials required but not supplied:

Description	*Catalog. No.*	*Description*	*Catalog. No.*
General Chemistry Calibrator	GL983	Photometer	N/A
General Chemistry Control Level 1	GL922	General Laboratory Equipment	N/A
General Chemistry Control Level 2	GL932	Saline solution 0.9 g/l NaCl	N/A

Assay procedure:
Wavelength: λ: 340 nm (Hg 334 - 365)
Temperature: 25°C (30°C or 37°C)
Optical path: 1 cm light path.

MONOREAGENT PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Working reagent	1000 µl	1000 µl	1000 µl
Sample	----	----	30 µl
Calibrator	----	30 µl	----
Gently mix and Incubate at 25°C, 30°C or 37°C for 1 minute. Measure the change of Optical Density per minute (ΔOD/min) over the next 3 minutes.			

Factor Calculation:
340 nm: U/l = ΔOD/min x 5450 334 nm: U/l = ΔOD/min x 5556 365 nm: U/l = ΔOD/min x 10098

BIREAGENT PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	30 µl
Calibrator	----	30 µl	----
Gently mix and Incubate at 37°C for 3 minutes			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and Incubate at 37°C for 1 minutes Measure the change of Optical Density per minute (ΔOD/min) over the next 4 minutes			

Factor Calculation:
340 nm: U/l = ΔOD/min x 6507 334 nm: U/l = ΔOD/min x 6633 365 nm: U/l = ΔOD/min x 12057
*The above factors should be validated using General Chemistry Calibrator (GL973).

Enzyme Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the laboratory Q.C. Programme.

CALCULATION:

MONOREAGENT PROCEDURE:
340 nm: U/l = ΔOD/min x 5450 334 nm: U/l = ΔOD/min x 5556 365 nm: U/l = ΔOD/min x 10098

BIREAGENT PROCEDURE
340 nm: U/l = ΔOD/min x 6507 334 nm: U/l = ΔOD/min x 6633 365 nm: U/l = ΔOD/min x 12057
*The above factors should be validated using General Chemistry Calibrator (AD973).
(Conversion Factor: Qty in µKat/l = Qty in U/l x 0.0167)

EXPECTED VALUES: [4]

	U/l	*µkat/L*
Adults	90 – 180	1.5 – 3

Each laboratory should establish its own reference range. HBDH results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linear up to 1008 U/L (16.8 µkat/L). For samples with a higher concentration: dilute 1:1 with 0.9% NaCl (9g/l) and reassay. Multiply result by 2.

Interfering substances:
Bilirubin (mixed isomers): Less than 10% interference up to 600 µmol/l Bilirubin.
Haemolysis: 22% interference up to 1.25 g/l Haemoglobin.
Lipemia: Less than 10% interference up to 2.5 g/l Intralipid.

Sensitivity:
The Lowest Detectable Level was estimated at 18 U/l.

Precision:

Within Run N = 20	Mean *(U/l)*	*SD*	*% CV*	*Between Run N = 20*	Mean *(U/l)*	*SD*	*% CV*
Level 1	155	4.66	3.00	Level 1	151	5.95	3.96
Level 2	258	4.77	1.85	Level 2	246	9.64	3.93

Method Comparison:
Using 50 samples, a comparison, between this HBDH test (y) and another commercially available test (x), gave the following results:

y = 0.921 + 7.957	r = 0.993	Sample range: 46 to 412 U/l

BIBLIOGRAPHY:

1. Burtis CA., Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed.; 30-54.
2. Bergmeyer HU. Empfehlungen der DGKC. Clin Chem Biochem 1970; 8: 658-660 & 1972; 10: 182-192
3. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of Anticoagulants in Diagnostic Laboratory Investigations and Stability of Blood, Plasma and Serum Samples. WHO/DIL/LAB/99.1 Rev.2:36pp.
4. Thomas L. Enzyme. Labor und Diagnose, 4 Auflage. Marburg: Die Medizinische Verlagsgesellschaft, 1992:136pp

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	CAL	Calibrator
CE	CE Mark - Device comply with the Directives 98/79/EC		
	Storage temperature		Consult Instruction for Use
	Expiry Date (Last day of the month)		Manufactured By

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