



CATACHEM

GLUTAMATE DEHYDROGENASE (GLDH) REAGENT KIT
C552-0A
FOR VETERINARY USE ONLY

Contents	Product No.	Package
GLDH REAGENT KIT GLDH Reagent Diluent GLDH Reagent Powder	C552-0A C552-01 C552-02	3 x 25 mL 3 x 67.5 mg

Reagent Preparation

To prepare the Catachem GLDH reagent, empty the entire contents of one bottle of diluent into one bottle of powder. Swirl gently to ensure total solubilization.

Storage and Stability

Store unopened reagents at 2-8°C. When stored as directed, unopened reagents are stable until the expiry date stated on the label.
Once activated, the GLDH reagent is stable for at least 5 days when stored at 2-8°C and capped tightly when not in use.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
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GLUTAMATE DEHYDROGENASE (GLDH) REAGENT KIT C552-0A FOR VETERINARY USE ONLY MANUAL/AUTOMATED APPLICATIONS

Intended Use

For the quantitative determination of Glutamate Dehydrogenase (GLDH).

Clinical Significance

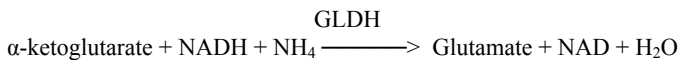
Measurements of GLDH activity are primarily used for diagnosing liver disorders and particularly to assess the severity of damage to specific cells such as in acute hepatic dystrophy. GLDH levels are most commonly employed in assessing health in large animals although the enzyme is also used in testing rodent samples. The *in vivo* half-life of GLDH in dogs is approximately 8 hours, horses approximately 12-24 hours and cattle approximately 14 hours.

Method History

The Catachem GLDH method for manual or automated chemistry instruments measures GLDH utilizing α -ketoglutarate as substrate and NADH as cofactor and reaction indicator. It is formulated as a single reagent once reconstituted.

Method Principle

Glutamate Dehydrogenase (EC 1.4.1.3) is an enzyme which catalyzes the oxidation of α -ketoglutarate to glutamate with the mediation of NADH as a hydrogen donor and ADP as GLDH activator. The reaction is reversible and strongly favors the conversion of α -ketoglutarate to glutamate. The rate of decrease in absorbance of the reaction mixture due to the formation of NAD is proportional to the GLDH concentration present in the serum sample. The Catachem method is based on the work of Ellis and Goldberg.⁽¹⁾ The reaction scheme below illustrates the reaction that occurs in this method.



Reagent Content

The concentrations of the active ingredients are approximately as follows:

GLDH Reagent

Each vial contains:

Buffer	
α -ketoglutarate	7.5 mmol/L
ADP	0.20 mmol/L
Ammonium Chloride	0.2 mmol/L
LDH	≥ 5000 units

Nonreactive ingredients and stabilizers

Preparation of Working Reagents

Catachem Glutamate Dehydrogenase (GLDH) reagent is prepared by adding the contents of one diluent bottle (C552-01) to one bottle of powder reagent (C552-02)

GLDH Reagent Storage and Stability

Store the GLDH reagent at 2-8°C. When stored as directed, this reagent is stable until the expiration date stated on the label. After opening and reconstitution, the GLDH Reagent is stable for at least 5 days when stored at 2-8°C and capped when not in active use.

Catachem GLDH Reagent has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures of up to 40°C for up to one week.

Specimen Collection and Preparation

The use of clear, un-hemolyzed serum that has been separated from the clot as soon after collection as possible is recommended. Although GLDH activity in serum has been shown by Armbruster⁽²⁾ to be stable for 3 days when stored at 4 °C, the enzyme can deteriorate after collection and samples should be stored on ice until assayed to minimize any loss of enzyme activity. Heparinized plasma is also an appropriate sample type.

Precaution

Avoid contact of specimen with skin. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Interfering Substances

A number of interfering substances can affect the GLDH values Tietz.⁽⁴⁾ A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.⁽³⁾

Expected Values

The range of expected values determined for this method varies with species and is approximately 0-4 U/L for human samples, 0-12 U/L for small animals and horses and up to 60 U/L for cattle and 20 U/L for alpacas. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located and for each species under test.

Procedure

These instructions are outlined for performing the GLDH assay on a manual spectrophotometer.

Materials Provided

GLDH reagent

Materials Required But Not Provided

- Spectrophotometer equipped with 340 nm wavelength
- Calibrator material with assigned GLDH value
- Quality Control material with assigned GLDH values

General Test Parameters

Sample volume	25 μ l
Reagent volume (R1)	500 μ l
Reaction Temperature	37°C
Wavelength	340 nm
Units	U/L

Calibration

Catachem GLDH Calibrator, (Product No. C550-40), with a known GLDH value is recommended.

Quality Control

To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's GLDH CALIBRATOR (C550-40). Assay performance should be monitored



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by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Procedure Limitations

Samples with GLDH values greater than 200 U/L should be diluted 1:2 with laboratory grade water and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.0003-0.0005 absorbance/minute/unit/L.

Linear Range: No significant nonlinearity over the range of 2-200 U/L.

Precision Study(as carried out on an AU 600):

GLDH MEAN U/L	TOTAL		WITHIN-RUN	
	SD U/L	CV %	SD U/L	CV %
7.30	0.45	6.2	0.55	7.5
62.20	1.45	2.3	1.65	2.6
119.93	1.62	1.3	1.72	1.4

Assay Procedure (When using a calibrator)

1. Pipette 500 uL of GLDH reagent into the number of cuvettes required, i.e. the number required for samples, calibrator & controls.
2. Incubate cuvettes until they reach 37C.
3. Add 25 uL of calibrator to a cuvette and place into the spectrophotometer.
4. Determine the rate of decrease of OD (optical density) over a 10-minute period at 340 nm for this calibrator.
5. Use the value of this calibrator and its OD change over 10 minutes to determine a factor. Use this factor to calculate the values for any subsequent samples (controls or patient) after performing the same action.

References

(1) Ellis & Goldberg; Optimal Conditions for the Kinetic Assay of Serum Glutamate Dehydrogenase Activity at 37°C Clin. Chem., 18, 6 (1972)
(2) Armbruster; Optimized Kinetic Glutamate Dehydrogenase Assay Adapted to the Cobas Bio Centrifugal Analyzer Clin. Chem. 33, 9 (1987)
(3) 4. Young DS, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21 No. 5(1975).
(4) Fundamentals of Clinical Chemistry. Edited by Norbert Tietz, WB Saunders, Philadelphia (1976).