CATACHEM

GLUTAMATE DEHYDROGENASE (GLDH)
MANUAL OR AUTOMATED APPLICATIONS
C550-series

Intended Use
For **IN VITRO** 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.

Method History
The Catachem GLDH method for manual or automated chemistry systems measures GLDH utilizing α-ketoglutarate as substrate and reduced NADH as cofactor and reaction indicator. It is formulated as a single, stable liquid reagent.

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. 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 reaction scheme below illustrates the reaction that occurs in this method.

$$\alpha\text{-ketoglutarate} + NADH + NH_4 \xrightarrow{GLDH} \text{Glutamate} + NAD + H_2O$$

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 salt	0.2 mmol/L
NADH	3.0 mmol/L
LDH	≥ 5000 units
Nonreactive ingredients and stabilizer	

Preparation of Working Reagents
The Catachem Glutamate Dehydrogenase (GLDH) reagent is packaged ready for use. No preparation is required. Store the Catachem GLDH Reagent at 2-8ºC.

Reagent Storage and Stability
Store the unopened GLDH reagent (liquid) at 2-8˚C. When stored as directed, the reagent is stable until the expiration date stated on the label. After opening, the reagent is stable for a minimum of 30 days when stored at 2-8˚C in a tightly closed bottle.

Specimen Collection and Preparation
The use of clear, unhemolyzed serum that has been separated from the clot as soon after collection as possible is recommended. The half-life for GLDH in dog serum is approximately 8 hours, in horse serum approximately 12-24 hours and in cattle serum approximately 14 hours.

Precaution
Avoid contact of reagent or specimens with skin. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE REAGENT OR SPECIMENS BY MOUTH.**

Interfering Substances
A comprehensive discussion has been reported on the effect of interfering substances on various GLDH methods. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.

Expected Values
The range of expected values determined for this method varies with species and is approximately 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 species and the area in which it is located.

Procedure
Important: Read the entire procedural instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	To time incubation time
Pipette	1.0 mL for reagent
Pipette	0.060 mL for sample

Materials Provided: GLDH Reagent

Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	zero-order rate, direct
Reaction Time	10 min. 37˚C
Reagent Volume	1.0 ml
Sample Volume	0.06 ml
Total Volume	1.06 ml
Sample-to-Reagent Ratio	approx. 1:18

Assay Procedure
1. Into separate cuvettes, pipette 1 mL Working Reagent.
2. Pipette 0.06 ml of “Control” or “Sample” into their respective cuvettes. Mix all cuvettes well.

3. Incubate all cuvettes for approximately 30 seconds, then read rate of decrease of O.D. over a 10 minute period at 340nm.

Quality Control
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of normal and abnormal control serum.

Calibration
A reference calibration is optional but not required to calibrate the GLDH procedure. Results can be obtained by using the following calculation:

Calculations and Results

$$\text{GLDH U/L} = \frac{\Delta \text{ A/min} \times 1.06 \text{ ml} \times 1000}{6.22 \times 1 \text{ cm light path} \times 0.06 \text{ml}}$$

GLDH U/L = Δ A/min x 2840.30
Δ A/min = Change in absorbance per minute
1.06 mL = Total assay volume in mL
1000 = Converts U/mL to U/L
6.22 = Absorbance coefficient of NADH at 340nm
Light Path = 1 cm
0.06 mL = Sample volume

(2840.3 = Factor derived from constants in the equation)

Example:
Sample Δ A/min = 0.01
GLDH U/L = 0.01 x 2840.30 = 28.4 u/L

Method Performance Characteristics
Sensitivity: The sensitivity of this method is 0.0003-0.0005 absorbance/minute/unit/L.
Linear Range: This method is linear over the range of 0-200 U/L at 37°C.

Precision Study (as carried out on an AU 600)

GLDH	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
7.3	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

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353 Christian Street, Suite #2, Oxford, Connecticut 06478 USA ● Phone (203) 262-0330 ● Fax (203) 262-9836 ● email: catachem@catacheminc.com