## GLUTAMATE DEHYDROGENASE

### [Summary and Explanation]

Glutamate dehydrogenase (GDH, EC 1.4.1.2) converts glutamate to alpha-ketoglutarate yielding ammonia in the presence of its coenzyme NAD(P)$^+$. It is an oxidoreductase using both NAD$^+$ or NADP$^+$ as acceptor. GDH was known as a mitochondrial indicator and because of the crucial function of linking catabolic-anabolic pathways, it is ubiquitously found in whole tissues, mostly in liver. While most mammals have only GDH1, humans have been shown to acquire the second isoform, hGDH2 (h for human) displaying tissue specificity. Intracellular localizations of GDH were identified as mitochondrial matrix, membrane and nuclear fractions (1).

GDH plays an important role in the amino acid, carbohydrate, oxidative phosphorylation metabolisms and has been associated with several cellular processes such as balancing ammonia and acid-base concentrations, redox homeostasis, lipid and lactate biosynthesis (1). Previous reports proved that enhanced activity of GDH aids tumor cells for proliferation through supporting nitrogen sources (1-3). Besides, GDH serum level may show the liver tissue damage, particularly mitochondrial dysfunction considering the cellular sublocalization of the enzyme (4). Thus, measuring serum GDH activity may provide great advantages for clinical and diagnostic purposes.

### [Principle of Assay]

The Glutamate Dehydrogenase Activity Assay kit provides a simple and direct procedure for measuring GDH activity in serum. The enzyme activity is determined using alpha-ketoglutaric acid as the substrate and NADH+H$^+$ as the coenzyme in the reaction. Decreasing concentration of NADH+H$^+$ causes an absorbance change at 340 nm that is proportional to the enzymatic activity of GDH. One unit of GDH is the amount of enzyme that will generate 1.0 µmole of NAD$^+$ per minute at pH 8.0 and 37 °C.

$$alpha - ketoglutarate + NADH + H^+ + NH_4^+$$

$$\rightarrow glutamate + NAD^+ + H_2O$$

### [Reagent Composition]

**Reagent:** Buffer Solution
Cofactors
Coenzymes
Surfactants
Substrate
Preservatives

### [Intended Use]

This product can be used for quantitative *in vitro* determination of total GDH activity from human serum and plasma.

### [Stability and Preparation of Reagents]

**Reagent**:

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

### CAL

The assay can be calibrated with deionized water. Daily calibration is recommended.

The procedure is standardized by means of the molar absorptivity of NADH+H$^+$ taken as 6300 at 340 nm under the test conditions described (Optical path: 1cm).

### Calculation

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions (Optical path: 1cm).

GDH (U/L) = ΔAbs/ min. x 4126

If your optical path is different from 1 cm, the factor (4126) must be divided into optical path.

### [Performance Characteristics]

*Low linearity:* 0 IU - *High linearity:*800 IU

If the results obtained are greater than linearity limit, dilute the sample 1/5 with deionized water and multiply the result by 5.

### [Precision]

Intra-assay CV was determined with 30 replicates of 20 samples. The %CV result was lower than 1 and inter-assay %CV result was 1.5.

### [Normal range]

Adult Serum: ≤ 8 IU

Each laboratory is recommended to establish their own reference values.

Serum samples collected after at least 8-10 hours of fasting must be used for the test.

### [Components]

All reagents are ready to use.

| Content | | Amount |
|---|---|---|
| Reagent | | 60 ml |
| Level 1 | 90 IU (72-108) | 3 ml |
| Level 2 | 300 IU (240-360) | 3 ml |

**[Storage and Validity Date]**

*Storage:* This kit should be stored at 2-8°C.

*Validity date:* The validity of reagent is indicated on the bottle and on the kit box.

(This kit is stable for up to expiry date when stored at 2-8°C after opened.)

**[Sample]**

Blood serum, plasma and urine could be used as sample.

Serum samples are stable up to 1 week stored at 4˚C, 2 months at -20˚C and 6 months at -80˚C.

Collect samples in collection tubes produced for serum and plasma.

Serum samples collected after at least 8-10 hours of fasting must be used for the test.

**[Procedure and Calculation]**

**Serum and plasma application**

| Assay type | Rate Down | |
|---|---|---|
| Wavelength | 340 | |
| Optical path | 1cm | |
| | R1 | R2 |
| Reagent pipetting | 170 µL | 30 µL |
| Sample | 8 µL | |

**[Interferences]**

No interferences were observed with bilirubin up to 18 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

In order to obtain best results, the pipettes and cuvettes used for the assay must be cleaned thoroughly. Make sure that no wash solution is left on the pipettes and in cuvettes.

**[Recovery]**

Serum pool was prepared and GDH activity was measured. Then enzyme activity was re-measured following addition of 1200 IU/mL GDH enzyme solution in ratios of 1/10 and 1/100 into the same serum pool. As a result, recovery was found between the %95 and %105 range.

**[Safety precautions and warning]**

1. For *research* use only.
2. Do not pipette by mouth.
3. Exercise the routine precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose cleaning liquid and such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.
9. The Reagent contains sodium azide (0.01%) as a preservative. Do not ingest.

***References***

1. Plaitakis A, Kalef-Ezra E, Kotzamani D, Zaganas I, Spanaki C. The glutamate dehydrogenase pathway and its roles in cell and tissue biology in health and disease. Biology. 2017;6(1):11.
2. Spinelli JB, Yoon H, Ringel AE, Jeanfavre S, Clish CB, Haigis MC. Metabolic recycling of ammonia via glutamate dehydrogenase supports breast cancer biomass. Science. 2017;358(6365):941-6.
3. Moreno-Sánchez R, Marín-Hernández Á, Gallardo-Pérez JC, Pacheco-Velázquez SC, Robledo-Cadena DX, Padilla-Flores JA, et al. Physiological role of glutamate dehydrogenase in cancer cells. Frontiers in oncology. 2020;10.
4. Plaitakis A, Kalef-Ezra E, Kotzamani D, Zaganas I, Spanaki C. The Glutamate Dehydrogenase Pathway and Its Roles in Cell and Tissue Biology in Health and Disease. Biology (Basel). 2017;6(1).