QuantiChrom™ Glutamate Dehydrogenase Kit (DGLDH-100)

Quantitative Colorimetric Kinetic Glutamate Dehydrogenase Activity Determination

DESCRIPTION

GLUTAMATE DEHYDROGENASE (GLDH) is an enzyme which catalyzes the interconversion of glutamate and α-ketoglutarate. Elevated blood serum GLDH levels indicate liver damage; thus, GLDH plays an important role in the diagnosis of liver disease, especially in combination with aminotransferases. Transgenic plants expressing microbial GLDHs are improved in tolerance to herbicide, water deficit, and pathogen infections. BioAssay Systems' non-radioactive, colorimetric GLDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.4 to 80 U/L for 30 min reaction. Detection Limit of 0.1 U/L for 120 min reaction.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

GLDH activity determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Diaphorase:**	120 µL
NAD Solution:	1 mL	**Calibrator:**	1.5 mL
MTT Solution:	1.5 mL	**Substrate:**	1.5 mL 1 M Glutamate

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

The Working Reagent (WR) is prepared by mixing, for each 96-well assay, 10 µL Substrate, 14 µL MTT Solution, 9 µL NAD Solution, 1 µL Diaphorase and 50 µL Assay Buffer.

The Blank Working Reagent (BWR) is prepared by mixing, for each 96-well assay, 14 µL MTT Solution, 9 µL NAD Solution, 1 µL Diaphorase and 60 µL Assay Buffer (*i.e. no Substrate*). Fresh reconstitution of the WRs is recommended.

Reaction Preparation:

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL sample into 2 separate wells. Add 80 µL WR to one sample well and 80 µL BWR to the other sample well. Tap plate briefly to mix.
3. Read OD_{565nm} (OD_0), and again after 30 min (OD_{30}) on a plate reader.

CALCULATION

Subtract the OD_0 from OD_{30} for each sample and sample blank well to compute the ΔOD_S and ΔOD_B values respectively. GLDH activity can then be calculated as follows:

$$\text{GLDH Activity} = \frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{273}{t\,\text{(min)}} \times \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H20}} \times n \quad \textit{(U/L)}$$

where ε_{mtt} is the molar absorption coefficient of reduced MTT. *l* is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_0) values of the Calibrator and water. *t* is the reaction time (30 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. *n* is the dilution factor.

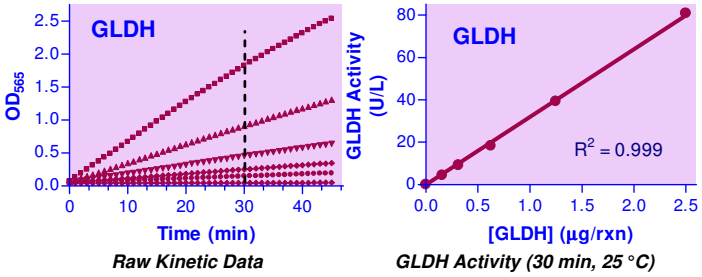
Unit definition: 1 Unit (U) of GLDH will catalyze the conversion of 1 µmole of glutamate to α-ketoglutarate per min at pH 8.2.

Note: If sample GLDH activity exceeds 80 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with GLDH activity < 1 U/L, the incubation time can be extended to 2 hours.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.

GLDH Titration Curves



Raw Kinetic Data *GLDH Activity (30 min, 25 °C)*

LITERATURE

1. Chou, KH and WE Splittstoesser, WE (1972) Glutamate dehydrogenase from pumpkin cotyledons characterization and isoenzymes. Plant Physiol. 49: 550-4.
2. Yamaya, T et. al. (1984). Characteristics of glutamate dehydrogenase in mitochondria prepared from corn shoots. Plant Physiol. 76: 1009-13.
3. Cho, SW et. al. (1995). Two soluble forms of glutamate dehydrogenase isoproteins from bovine brain. Eru. J. Biochem. 233: 340-6.