Technical Bulletin

# Glutamate Dehydrogenase Assay Kit

**Catalogue number MAK499**

## Product 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.

The 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.

The linear detection range of the kit is 0.4 to 80 U/L for a 30 minute reaction. The limit of detection is 0.1 U/L for a 120 minute reaction. The kit is suitable for GLDH determination in biological samples such as plasma, serum, urine, tissue, and culture media.

## Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK499A
- Diaphorase 120 µL
  Catalogue Number MAK499B
- NAD Solution 1 mL
  Catalogue Number MAK499C
- MTT Solution 1.5 mL
  Catalogue Number MAK499D
- Substrate (1 M Glutamate) 1.5 mL
  Catalogue Number MAK499E
- Calibrator 1.5 mL
  Catalogue Number MAK499F

## Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Phosphate Buffered Saline (PBS) (Catalogue Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalogue Number P0662 or equivalent)

## Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

## Preparation Instructions

Briefly centrifuge small vials prior to opening. Assays can be executed at any desired temperature 25 °C or 37 °C. Equilibrate all components to desired temperature prior to use.

## Procedure

All Samples and Standards should be run in duplicate.

## Sample Preparation

Serum and plasma are assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize 50 mg of tissue in ~200 µL of buffer containing 50 mM potassium phosphate (pH 7.5)
3. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5)
4. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
5. Remove supernatant for assay.

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

## Working Reagent

**Note:** Fresh reconstitution of the Working Reagent is recommended

Mix enough reagent for the number of assays to be performed. For each Sample well, prepare 84 µL of Working Reagent according to Table 1. For each Sample Blank well, prepare 84 µL of Blank Working Reagent according to Table 1.

**Table 1.**

Preparation of Working Reagents

| Reagent | Working Reagent | Blank Working Reagent |
|---|---|---|
| Substrate | 10 µL | - |
| MTT Solution | 14 µL | 14 µL |
| NAD Solution | 9 µL | 9 µL |
| Diaphorase | 1 µL | 1 µL |
| Assay Buffer | 50 µL | 60 µL |

## Assay Reaction

**Note:** This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Set the plate reader for the desired assay temperature 25 °C or 37 °C
2. Transfer 100 µL of purified water ($OD_{H2O}$) and 100 µL of Calibrator ($OD_{CAL}$) solution into separate wells of a clear flat bottom 96-well plate.
3. Transfer 20 µL of each sample into separate wells of the plate.
4. Add 80 µL of Working Reagent to one sample well and 80 µL of Blank Working Reagent to the other sample well. Tap plate lightly to mix.

## Measurement

Read the optical density (OD) of each well at 565 nm at zero minutes and again at 30 minutes ($OD_{0Min}$ and $OD_{30Min}$ respectively). Alternatively, using the plate reader's kinetic mode, monitor the OD for 30 minutes.

# Results

1. Subtract the $OD_0$ from $OD_{30}$ for each sample and sample blank well to compute the $\Delta OD_S$ and $\Delta OD_B$ values, respectively.
2. GLDH activity can then be calculated as follows:

GLDH Activity (U/L) =

$$\frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{MTT} \times l} \times \frac{\text{Reaction Vol (µL)}}{t\ (\text{min}) \times \text{Sample Vol (µL)}} \times DF$$

$$= \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H2O}} \times \frac{273}{t\ (\text{min})} \times DF$$

Where:

$\varepsilon_{mtt}$ = the molar absorption coefficient of reduced MTT
l = the light pathlength which is calculated from the calibrator
$OD_{CAL}$ = $OD5_{65nm}$ values of the Calibrator
$OD_{H20}$ = $OD5_{65nm}$ values of the water
t= the reaction time (30 minutes)
Reaction Vol =100 µL
Sample Vol = 20 µL
DF = the dilution factor

**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.

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.

**Figure 1:**

Typical GLDH Raw Kinetics data

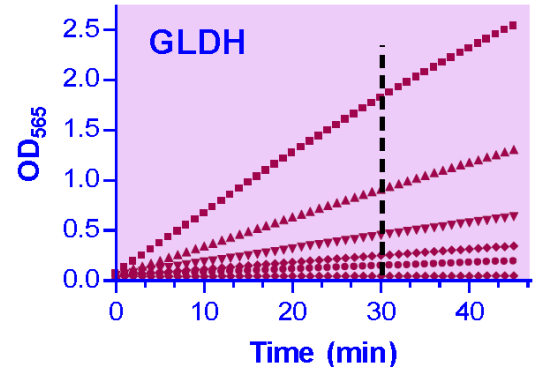
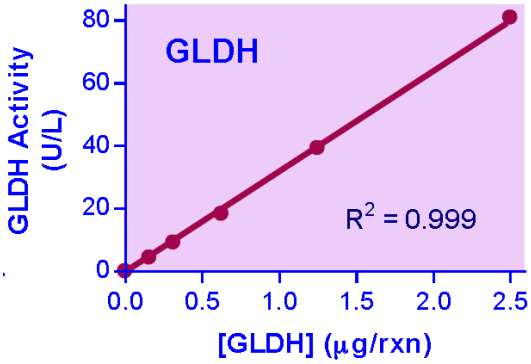



**Figure 2:**

Typical GLDH Activity (30 min, 25 °C)



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