



Glutamate Dehydrogenase Assay (GDH)

Cat. No. 8358
100 Tests in 96-well plate

Introduction

Glutamate dehydrogenase (GDH) is an important branch-point enzyme between carbon and nitrogen metabolism. GDH catalyzes the reversible NAD (P)⁺-linked oxidative deamination of glutamate into α - ketoglutarate and ammonia. This colorimetric assay is based on GDH catalyzed oxidation of glutamate, in which the formed NADH reduces a tetrazolium salt, MTT. The intensity of the product color, which exhibits maximum absorbance at 570 nm, is proportional to the GDH activity in the sample.

Kit Components

| Cat. No. | # of vials | Reagent | Quantity | Storage |
|----------|------------|--------------------|----------|---------|
| 8358a | 1 | Assay buffer | 10 mL | 4°C |
| 8358b | 1 | Glutamate solution | 1 mL | 4°C |
| 8358c | 1 | NAD | 0.4 mL | -20°C |
| 8358d | 1 | Substrate mix | 1.6 mL | -20°C |
| 8358e | 1 | GDH standard | 0.1 mL | -20°C |

Product Use

This kit measures GDH activity of samples from cells, serum, plasma, tissue and urine. It is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Glutamate Dehydrogenase Assay of GDH standard with concentrations ranging from 0.25 to 8 U/ml show a linear relationship between OD_{570nm} and GDH activity (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of GDH standard

1. Add 1 μL of GDH standard (8358e) to 74 μL of assay buffer (8358a) to make a 75 μL solution of 16 U/ml GDH standard.
2. Obtain 7 test tubes, add 25 μL of assay buffer (8358a) into each tube and label them #1 through #7.
3. Add 25 μL of the 16 U/ml GDH into tube #1 and mix well to get the 8 U/ml GDH standard.
4. Transfer 25 μL of the 8 U/ml GDH standard from tube #1 to tube #2 and mix well to get the 4 U/ml GDH standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the GDH standards. Do not add any GDH to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each GDH standard by aliquoting 10 μL /well of each GDH standard into duplicate wells of the 96-well test plate, according to the following plate format:

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|--------|--------|--------|--------|----------|-----------|-------|
| A | 8 U/ml | 4 U/ml | 2 U/ml | 1 U/ml | 0.5 U/ml | 0.25 U/ml | Blank |
| B | 8 U/ml | 4 U/ml | 2 U/ml | 1 U/ml | 0.5 U/ml | 0.25 U/ml | Blank |

B. Preparation of test samples

1. Cells or tissues can be homogenized in 4 volumes of the assay buffer (8358a). Centrifuge the samples at $13,000 \times g$ for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serially diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 μL /well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare the working reagent by mixing 60 μL assay buffer (8358a), 10 μL glutamate solution (8358b), 4 μL NAD (8358c), and 16 μL substrate mix (8358d).
2. Add 90 μL of working reagent mix into each well of the 96-well plate containing GDH standard, samples, and blank. Incubate for 20 minutes at room temperature in dark.
3. Read the absorbance at 570 nm with an ELISA plate reader.

D. Calculations

1. Average the $\text{OD}_{570\text{nm}}$ of duplicate wells of each GDH standard, test sample and blank. Subtract the $\text{OD}_{570\text{nm}}$ value of the blank from the $\text{OD}_{570\text{nm}}$ values obtained with all other standard and samples to get $\Delta\text{OD}_{570\text{nm}}$ value.
2. Based on the calibrated $\Delta\text{OD}_{570\text{nm}}$ of the GDH standard, make a standard curve by plotting $\Delta\text{OD}_{570\text{nm}}$ as a

function of GDH concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.

3. Because the equation of the trend line of the standard curve is $y = Ax + B$, calculate the GDH concentration of test samples as follows:

$$[\text{GDH}] = \frac{\Delta\text{OD}_{570\text{nm}} - B}{A}$$

Unit definition: One unit would generate 1.0 μmole NADH per minute at pH 8.6 at 25 $^{\circ}\text{C}$

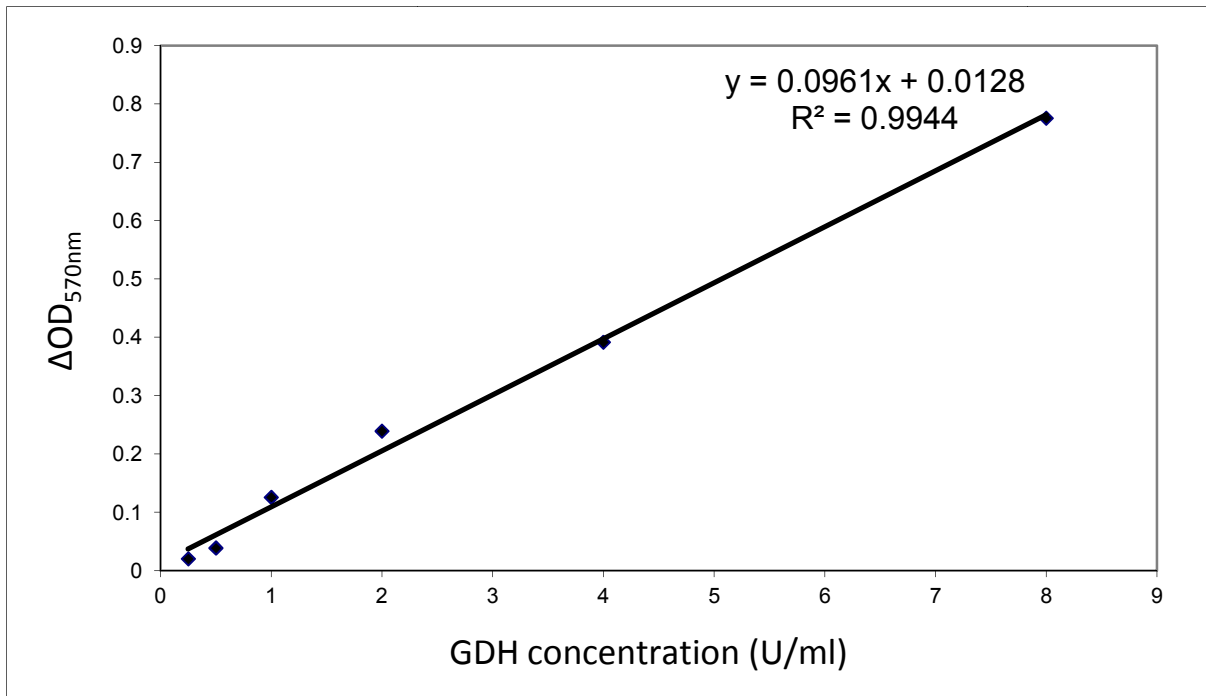


Figure1. A typical GDH standard curve measured by ScienCell™ Glutamate Dehydrogenase Assay kit