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	8 mg	-20°C
8358e	1	Substrate solvent	1.6 mL	-20°C
8358f	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 (8358f) 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 substrate solution

1. Combine substrate (8358d) and substrate solvent (8358e) and mix thoroughly to make substrate solution.
2. Store prepared substrate solution at -20°C.

C. 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 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous NADH from cell or tissue extracts generates background in the glutamate dehydrogenase assay. As a control to remove the endogenous NADH background, the same amount of sample can be tested in the absence of glutamate solution (8358b).
3. Samples should be serial 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.

D. 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 solution (prepared in step B). When a control is required to remove endogenous NADH background from a sample, prepare a control working reagent by mixing 70 µL assay buffer (8358a), 4 µL NAD (8358c) and 16 µL of substrate solution (prepared in step B).

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.

E. Calculations

1. Average the OD_{570nm} of duplicate wells of each GDH standard, test sample and blank. Subtract the OD_{570nm} value of the blank from the OD_{570nm} values obtained with all other standard and samples to get ΔOD_{570nm} value.
2. Based on the calibrated ΔOD_{570nm} of the GDH standard, make a standard curve by plotting ΔOD_{570nm} 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. For samples requiring control without glutamate, subtract the ΔOD_{570nm} without glutamate value from the ΔOD_{570nm} with glutamate value and use this $\Delta\Delta OD_{570nm}$ value to determine the sample glutamate dehydrogenase activity from the standard curve.
4. Because the equation of the trend line of the standard curve is $y = Ax + B$, calculate the GDH concentration of test samples as follows:

$$[GDH] = \frac{\Delta\Delta OD_{570nm}}{A}$$

Unit definition: One unit would generate 1.0 µmole NADH per minute at pH 8.6 at 25 ºC

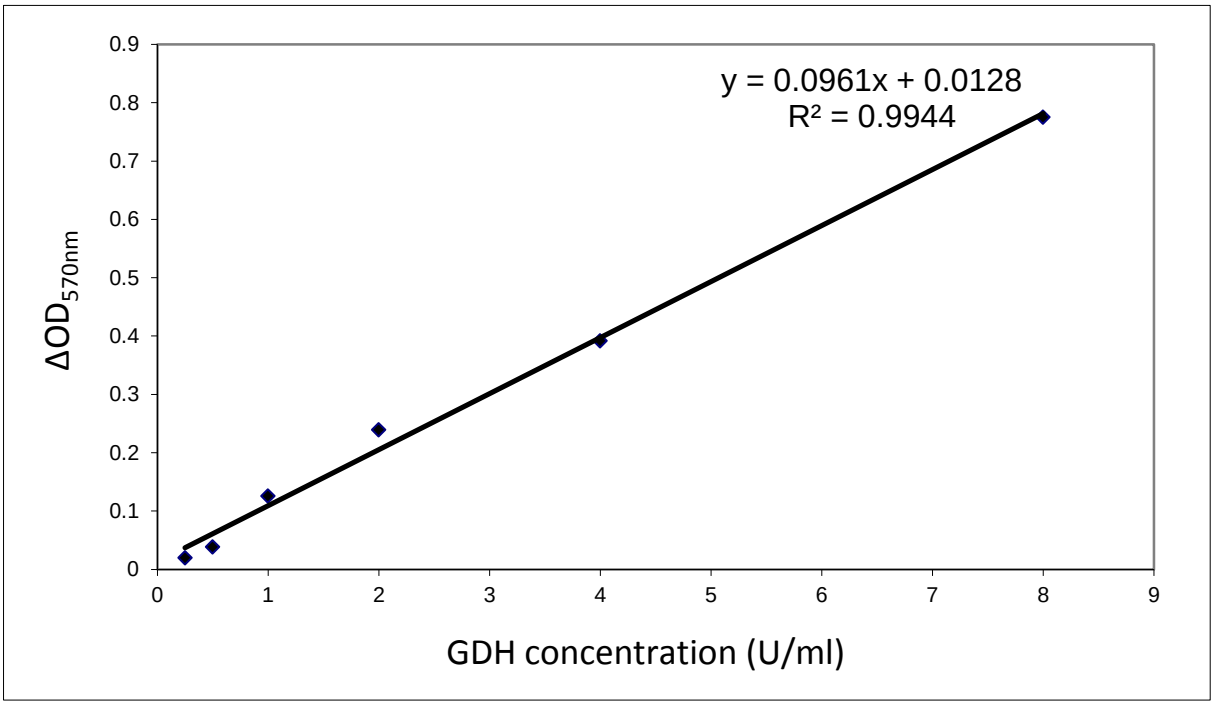

Figure1. A typical GDH standard curve measured by ScienCell™ Glutamate Dehydrogenase Assay kit