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Product Information

Glutamate Dehydrogenase (GDH) Activity Assay Kit

Catalog Number **MAK099**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glutamate Dehydrogenase (GDH) is a mitochondrial enzyme that catalyzes the reversible oxidative deamination of glutamate to α-ketoglutarate and serves as a key link between anabolic and catabolic pathways. In mammals, GDH is subject to allosteric regulation and has high activity in liver, kidney, brain, and pancreas. GDH activity in serum can be used to differentiate between liver diseases due to liver inflammation, which do not show elevated serum GDH activity, and diseases that result in hepatocyte necrosis, which results in elevated serum GDH.

GDH activity is determined by a coupled enzyme assay in which glutamate is consumed by GDH generating NADH, which reacts with a probe generating a colorimetric (450 nm) product proportional to the GDH activity present. One unit of GDH is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 7.6 at 37 °C.

Components

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

GDH Assay Buffer Catalog Number MAK099A	25 mL
Glutamate, 2 M Catalog Number MAK099B	1 mL
GDH Developer Catalog Number MAK099C	1 vl
GDH Positive Control Catalog Number MAK099D	1 vl
NADH, 0.5 μmole Catalog Number MAK099E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

GDH Assay Buffer – Allow buffer to come to room temperature before use.

GDH Positive Control – Reconstitute with 220 μL of GDH Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

GDH Developer – Reconstitute with 900 μL of water. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

NADH – Reconstitute with 50 μL of water to generate a 10 mM (10 nmole/μL) NADH Stock Solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Dilute 10 µL of the 10 mM NADH Stock Solution with 90 µl of GDH Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add GDH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 µL of ice-cold GDH Assay Buffer. Incubate for 10 minutes on ice and then centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples (5–50 µL) can be added directly to the wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 µL with GDH Assay Buffer.

Add 2 µL of the positive control into wells and bring to a final volume of 50 µL with GDH Assay Buffer.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Volume**
GDH Assay Buffer	82 µL
GDH Developer	8 µL
Glutamate	10 µL

2. Add 100 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation

3. Incubate the plate at 37 °C. After 3 minutes, take the initial measurement ($T_{initial}$). Measure the absorbance at 450 nm at the initial time $(A_{450})_{initial}$. Note: It is essential $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C taking measurements (A_{450}) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (10 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement $[(A_{450})_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH standard from the final measurement [$(A_{450})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of NADH generated between $T_{initial}$ and T_{final} (B).

The GDH activity of a sample may be determined by the following equation:

$$\text{GDH Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

GDH activity is reported as nmole/min/mL = milliunit/mL
One unit of GDH is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 7.6 at 37 °C.

Example:
NADH amount (B) = 5.84 nmole
First reading ($T_{initial}$) = 3 minute
Second reading (T_{final}) = 33 minutes
Sample volume (V) = 0.01 mL
Sample dilution is 1

GDH activity is:

$$\frac{5.84 \times 1}{(33-3) \times 0.01} = 19.47 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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