Glutamate Dehydrogenase (GDH) Activity Assay Kit

(Catalog #K729-100; 100 reactions; Store kit at -20 °C)

I. Introduction:

Glutamate dehydrogenase (GDH) is an enzyme that converts glutamate to α-Ketoglutarate, and vice versa. It represents a key link between catabolic and metabolic pathways, and is therefore ubiquitous in eukaryotes. BioVision's GDH Assay Kit provides a convenient tool for sensitive detection of GDH in a variety of samples. GDH in sample will consume glutamate as a specific substrate and generate NADH stoichiometrically, resulting in a proportional color development. The GDH activity is easily quantified colorimetrically (λ = 450 nm). This assay detects GDH activity as low as 0.01mU in serum or tissue and cell extracts.

II. Kit Contents:

Components	K729-100	Cap Code	Part Number
GDH Assay Buffer	25 ml	WM	K729-100-1
Glutamate (2 M)	1.0 ml	Blue	K729-100-2
GDH Developer (lyophilized)	1 vial	Red	K729-100-3
GDH Positive Control (lyophilized)	1 vial	Green	K729-100-4
NADH (0.5 µmol; lyophilized)	1 vial	Yellow	K729-100-5

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

- Ensure that the Assay Buffer is at room temperature before use.
- Reconstitute the Glutamate Dehydrogenase (GDH Positive Control) with 220 µl Assay Buffer. Keep the GDH Positive Control on ice during the preparation and protect from light. Aliquot and store -20°C.
- Reconstitute the GDH developer with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution (DO NOT VORTEX).
- Reconstitute the NADH with 50 µl ddH_2O to generate a 10 mM NADH stock solution.
- The GDH Positive Control and GDH Developer are stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times). Reconstituted NADH (10 mM) and the supplied Glutamate (2 M) solution are stable for up to 6 months at -20°C.

V. Glutamate Dehydrogenase Assay Protocol:

1. **NADH Standard Curve:** Dilute 10 µl of the 10 mM NADH stock solution with 90 µl of GDH Assay Buffer to generate a 1 mM NADH standard. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM NADH standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Adjust the final volume to 50 µl with Assay Buffer.
2. **Sample Preparations**: Tissues (50 mg) or cells (1 x 10^6) can be homogenized in ~ 200 µl ice-cold Assay Buffer then centrifuged (13,000 x g for 10 min.) to remove insoluble material. Add test sample into 96-well plate, bring volume to 50 µl/well with Assay Buffer. 5 - 50 µl serum samples can be directly diluted in the Assay Buffer. For the positive control (optional), add 2 µl positive control solution to wells and adjust to a final volume of 50 µl with Assay Buffer.
3. **Reaction Mix**: Mix enough reagents for the number of assays to be performed. For each well, prepare a Reaction Mix (100 µl) containing:

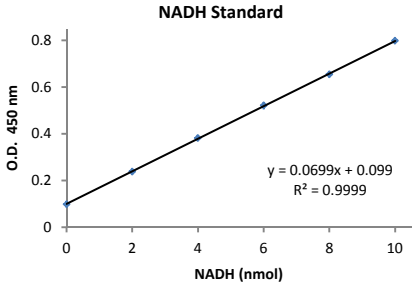
   82 µl Assay Buffer
   8 µl GDH Developer
   10 µl Glutamate (2 M)

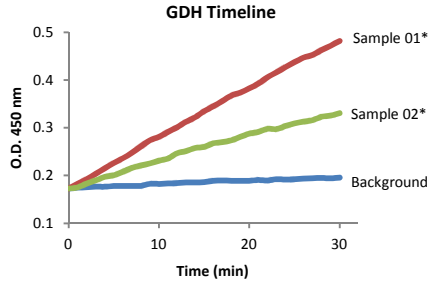
   Add 100 µl of the Reaction Mix to each well containing the test samples, positive controls and standards. Mix well. For the samples and positive controls, incubate the mix for 3 min at 37°C, then measure OD at 450 nm in a microplate reader (A0), incubate for another 30 min. to 2 hrs at 37°C to measure OD at 450 nm again (A1); incubation times will depend on the GDH activity in the samples. We recommend measuring the OD in a kinetic method (preferably every 3 – 5 min.) and choose the period of linear range (e.g. $\mathbf{A_n}$ **to** $\mathbf{A_{n+1}}$) to calculate the GDH activity of the samples.
4. **Calculation:** Plot Glutamate Standard Curve. Apply Δ OD = A1 - A0 (or $\mathbf{A_{n+1} - A_n}$) to the Glutamate Standard Curve to get B nmol of NADH produced by GDH in the given time.

   GDH Activity = $\frac{\mathbf{B}}{\mathbf{T} \times \mathbf{V}}$ × Sample Dilution Factor = nmol/min/ml = mU/ml

   Where: **B** is the NADH amount from Standard Curve (in nmol).
   T is the time incubated (in min).
   V is the sample volume added into the reaction well (in ml).

   Unit Definition: One unit is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 7.6 and 37°C.





*Sample 01: Bovine Liver extraction (2 µg protein), Sample 02: 5 µl Rabbit serum

VI. Related Products:

NAD/NADH Quantification Kit
ADP/ATP Ratio Assay Kit
Glucose Assay Kit
Ethanol Assay Kit
Pyruvate Assay Kit
Creatine Assay Kit
Ammonia Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Sarcosine Assay Kit
Nitric Oxide Assay Kit
NADP/NADPH Quantification Kit
Ascorbic Acid Quantification Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit
Lactate Assay Kit/ II
Creatinine Assay Kit
Free Glycerol Assay Kit
Hemin Assay Kit
Total Antioxidant Capacity (TAC) Assay Kit
L-amino Acid Assay Kit
Glutamate Kit

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		