Product Information

Glutamate Dehydrogenase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2229-100 100 assays	Cap Color	Part Number
GDH Assay Buffer	25 ml	WM	K2229-C-1
Glutamate (2 M)	1.0 ml	Blue	K2229-C-2
GDH Developer (lyophilized)	1 vial	Red	K2229-C-3
GDH Positive Control (lyophilized)	1 vial	Green	K2229-C-4
NADH (0.5 µmol; lyophilized)	1 vial	Yellow	K2229-C-5

II. Introduction:

Glutamate dehydrogenase (GDH) is an enzyme that converts glutamate to α-ketoglutarate and is present in the mitochondria of eukaryotes and most microbes. Since increased serum GDH levels indicate liver damage, GDH often used as an indicator of liver disease, often in combination with aminotransferases.

The Glutamate Dehydrogenase Activity Colorimetric Assay Kit provides a sensitive, simple, fast and convenient way for detection of GDH activity in various samples based on colorimetric method. In the assay, GDH in sample consumes glutamate as a specific substrate and produces NADH stoichiometrically, resulting in a proportional color development that can be easily quantified colorimetrically (λmax = 450 nm). The kit can detect GDH activity as low as 0.01 mU in serum or tissue and cell extracts.

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 icecold 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:

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

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. An to An+1) to calculate the GDH activity of the samples.

4. Calculation: Plot Glutamate Standard Curve. Apply Δ OD = A1 - A0 (or An+1 - An) to the Glutamate Standard Curve to get B nmol of NADH produced by GDH in the given time.

GDH Activity = B/ (T × 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

IV. Reagent Reconstitution and General Consideration:

Substrate Mix: Dissolve with 1.1 ml ddH_2O for 10 min, sufficient for 500 reactions.

NADH Standard Solution: Dissolve NADH Standard into 0.4 ml ddH_2O to generate 1.25 mM NADH Standard Solution.

LDH Positive Control: Dilute 1:9 with Assay Buffer before use, and use 2 - 5 µl diluted LDH as Positive Control. Keep on ice when using.

1. Sample Preparations:

Homogenize 0.1 g Tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.5 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 min at 4°C Collect the supernatant for assay and store on ice. Serum can be tested directly. Add 2 - 50 µl samples into a 96-well plate; bring the volume to 50 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

2. NADH Standard Curve:

Add 0, 2, 4, 6, 8, 10 µl of the 1.25 mM NADH Standard into 96-well plate in duplicate to generate 0, 2.5, 5.0, 7.5, 10.0, 12.5 nmol/well standard. Bring the final volume to 50 µl with Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays and standards to be performed. For each well, prepare a total 50 µl Reaction Mix:

Assay Buffer	48 µl
Substrate Mix Solution	2 µl

Mix well. Add 50 µl of the Reaction Mix to all samples, Positive Control, and Standard, mix well.

4. Measure OD 450 nm at T1 to read A1, measure again at T2 after incubating the reaction at 37°C for 30 min (or longer if the LDH activity is low) to read A2, protect from light. ΔA450 nm = A2 - A1.

Note: (A) It is essential to read A1 and A2 in the reaction linear range. It is more accurate if you observe the reaction progress, then choose A1 and A2 in the linear portion. (B) For Standard Curve, use A2 reading after 30 min incubation, do not subtract the A1 reading. The Standard reading is stable for a few hours.

5. Calculation: Subtract 0 nmol/well NADH background from all readings, plot NADH Standard Curve. Apply the sample ΔA450nm to the NADH standard curve to get B (the NADH amount that was generated between T1 and T2).

LDH Activity = B/[(T2-T1) × V] ×Sample dilution = nmol/min/ml = mU/ml

Where: B is the NADH amount that was generated between T1 and T2 (in nmol).

T1 is the time of first reading (A1) (in min).

T2 is the time of second reading (A2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

NADH molecular weight: 763.0 g/mol.

Unit definition: One unit of LDH is the amount of enzyme that generates 1.0 µmol NADH per minute at 37℃ in our buffer system.

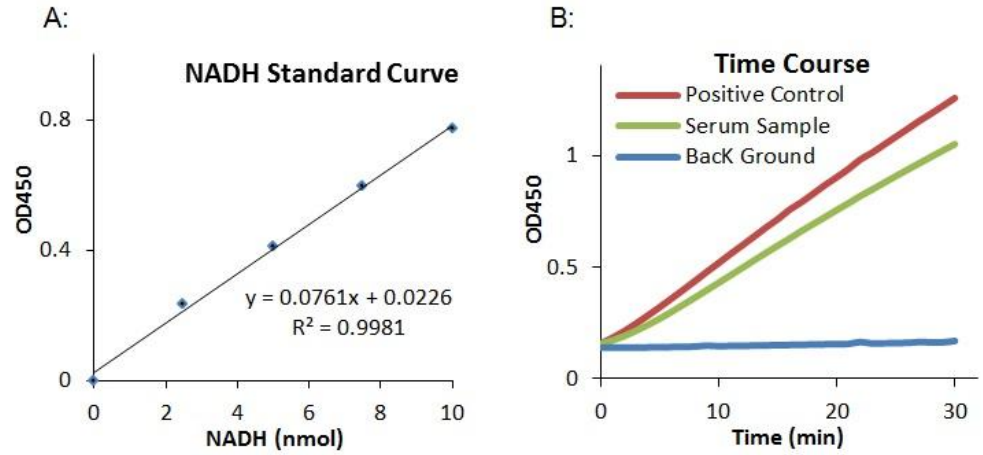

Figure: (A) NADH Standard curve. (B) Kinetic profiles of approx 0.5 mU of a sample of pure LDH (Positive control) and 2 µl frozen human serum from a commercial source using buffer as a background control.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher	• Improperly thawed components	• Thaw all components completely and mix gently before use

readings in Samples and Standards	• Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com