RayBio® Glucose Dehydrogenase Activity Assay Kit

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RayBio® Glucose Dehydrogenase Activity Assay Kit Protocol

(Cat#: 68AT-GluD-S100)

RayBiotech, Inc.

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I. INTRODUCTION

Glucose 1-dehydrogenase (NAD^+) (EC 1.1.1.118) is an enzyme that catalyzes the chemical reaction: D-glucose + NAD^+ ↔ D-glucono-1,5-lactone + NADH + H^+. This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with NAD^+ or $NADP^+$ as acceptor. RayBiotech's Glucose Dehydrogenase (GDH) Assay Kit provides a convenient tool for sensitive detection of the GDH in a variety of samples. The GDH present in sample will recognize D-glucose as a specific substrate leading to a proportional color development. The activity of GDH can be easily quantified colorimetrically (λ = 450 nm). This assay detects GDH activity as low as 0.01 mU with our unit definition.

II. REAGENTS

Components	GluD-S100	Cap Code	Part Number
GDH Assay Buffer	25 ml	WM	Item A
Glucose (2 M)	1 ml	Blue	Item B
Developer (lyophilized)	1 vial	Red	Item C
GDH Positive Control (lyophilized)	1 vial	Green	Item D
NADH Standard (0.5 µmol, Lyophilized)	1 vial	Yellow	Item E

III. STORAGE AND HANDLING

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

IV. REAGENT RECONSSTITUTION AND GENERAL CONSIDERATION

- Reconstitute 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 GDH Positive Control with 220 µl Assay Buffer; Keep on ice during the preparation and protect from light. Aliquot and store -20°C.
- Reconstitute the NADH with 50 µl ddH_2O to generate a 10 mM NADH stock solution.
- The GDH Positive Control and the Developer are stable for up to 2 months at -20^{o}C after reconstitution or freeze-thaw cycles (< 5 times). Reconstituted NADH (10 mM) and the supplied Glucose (2 M) solution are stable for up to 6 months at -20°C.

V. GLUCOSE DEHYDROGENASE ASSAY PROTOCOL

1. **NADH Standard Curve:** Dilute 10 µl of the 10 mM NADH stock solution with 90 µl of 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 in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well standards. 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 ~ 200µl ice-cold Assay Buffer then centrifuged (13,000 x g, 10 min.) to remove insoluble material. 5 - 50µl serum samples can be directly diluted in the Assay Buffer. Adjust the final volume of test samples to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve and set up the background control group to avoid interference of the NADH in the sample. 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:

Reaction Mix	Background Control Mix
82 µl Assay Buffer	92 µl Assay Buffer
8 µl GDH Developer	8 µl GDH Developer
10 µl 2 M Glucose	

4. **Measurement:** Incubate the mix for 3 min at 37°C, then measure OD at 450 nm in a microplate reader (A_0), incubate for another 30 mins to 2 hrs at 37°C and measure OD at 450 nm again (A_1), (Note: Incubation times depends on the GDH activity in your samples). We recommend measuring the OD in a kinetic method (preferably every 3 - 5 min) and choose the period of linear range to calculate the GDH activity of the samples. The NADH Standard Curve can read in Endpoint Mode (i.e., at the end of the incubation time).
5. **Calculation:** Subtract the 0 Standard value from all readings (standards and test samples). Plot the NADH standard Curve, then calculate the GDH activity of the test samples: $\Delta OD = A_1 - A_0$, apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by GDH during the reaction time ($\Delta T = T_2 - T_1$).

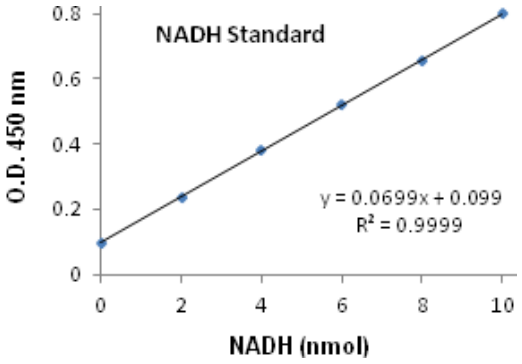
GDH Activity = $\frac{B}{\Delta T \times V}$ **Sample Dilution Factor = nmol/min/ml = mU/ml**

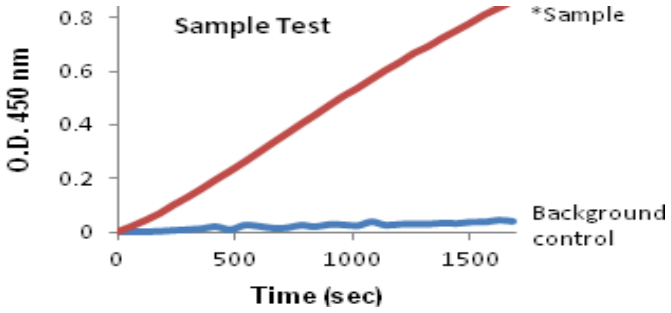
Where: **B** is the glutamate amount from standard curve (in nmol).

T is the time incubated.

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 8 at 37°C.





IV. General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay 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 (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • 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 the 10 kDa spin cut-off filter or PCA precipitation as indicated • 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 • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • 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	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & 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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