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Glutamate Dehydrogenase Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Glutamate Dehydrogenase activity in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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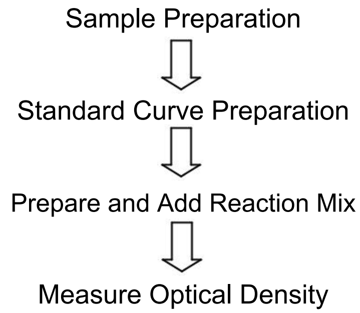
1. Overview

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.

Abcam's Glutamate Dehydrogenase Detection 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
GDH Assay Buffer	25 mL
Glutamate Solution/Glutamate (2M)	1 mL
Developer Solution III/GDH Developer (Lyophilized)	1 vial
GDH Positive Control/GDH Positive Control (Lyophilized)	1 vial
NADH Standard I/NADH (0.5 µmol; Lyophilized)	1 vial

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

GDH POSITIVE CONTROL: Reconstitute with 220 µl Assay Buffer. Keep the GDH Positive Control on ice during the preparation and protect from light. Aliquot and store -20°C. Stable for up to 2 months after reconstitution or freeze-thaw cycles (< 5 times).

DEVELOPER SOLUTION IIII/GDH DEVELOPER: Reconstitute with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution (DO NOT VORTEX). Stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times).

NADH STANDARD I/NADH: Reconstitute with 50 µl ddH_2O to generate a 10 mM NADH Standard I/NADH stock solution.

Reconstituted NADH Standard I/NADH (10 mM) and the supplied Glutamate Solution/Glutamate (2 M) solution are stable for up to 6 months at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. **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. Sonicating a few pulses on ice or using a Dounce homogenizer can be used in order to facilitate lysis.

b. **5-50 µl serum samples** can be directly diluted in the Assay Buffer.

c. Add test sample (**5-50uL**) into the Sample wells and bring volume up to 50 µl/well with 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.

2. NADH Standard Curve:

Dilute 10 µl of the NADH Standard I/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.

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
Developer Solution III/GDH Developer	8 µl
Glutamate Solution/Glutamate (2M)	10 µl

Add 100 µl of the Reaction Mix to each well containing the test samples, positive controls and standards. Mix well.

4. 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 (A_0), incubate for another 30 min. to 2 hrs at 37°C to measure OD at 450 nm again (A_1).

Note:

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. A_n to A_{n+1}) to calculate the GDH activity of the samples.

5. Data Analysis

Plot the Glutamate standard curve. Apply OD = $A_1 - A_0$ (or $A_{n+1} - A_n$) to the Glutamate Standard Curve to get B nmol of NADH produced by GDH in the given time.

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

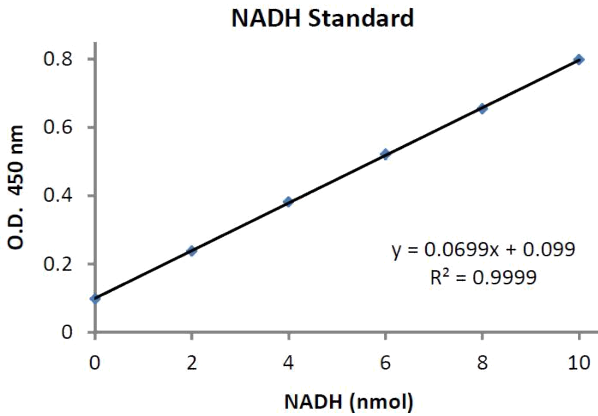
Where:

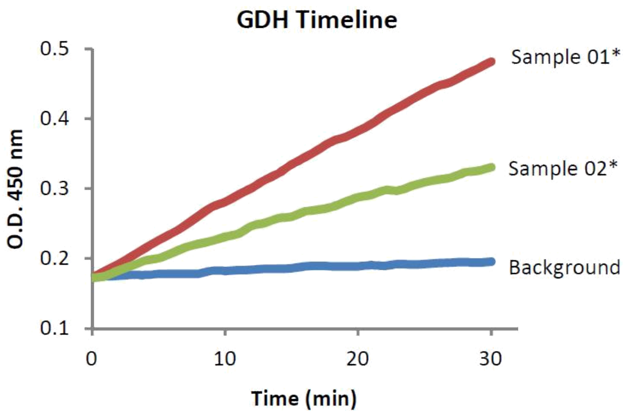
B is the NADH amount from standard curve (in nmol).

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

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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