

ab102527

**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.

Table of Contents

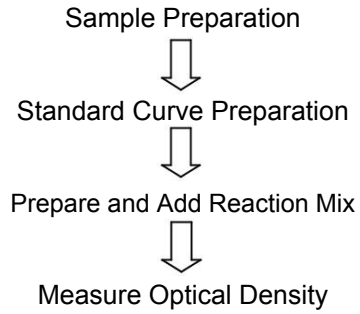
1. Overview	3
2. Protocol Summary	4
3. Components and Storage	5
4. Assay Protocol	7
5. Data Analysis	9
6. Troubleshooting	11

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 ($\lambda = 450 \text{ nm}$). 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	1 mL
Developer Solution III	1 vial
GDH Positive Control	1 vial
NADH Standard I	1 vial

PLEASE NOTE: Developer Solution III was previously labelled as GDH Developer (Lyophilized), and Glutamate Solution as Glutamate (2M), and NADH Standard I was previously labelled as NADH (0.5 μ mol; Lyophilized). The composition has not changed.

* 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 μ l 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 III: Reconstitute with 0.9 ml of ddH₂O. 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: Reconstitute with 50 μl ddH₂O to generate a 10 mM NADH Standard I stock solution.

Reconstituted NADH Standard I (10 mM) and the supplied Glutamate 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 (1×10^6)** 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 (**5-50 μL**) 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 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 μ l) containing:

Assay Buffer	82 μ l
Developer Solution III	8 μ l
Glutamate Solution	10 μ l

Add 100 μ l 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.

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

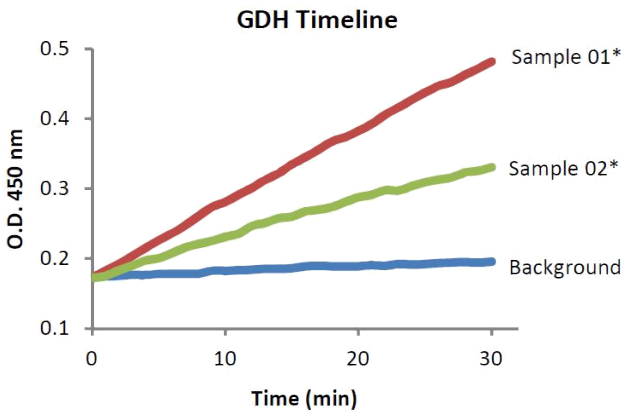
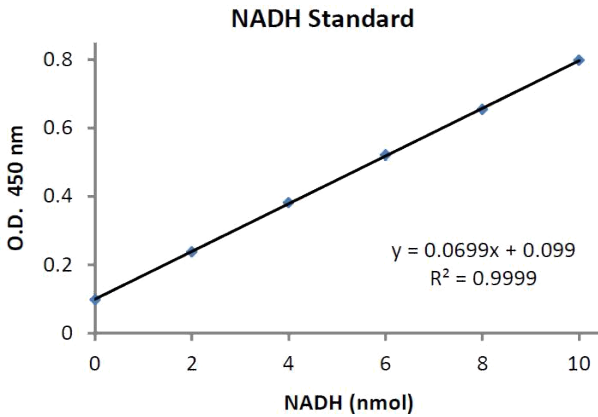
Where:

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 μ g protein), Sample 02: 5 μ l 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

Technical Support

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