ab102532

Glucose Dehydrogenase Assay Kit (Colorimetric)

Instructions for Use

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

View kit datasheet: www.abcam.com/ab102532
(use www.abcam.cn/ab102532 for China, or www.abcam.co.jp/ab102532 for Japan)

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

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1. Overview

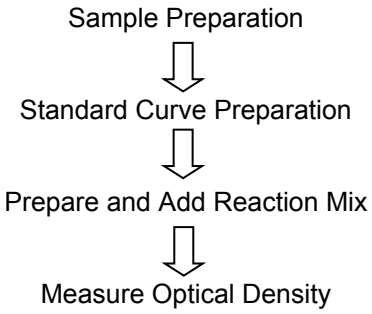
Glucose 1-dehydrogenase (NAD^+) (EC 1.1.1.118) is an enzyme that catalyzes the chemical reaction:

$$\text{D-glucose} + NAD^+ \leftrightarrow \text{D-glucono-1,5-lactone} + NADH + H^+$$

This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with NAD^+ or $NADP^+$ as acceptor.

Abcam's Glucose Dehydrogenase Assay Kit (Colorometric) 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.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
GDH Assay Buffer	25 mL
Glucose (2 M)	1 mL
Developer (Lyophilized)	1 vial
GDH Positive Control (Lyophilized)	1 vial
NADH Standard (0.5 µmol, Lyophilized)	1 vial

4. Storage and Stability

Upon arrival, store kit at -20°C, protected from light.

Allow the Assay Buffer to warm up 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 in 220 µl of Assay Buffer; keep on ice during the preparation. Aliquot and store at -20°C. Reconstituted control is stable for up to 2 months at -20°C or < 5 freeze/thaw cycles.

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)**. Protect from light during preparation**.** Reconstituted developer is stable for up to 2 months at -20°C or < 5 freeze/thaw cycles.

NADH STANDARD: Reconstitute with 50 µl of ddH_2O to generate a 10 mM NADH stock solution. Reconstituted NADH 10 mM stock is stable for up to 6 months at -20°C.

GLUCOSE: it is already supplied in a 2 M solution. It is stable for up to 6 months at -20°C.

5. Materials Required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

6. Assay Protocol

1. **Sample Preparation:**

a. **For tissue or cell samples:**

Tissues (50 mg) or cells (1 x 10^6) can be homogenized in ~200 µl of ice-cold Assay Buffer then centrifuged (13,000 x g, 10 min.) to remove insoluble material.

b. **For liquid samples (cell culture media, cell culture supernatant, plasma, serum, urine and other biological fluids):**

Liquid samples can be assayed directly (use 5 – 50 µl) or after dilution 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.

c. **Positive control**:

Add 2 µl of reconstituted GDH positive control to wells and adjust to a final volume of 50 µl with Assay Buffer.

d. Set up the **background control** group to avoid interference of the NADH in the sample.

2. **Standard Curve Preparation:**

   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.

3. **Reaction Mix Preparation:**

   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
Assay Buffer	82 µL	92 µL
Developer	8 µL	8 µL
2 M Glucose	10 µL	---

   Add 100 µL of the Reaction Mix to each well containing the test samples, positive controls, and standards; add 100 µL of the Background Control Mix to each well containing the background control sample. Mix well. The reaction mix is stable for 4 hours on ice and 1 hour at room temperature. Reaction mix needs to be protected from light so prepare in an amber colored vial.

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 depend 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 be read in Endpoint Mode (i.e., at the end of the incubation time).

7. Data Analysis

Subtract the zero Standard value from all readings (standards and test samples). Plot the NADH standard Curve, then calculate the GDH activity of the test samples: $\mathbf{\Delta OD = A_1 - A_0}$

Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by GDH during the reaction time ($\Delta T = T_2 - T_1$).

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

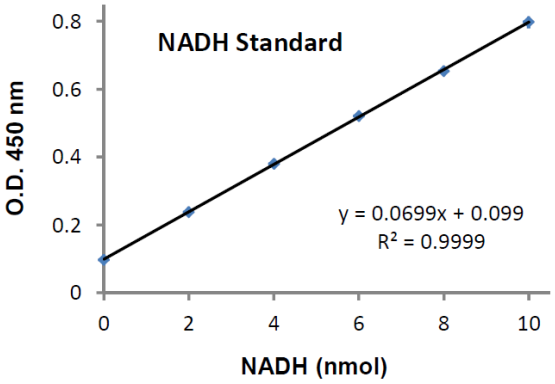
Where:

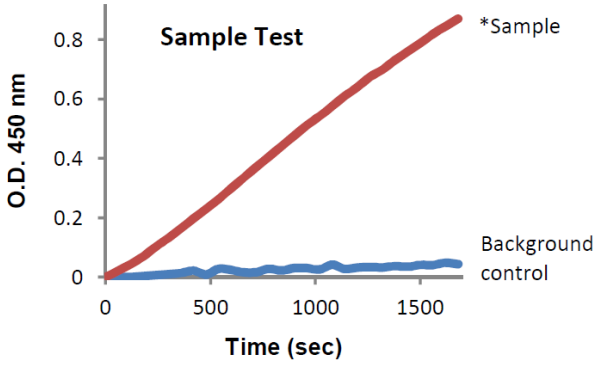
B is the glutamate amount from standard curve (in nmol).

T is the time incubated.

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

One unit is defined as the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 8 at 37°C.





*Sample: Bovine Liver extraction (36µg protein)

8. 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 consider different dilutions/purification of the sample
	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)
	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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