ab174095

Glycerol-3-Phosphate Dehydrogenase (G3PDH) Assay Kit (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of Glycerol-3-Phosphate Dehydrogenase activity in a variety of samples

Version: 3c Last Updated: 4 January 2021

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

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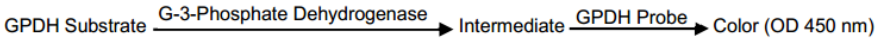
1. Overview

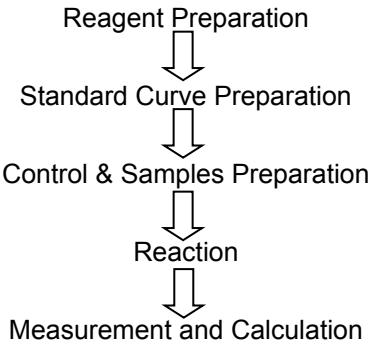
Glycerol-3-Phosphate Dehydrogenase (EC 1.1.1.8) is an important enzyme for lipid metabolism. It catalyzes the reversible conversion between dihydroxyacetone phosphate and glycerol-3-phosphate. GPDH plays multiple functions inside cells; it links carbohydrate and lipid metabolism, and provides electrons through the Glycerol-3-Phosphate Shuttle. When progenitor adipocytes differentiate into mature adipocytes, GPDH activity increases significantly. Analysis of glycerol-3-phosphate dehydrogenase activity is crucial for the study of fatty acid metabolic pathways.

In Abcam's Glycerol-3-Phosphate Dehydrogenase (G3PDH) Assay Kit (Colorimetric) (ab174095), Glycerol-3-phosphate dehydrogenase reacts with the substrate to forms an intermediate, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and rapid and can detect Glycerol-3-Phosphate dehydrogenase activity less than 1 mU/well.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
GPDH Assay Buffer	27 mL
GPDH Substrate (Lyophilized)	1 vial
GPDH Probe (Lyophilized)	1 vial
NADH Standard (Lyophilized)	1 vial
GPDH Positive Control (Lyophilized)	1 vial

4. Storage and Stability

Upon arrival, store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96-well clear plate with flat bottom (for colorimetric reading)
- Multi-well spectrophotometer (ELISA reader)
- Multi-channel pipette
- Distilled water

6. Reagent Preparation

1. **GPDH Assay Buffer:**
   Provided as ready to use. Warm GPDH Assay Buffer to room temperature before use.

2. **GPDH Substrate:**
   Reconstitute with 220 µL GPDH Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.

3. **GPDH Probe**:
   Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

4. **GPDH Positive Control:**
   Reconstitute with 100 µL GPDH Assay Buffer and mix thoroughly. Aliquot and store at –20°C. Use within two months.

5. **NADH Standard:**
   Reconstitute Standard with 100 µL GPDH Assay buffer to generate 5 mM NADH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.

7. Assay Protocol

1. Sample Preparation and Control:

a) Cells – adipocytes, preadipocytes, etc (starting material: 1 x 10^6 cells)

Harvest cells and spin down briefly and discard supernatant. Resuspend the cell pellet in 200 µL ice cold GPDH Assay Buffer and put on ice. Homogenize with a Douncer homogenizer (10 – 15 passes) on ice, or by pipetting up and down using a smaller tip, until efficient lysis is confirmed by viewing the cells under the microscope. Centrifuge homogenate at 12000 rpm for 5 minutes at 4°C to remove cell debris and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 1 – 50 µL test sample to the wells of a 96-well plate. If volume need is <50 µL, bring it up to 50 µL with Assay Buffer.

b) Tissue Sample (starting material: 10 mg tissue)

Cut tissue in small pieces, add 200 µL ice cold GPDH Assay Buffer and put on ice. Homogenize using a Douncer homogenizer (10 – 15 passes) on ice, until efficient lysis is confirmed, by viewing the cells under the microscope. Spin down the samples and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 1 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with GPDH Assay Buffer.

NOTE: For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

c) BACKGROUND CONTROL:

Prepare a parallel sample well as the background control to avoid interference from NADH in the sample.

d) POSITIVE CONTROL:

Use 1 – 10 µL of reconstituted GPDH Positive Control and adjust the final volume to 50 µL with GPDH Assay Buffer.

2. NADH Standard Curve

a) Prepare a 1mM NADH Standard by adding 30 µL of 5mM NADH Standard to 120 µL GPDH Assay buffer.

b) Using the 1 mM NADH Standard prepare a standard curve as follows, in a microplate or microfuge tubes:

NADH 1 mM amount (µL)	GPDH Assay buffer (µL)	Amount in well	End concentration NADH in well
0	150	50 µL	0 nmol/well
7.5	142.5	50 µL	2.5 nmol/well
15	135	50 µL	5 nmol/well
22.5	127.5	50 µL	7.5 nmol/well
30	120	50 µL	10 nmol/well
37.5	112.5	50 µL	12.5 nmol/well

Add 50 µL of each standard dilution into a 96-well plate to set up standard. Each dilution has enough amount of standard to set up 2 duplicates x 50 µL/well.

3. **Reaction Mix:**

Prepare Reaction Mix for each reaction containing:

	Reaction Mix	Background Control Mix
GPDH Assay Buffer	46 µL	48 µL
GPDH Substrate	2 µL	---
GPDH Probe	2 µL	2 µL

Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a Master Mix of the

Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
GPDH Assay Buffer	46 µL x (Nb samples + Standards + positive controls + 1)
GPDH Substrate	2 µL x (Nb samples + Standards + positive controls + 1)
GPDH Probe	2 µL x (Nb samples + Standards + positive controls + 1)

We also recommend preparing enough **Background Control Mix** to set up duplicate readings for the NADH background control using the same calculation.

4. Plate set up and Detection:

a) Add 50 µL of standard, sample and positive control to wells.

b) Add 50 µL of the Reaction Mix to each well containing the Standard, positive control and test samples. Mix well.

c) Add 50µL of Background Control Mix to 50 µL of background control well(s). Mix well.

d) Incubate for 20-60 min at 37ºC and measure OD at λ = 450 nm

NOTE: Incubation time depends on the Glycerol-3-Phosphate Dehydrogenase activity in the samples. We recommend

measuring the OD in a kinetic mode, and choose two time points (T1 & T2) in the linear range to calculate the GPDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

8. Data Analysis

Calculations:

a) Correct background by subtracting the value derived from the zero standard from all sample readings.

b) Plot the NADH Standard Curve.

c) If Background Control reading is significantly high, subtract the Background Control reading from the sample reading.

Calculate the GPDH activity of the test sample:

$$\Delta \mathbf{OD = A2 - A1}$$

Apply the ΔOD to the NADH Standard Curve to get **B** (nmol of NADH) generated by Glycerol-3-Phosphate Dehydrogenase during the reaction time (ΔT = T2 - T1).

$$\mathbf{Sample\ GPDH\ activity = B/(\Delta T\ X\ V)\ x\ Dilution\ Factor}$$

$$\mathbf{= nmol/min/mL = mU/mL}$$

Where:

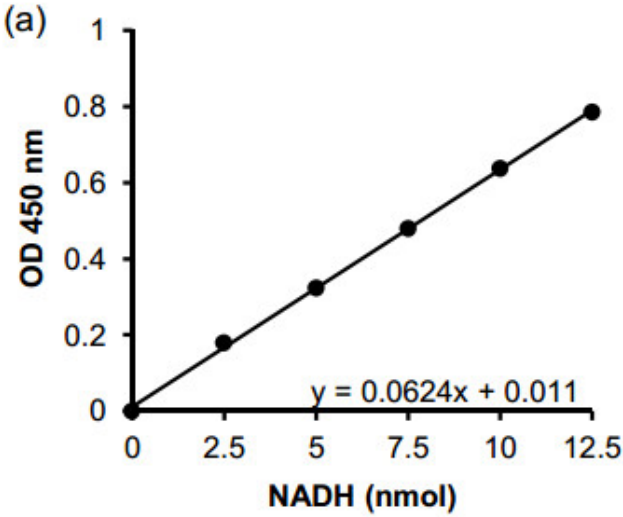
B = NADH amount from Standard Curve (nmol)

ΔT = reaction time (min)

V = volume of sample used in the reaction well (mL)

Unit Definition: One unit of Glycerol-3-Phosphate Dehydrogenase (GPDH) is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 8 at 37ºC.

1 GPDH U (µmol/min) = 1 mU (nmol/min)



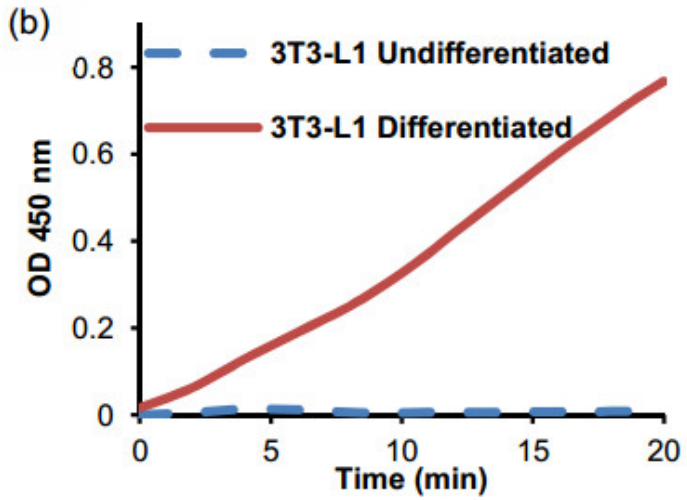


Figure 2. : (a) NADH Standard Curve. **(b)** Measurement of Glycerol-3-Phosphate Dehydrogenase activity in 3T3-L1 pre- adipocyte (60 µg) and differentiated 3T3-L1 adipocytes (60 µg). Assays were performed following kit protocol.

9. 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

Problem	Reason	Solution
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

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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