ab155892

Glucose-1-Phosphate Colorimetric Assay Kit

Instructions for Use

For the sensitive and accurate measurement of Glucose-1-Phosphate in animal tissues and cell culture samples (adherent and suspension).

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

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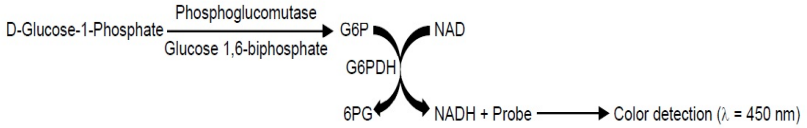
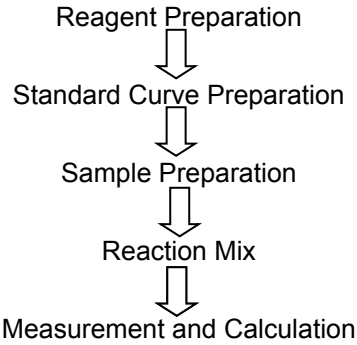
1. Overview

Glucose-1-phosphate (G1P) is an important carbohydrate intermediate in glucose metabolism and storage. In response to hormonal or neural signals, glycogenolysis occurs in liver and muscle tissues where Glucose-1-phosphate is released as the rate-limiting step in glycogen breakdown. Glucose-1-phosphate is subsequently converted to Glucose-6-phosphate by phosphoglucomutase and enters glycolysis. In glycogen synthesis, glucose is transferred to glycogen through the actions of Phosphoglucose isomerase (G6P→G1P), UDPG-pyrophosphorylase (G1P→UDP-glucose) and glycogen synthase (UDP-glucose + glycogen[n] →UDP + glycogen[n+1]). Glucose-1-phosphate is found in virtually all organisms from bacteria to higher plants and animals. Measurement of intracellular G1P levels is crucial for analyzing the carbohydrate metabolic pathways and their kinetic properties

In Abcam's Glucose-1-phosphate assay, G1P is converted to glucose-6-phosphate by phosphoglucomutase in the presence of Glucose 1,6-biphosphate; glucose-6-phosphate is subsequently oxidized by glucose-6-phosphate dehydrogenase to form NADH which reduces a colorless probe to a colored product with strong absorbance at 450 nm. BioVision's Glucose-1-phosphate assay kit is rapid, sensitive and easy to use and can detect 1µM to 10 mM G1P. This G1P assay kit can be used for a variety of sample types.



2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer	27 mL
Enzyme Mix (Lyophilized)	1 vial
Developer (Lyophilized)	1 vial
Substrate Mix (Lyophilized)	1 vial
Standard (Lyophilized)	1 vial

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

Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

4. Assay Protocol

A. Reagent Preparation

1. G1P Enzyme Mix:

Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months at -20°C.

2. G1P Developer:

Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months at -20°C.

3. G1P Substrate Mix:

Reconstitute with 220 µl dH2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.

4. G1P Standard:

Reconstitute with 100 µl dH2O to generate 100 mM (100 nmol/µl) G1P Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

B. G1P Assay Protocol

1. Standard Curve Preparation:

Dilute G1P standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM G1P Standard to 990 µl dH_2O & mix well. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM G1P Standard into a series of wells in duplicate in 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of G1P standard. Adjust volume to 50 µl/well with Assay Buffer.

2. Sample Preparation:

Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold G1P Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (40 µg) per well, adjust final volume to 50 µl with G1P Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from the NADH in the sample.

Note: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

3. Reaction Mix:

Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix
G1P Assay Buffer	44 µl	46 µl
G1P Enzyme Mix	2 µl	----
G1P Developer	2 µl	2 µl
G1P Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples and 50 µl of Background Control mix to each well containing the Background Control sample. Mix well.

4. Measurement:

Incubate for 30 min at room temperature and measure OD450nm.

5. Data Analysis

Calculation: Subtract the 0 standard reading from all standard readings. Plot the G1P standard curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Apply the corrected sample reading to the standard curve to get B nmol of G1P amount in the sample wells.

The GIP concentration in the sample:

$$\mathbf{C = \frac{B}{V} \times \text{Dilution Factor} = nmol/\ ml/ = \mu M}$$

Where:

B is the G1P amount from standard curve (nmol).

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

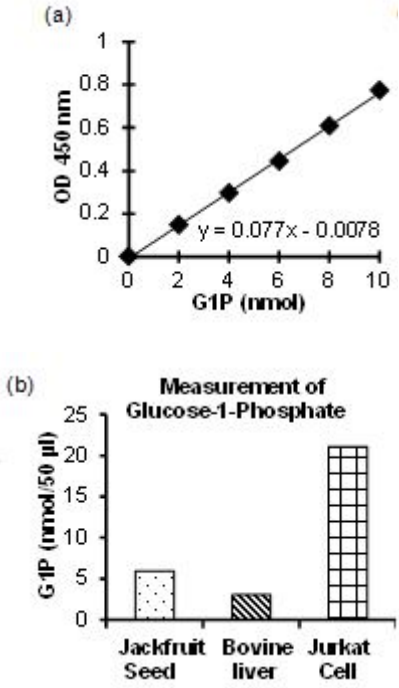


Figure 1: G1P standard curve (a). Measurement of glucose-1-phosphate in jackfruit seed, bovine liver and jurkat cell lysate (induced with 2 µM camptothecin) (b). Assays were performed following kit protocol.

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

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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