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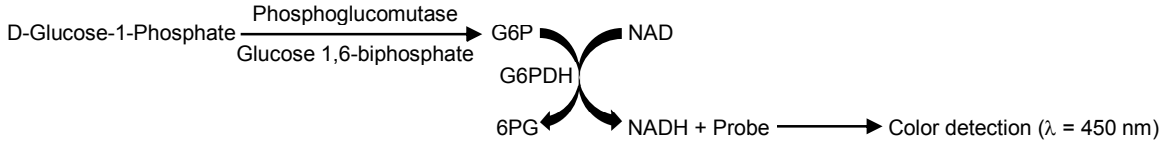
Glucose-1-Phosphate Colorimetric Assay Kit

rev. 08/12

(Catalog # K697-100; 100 assays; Store at -20°C)

I. Introduction:

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 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 & easy to use & can detect 1µM to 10 mM G1P. This G1P assay kit can be used for a variety of sample types.



II. Application:

- Measurement of Glucose-1-Phosphate in various tissues/cells.
- Analysis of glucose metabolism and cell signaling in various cells.
- Screening anti-diabetic drugs.

III. Sample Type:

Animal tissues: Liver, muscle and heart etc.
Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K697-100	Cap Code	Part Number
G1P Assay Buffer	27 ml	WM	K697-100-1
G1P Enzyme Mix (Lyophilized)	1 vial	Purple	K697-100-2
G1P Developer (Lyophilized)	1 vial	Green	K697-100-3
G1P Substrate Mix (Lyophilized)	1 vial	Red	K697-100-4
G1P Standard (Lyophilized)	1 vial	Yellow	K697-100-5

V. User Supplied Reagents and Equipments:

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

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **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.
- **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.
- **G1P Substrate Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **G1P Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) G1P Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

VIII. 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:** Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 200 µl ice cold G1P Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add1-50 µl (~100 µg) samples per well and adjust the 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 reaction 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 OD_{450nm}.

5. **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 G1P concentration in the sample:

C= B/V x Dilution Factor = nmol/ml = µM

Where: **B** is the amount of G1P from the standard curve (nmol).

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

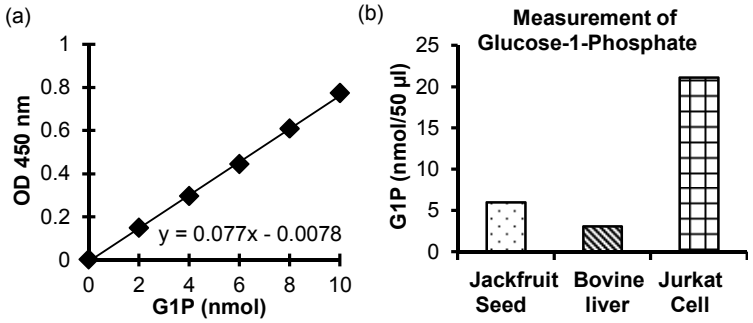


Figure. G1P standard curve (a). Measurement of glucose-1-phosphate in jackfruit seed, bovine liver & jurkat cell lysate (induced with 2 µM camptothecin) (b). Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Glucose Assay kit
Glucose Dehydrogenase Activity Assay Kit
PicoProbe™ Glucose-6-Phosphate Assay Kit
Glucose Uptake Fluorometric Assay Kit
Hexokinase Colorimetric Assay Kit
NAD/NADH Quantification Kit
Starch Assay Kit
Glucose and Sucrose Assay Kit
Glucose-6-Phosphate Dehydrogenase Assay Kit
Glucose Uptake Colorimetric Assay Kit
Glycogen Assay Kit
Maltose and Glucose Assay Kit
NADP/NADPH Quantification Kit

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