Glycerol-3-Phosphate (G3P) Colorimetric Assay Kit *(#BN00865)*

(Catalog # BN00865; 100 assays; Store at -20°C)

I. Introduction:

Glycerol-3-phosphate (G3P) is an important intermediate for all living organisms. Glycerol-3-Phosphate is produced either by glycerol via glycerol kinase or by dihydroxyacetone phosphate through glycerol-3-phosphate dehydrogenase. In response to cellular signals, glycerol-3-phosphate can be utilized in multiple pathways: it can be further converted into glyceraldehyde-3-phosphate and enter glycolysis or rapidly generate NAD^+ in brain or muscle tissues through the G3P shuttle or enter the lipid biosynthetic pathway. Recent studies have found that glycerol-3-phosphate is a novel regulator and plays a fundamental defense role in plant pathogenesis. Assay Genie's Glycerol-3-Phosphate Assay kit is a sensitive, fast and easy-to-use kit. In this assay, G3P is oxidized by G3P Enzyme Mix to form an intermediate, which reduces a nearly colorless probe to a colored product with strong absorbance at 450 nm. This assay kit can detect G3P less than 2 nmol/well and can be used for a variety of sample types.

Glycerol-3-Phosphate $\xrightarrow{\text{G3P Enzyme Mix}}$ Intermediate $\xrightarrow{\text{G3P Probe}}$ Color Detection (λ = 450 nm)

II. Application:

- Measurement of Glycerol-3-Phosphate in various tissues/cells
- Analysis of carbohydrate and lipid metabolism and cell signaling
- Drug screening

III. Sample Type:

- Animal tissues
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	BN00865	Cap Code	Part Number
G3P Assay Buffer	25 ml	WM	BN00865-1
G3P Enzyme Mix (Lyophilized)	1 vial	Green	BN00865-2
G3P Probe (Lyophilized)	1 vial	Red	BN00865-3
G3P Standard (Lyophilized)	1 vial	Yellow	BN00865-4

V. User Supplied Reagents and Equipment:

- 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 Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **G3P Enzyme Mix**: Reconstitute with 220 µl G3P 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.
- **G3P Probe**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **G3P Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) G3P Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

VIII. Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly. Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 200 µl ice cold G3P Assay Buffer on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl samples into a 96 well plate and bring the volume to 50 µl with G3P Assay Buffer.

   Notes:

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

   b. NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:** Dilute G3P Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM G3P Standard to 990 µl dH_2O, mix well. Add 0, 2, 4, 6, 8 & 10 µl of the 1 mM G3P Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well G3P Standard. Adjust volume to 50 µl/well with G3P Assay Buffer.

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
G3P Assay Buffer	46 µl	48 µl
G3P Enzyme Mix	2 µl	----
G3P Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples & 50µl of background control mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate for 40 minutes at 37°C. Measure OD_{450nm}.

5. **Calculation:** Subtract 0 nmol G3P Standard reading from all readings. Plot the G3P Standard Curve. If sample background control reading is significant, subtract the background control reading from sample reading. Apply the corrected sample reading to G3P Standard Curve to get B nmol of G3P in the sample wells.

Sample G3P concentration = B/V x Dilution Factor = nmol/ml = µM

Where: **B =** the amount of G3P in the sample well (nmol)
V = sample volume used in the reaction well (ml)

G3P in samples can also be expressed in nmol/mg of protein.
Glycerol-3-Phosphate molecular weight: 172.074 g/mol

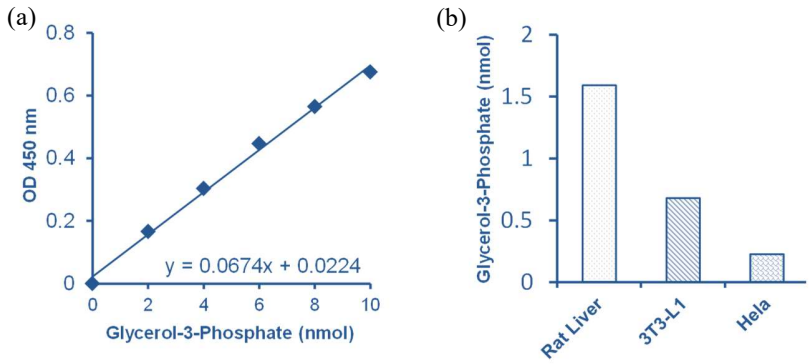

Figure: G3P Standard Curve (a). Measurement of G3P in rat liver (100 µg), 3T3-L1 (40 µg) and Hela (50 µg) lysate (b). Assays were performed following the kit protocol.

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