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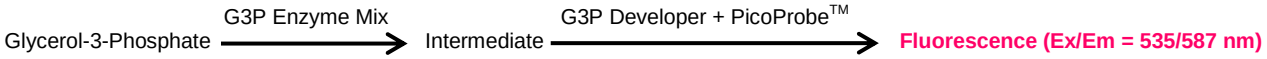
PicoProbe™ Glycerol-3-Phosphate Assay Kit (Fluorometric)

(Catalog # K196-100; 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 glycerol-3-phosphate dehydrogenase from dihydroxyacetone phosphate. 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, 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. BioVision's PicoProbe™ Glycerol-3- Phosphate Assay Kit is a sensitive, fast and easy-to-use method of measuring G3P concentration in various biological samples. In this assay, G3P is converted into an intermediate by the G3P Enzyme Mix. The G3P developer then utilizes the intermediate to generate a fluorometric signal with PicoProbe™, which can be used to calculate the concentration of G3P in the sample. This assay kit can detect G3P as little as 20 pmol/well and can be used for a variety of sample types.



II. Applications:

- Measurement of Glycerol-3-Phosphate content in biological fluids and extracts from cells and tissues

III. Sample Type:

- Biological fluids (such as plasma or serum)
- Tissue homogenates or cultured cell lysates

IV. Kit Contents:

Components	K196-100	Cap Code	Part Number
G3P Assay Buffer	25 ml	WM	K196-100-1
G3P Enzyme Mix	1 vial	Green	K196-100-2
G3P Developer	1 vial	Red	K196-100-3
PicoProbe™ (in DMSO)	400 µl	Blue	K196-100-4
G3P Standard	1 vial	Yellow	K196-100-5

V. User Supplied Reagents and Equipment:

- 96-well flat bottom white plate
- Multi-well spectrophotometer
- Dounce Tissue Homogenizer (Cat. #1998)
- 10K Spin Column (Cat. #1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Centrifuge vials prior to opening. Read the protocol before performing the assay.

- **G3P Assay Buffer:** Warm to room temperature before use.
- **G3P Enzyme Mix and G3P Developer:** Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute G3P Enzyme Mix and G3P Developer with 220 µl Assay Buffer each before use. Aliquot and store at -20°C (once reconstituted, vials contents are stable for two months).
- **PicoProbe™:** Aliquot and store at -20°C, **protected from light.** Thaw at room temperature before use.
- **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. Stable for at least two months.

VII. Glycerol-3-Phosphate Assay Protocol:

1. **Sample Preparation:**
   Liquid samples: biological fluids should be deproteinized by filtration through a 10 kDa MWCO spin column (Cat. #1997). Clarify liquid samples by centrifugation at 10,000 x *g* for 5 min at 4°C (to remove insoluble materials), then transfer supernatant to spin column. Centrifuge at 10,000 x *g* for 10 min at 4°C and collect the filtrate.
   For tissue or cell samples: 10 mg tissue or 1 x 10^6 cells should be rapidly homogenized with 100 µl of ice-cold G3P Assay Buffer; scale volume proportionally to amount of sample used. Centrifuge at 10,000 x *g* for 5 min at 4°C to remove insoluble materials. Transfer supernatant to a 10 kDa MWCO spin column, centrifuge at 10,000 x *g* for 10 min at 4°C and collect the filtrate.
   Add 2 – 50 µl sample filtrate into duplicate wells (one well for total signal and one for background signal) of a white, flat-bottom 96-well plate and bring the volume to 50 µl with G3P Assay Buffer. For unknown samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range.
2. **Glycerol-3-Phosphate Standard Curve Generation:** Dilute the provided 100 mM G3P standard 1:100 in G3P Assay Buffer to obtain 1 mM G3P. Further dilute the 1 mM G3P solution at a 1:20 ratio by adding 10 µl to 190 µl Assay Buffer to generate a 50 µM working solution. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM G3P standard to wells of the 96 well plate to obtain 0, 100, 200, 300, 400, and 500 pmol of Glycerol-3-Phosphate per well. Bring up the total volume in these wells to 50 µl with G3P Assay buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each sample and standard curve well, prepare 50 µl of Reaction Mix. For each sample background control well, prepare 50 µl of Background Mix. The total reaction volume after addition of Reaction Mix is 100 µl.

	Reaction Mix	Background Mix
G3P Assay Buffer	43 µl	45 µl
G3P Developer	2 µl	2 µl
G3P Enzyme mix	2 µl	–
PicoProbe™	3 µl	3 µl

Add 50 µl of the reaction mix to standard and sample total signal wells. Add 50 µl Background Mix to sample background (second) wells. Mix well. Incubate plate at 37°C for 30 minutes, **protected from light.**

4. **Measurement:** Record fluorescence of all sample and standard curve wells in end point mode at Ex/Em= 535/587 nm.

5. **Calculations:** Subtract the zero standard (0 pmol/well) reading from all Glycerol-3-Phosphate Standard readings and plot the Glycerol-3-Phosphate Standard Curve. For all test samples, calculate the corrected fluorescence by subtracting sample background control RFU readings from sample RFU readings. Apply the corrected RFU value to the Standard Curve to get B pmol G3P in the sample well.

G3P concentration in sample (C) = (B / V) x D = (pmol/µl) ≡ µM

Where **B** = Amount of Glycerol-3-Phosphate in the sample well from Standard Curve (in pmol)
V = Volume of sample added into the well (in µl)
D = Sample dilution factor (if applicable, D=1 for undiluted samples)

G3P concentrations can also be expressed as pmol G3P per µg tissue (nmol per mg)

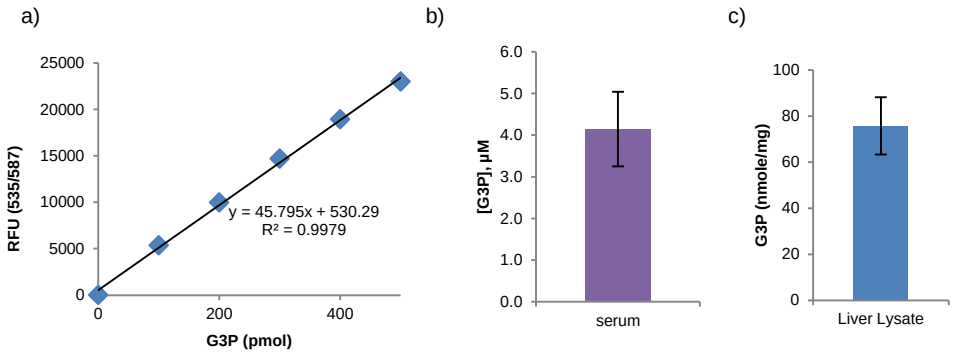


Figure: (a) Glycerol-3-Phosphate standard curve. (b) Glycerol-3-Phosphate content in pooled human serum. Serum was deproteinized by filtration through a 10 K MWCO spin column (Cat. #1997) and 20 µl of the filtrate was assayed in triplicate according to the kit protocol. (c) Glycerol-3-Phosphate determination in rat liver lysate (10 µg added per well). 100 mg of wet tissue was homogenized in 1 ml assay buffer using a dounce tissue homogenizer and deproteinized by filtration through a 10 kDa MWCO spin column.

VIII. RELATED PRODUCTS

PicoProbe™ Phosphatidic Acid Assay Kit (K748)
Phospholipid Assay Kit (K351)
Cardiolipin Assay Kit (K944)
Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Triglyceride Assay Kit (K622)
Sphingomyelin Quantification Colorimetric Assay Kit (K600)
Monoacylglycerol Lipase Activity Assay Kit (K561)
Glycerol-3-Phosphate Dehydrogenase Activity Assay Kit (K640)
Glycerol-3-Phosphate (G3P) Colorimetric Assay Kit (K641)
Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)
Free Glycerol Colorimetric Assay Kit II (K634)
Glycerol Cell-Based Assay Kit (K977)
Phosphatidylethanolamine Assay Kit (K499)
Monoacylglycerol Lipase Inhibitor Screening Assay Kit (K474)

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