ab174094

Glycerol-3-phosphate (G3P) Assay Kit (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of G3P activity in a variety of samples

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

Version 4a, Last Updated 4 June 2025

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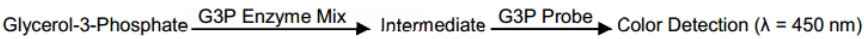
1. Overview

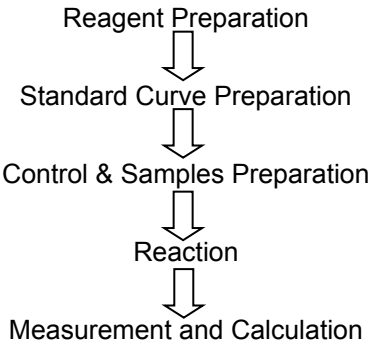
Glycerol-3-phosphate (G3P) is an important intermediate for all living organisms. Glycerol-3-Phosphate is produced either by glycerol via glycerol kinase or by dihydroxyacetone phosphate through glycerol-3-phosphate dehydrogenase. In response to cellular signals, glycerol-3-phosphate can be utilized 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.

Abcam's Glycerol-3-Phosphate Assay kit (ab174094) is a sensitive, fast and easy-to-use kit. In this assay, G3P is oxidized by Developer Mix F to form an intermediate, which reduces a nearly colorless Developer Solution III 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.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer 5	25 mL
Developer Mix F	1 vial
Developer Solution III	1 vial
G3P Standard	1 vial

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and G3P Assay Buffer, and Developer Mix F as Development Enzyme Mix VII and G3P Enzyme Mix (Lyophilized). The composition has not changed.

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. Reagents Preparation

1. **Developer Solution III**:
   Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.

2. **Developer Mix F:**
   Reconstitute with 220 µL Assay Buffer 5. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw.

3. **G3P Standard:**
   Reconstitute with 100 µL dH_2O to generate a 100 mM (100 nmol/µL) G3P Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

4. **Assay Buffer 5:**
   Warm Assay Buffer 5 to room temperature before use.

7. Assay Protocol

1. Control and Sample Preparation:

a) Cells (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 Assay Buffer 5 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 5.

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

Cut tissue in small pieces, add 200 µL ice cold Assay Buffer 5 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 Assay Buffer 5.

NOTE:

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

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

2. Standard Curve Preparation:

a) Prepare a 1mM (1nmol/µL) G3P Standard by adding 10 µL of 100 mM G3P Standard to 990 µL dH_2O. Mix well by pipetting up and down.

b) Using the 1 mM G3P Standard please prepare a standard curve as follows, in a microplate or microcentrifuge tubes:

G3P 1 mM amount (µL)	**Assay Buffer 5 (µL)**	**Amount in well**	**End concentration G3P in well**
0	150	50 µL	0 nmol/well
6	144	50 µL	2 nmol/well
12	138	50 µL	4 nmol/well
18	132	50 µL	6 nmol/well
24	126	50 µL	8 nmol/well
30	120	50 µL	10 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 x duplicates x 50 µL/well.

3. Reaction Mix:

Prepare Reaction Mix for each reaction containing:

	Reaction Mix	**Background Control Mix**
Assay Buffer 5	46 µL	48 µL
Developer Mix F	2 µL	---
Developer Solution III	2 µL	2 µL

Mix enough reagents for the number of assays to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
Assay Buffer 5	46 µL x (Nb samples + Standards + 1)
Developer Mix F	2 µL x (Nb samples + Standards + 1)
Developer Solution III	2 µL x (Nb samples + Standards + 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 or sample to wells

b) Add 50 µL of the Reaction Mix to wells containing standard and samples. Mix well.

c) If necessary, also add 50 µL of Background Control Mix to 50 µL of background control well(s). Mix well.

d) Incubate for 40 minutes at 37°C.

e) Measure OD450nm.

8. Data Analysis

Calculations:

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

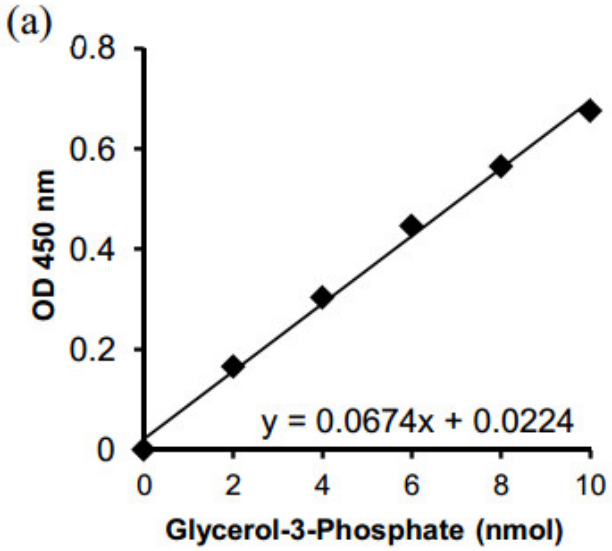
b) Plot the G3P Standard Curve.

c) 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 = amount of G3P in the sample well (nmol)
V = volume of sample 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


(a)
0.8
0.6
0.4
0.2
0
OD 450 nm
y = 0.0674x + 0.0224
0
2
4
6
8
10
Glycerol-3-Phosphate (nmol)

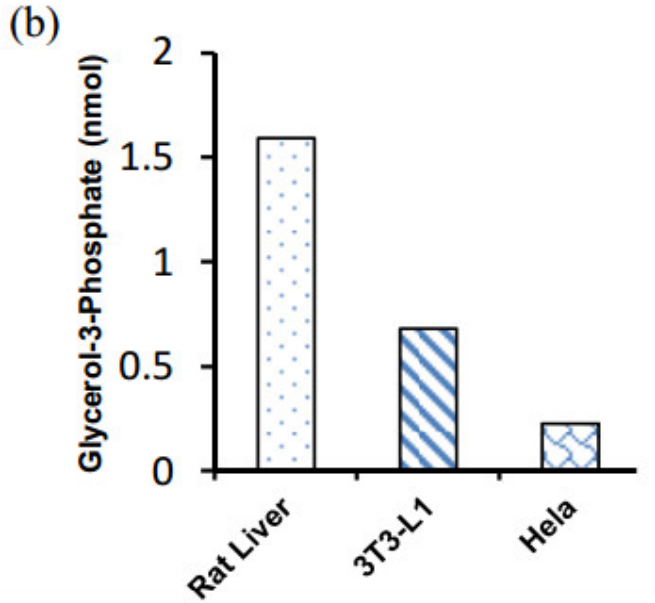


Figure 2: (a) G3P Standard Curve. **(b)** Measurement of G3P in rat liver (100 µg), 3T3-L1 (40 µg) and Hela (50 µg) lysate. Assays were performed following the kit protocol.

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).

Technical Support

For all technical or commercial enquiries please go to:

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