Technical Bulletin

Glycerol 3-Phosphate (G3P) Assay Kit

Catalogue number MAK546

Product Description

Glycerol 3-Phosphate (G3P) is an important intermediate in glycolysis metabolic pathway. Animals, fungi, and plants use G3P to produce ATP. It is used to regenerate NAD+ in brain and skeletal muscle cells. G3P has been linked to lipid imbalance diseases such as obesity.

The G3P Assay Kit uses red substrate to quantify the concentration of G3P, which is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. The kit is an optimized "mix and read" format that is compatible with HTS applications. The limit of detection for this assay is 12.5 µM G3P in 100 µL assay volume as shown in Figure 1. The assay can be performed in a convenient 96-well microtiter-plate format and easily adapted to automation without a separation step.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Red Substrate — 1 Vial
  Catalogue Number MAK546A
- Enzyme Mix — 1 Vial
  Catalogue Number MAK546B
- Assay Buffer — 10 mL
  Catalogue Number MAK546C
- Glycerol 3-Phosphate (G3P) Standard — 1 Vial
  Catalogue Number MAK546D
- DMSO — 100 µL
  Catalogue Number MAK546E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet Ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate reagents to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicates.

Preparation of Stock Solution

Red stock solution (200X): Add 50 µL of DMSO into the vial of Red Substrate to make 200X stock solution. Avoid exposure to light.

Note: The Red Substrate reagent is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol

in the reaction should be no higher than 10 µM. The Red Substrate is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH 7 – 8. The provided assay buffer (pH 7.4) is recommended.

G3P Standard Stock Solution (10 mM): Add 250 µL of purified water into the vial of G3P Standard to make 10 mM G3P Standard Solution.

Preparation of G3P Standard

1. Add 100 µL of 10 mM G3P Standard Stock Solution to 900 µL 1X PBS buffer to generate 1000 µM G3P Standard Solution.
2. Add 200 µL of 1000 µM G3P Standard Solution to 800 µL 1X PBS buffer to generate 200 µM G3P Standard (GP1).
3. Perform 1:2 serial dilutions to get the remaining G3P Standards (GP6 - GP1) as shown in Table 1.

Note: Diluted G3P Standard Solution is unstable and should be used within 4 hours.

Table 1.
Serial dilution of G3P Standard Solutions.

Dilution	G3P Std Vol (µL)	Serial Dilution Source	PBS Vol (µL)	Conc (µM)
GP1	300	200 µM stock	-	200
GP2	150	From GP1	150	100
GP3	150	From GP2	150	50
GP4	150	From GP3	150	25
GP5	150	From GP4	150	12.5
GP6	150	From GP5	150	6.25
GP7	150	From GP6	150	3.12

Preparation of G3P Working Solution

Note: Prepare immediately before use in assay reaction. The working solution is enough for one 96-well plate.

1. Add 5 mL of Assay Buffer to the bottle of Enzyme Mix and mix well.
2. Then add 25 µL of Red Substrate stock solution (200X) into the same bottle of Enzyme Mix to make the G3P Working Solution.

Note: This working solution is not stable, use it promptly and avoid direct exposure to light.

Assay Reaction

1. Add 50 µL of the G3P Working Solution to each well of G3P Standard, blank (Assay Buffer), and test sample to make the total G3P assay volume of 100 µL/well. For a 384-well plate, add 25 µL of G3P working solution and sample into each well instead, for a total volume of 50 µL/well.
2. Incubate the reaction mixture at room temperature for 30 - 60 minutes, protected from light.

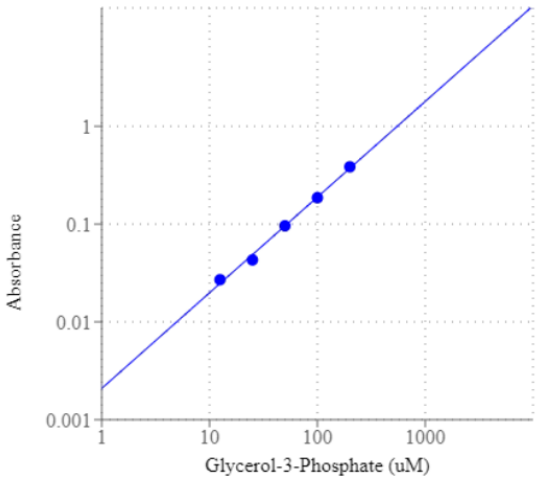
Measurement

Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 575 nm (or ratio of 570 nm/610 nm).

Results

1. The reading (Absorbance) obtained from the blank Standard well is used as a negative control.
2. Subtract the blank value from the Standards to obtain the base-line corrected values.
3. Plot the standards readings to obtain the Standard curve.
4. The concentration of G3P in the test Samples may be determined from the Standard curve.

Figure 1.
Typical Glycerol 3-Phosphate Standard Curve



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