3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Glycerol 3-Phosphate Colorimetric Assay Kit**

Catalog Number **MAK207**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Glycerol 3-phosphate (G3P) is an important intermediate of carbohydrate and lipid metabolic pathways. It is produced from glycerol by glycerol kinase or from dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. G3P may enter the G3P shuttle to generate $NAD^+$, or may be converted to glyceraldehyde 3-phosphate and enter glycolysis or the lipid biosynthesis pathway.[1,2]

The Glycerol 3-Phosphate Colorimetric Assay Kit provides a simple assay for measuring G3P in various tissues and cells (ranging from 2–10 nmole/well). G3P is determined by measuring a colorimetric product with absorbance at 450 nm ($A_{450}$) proportional to the amount of G3P present.

### Components

The kit is sufficient for 100 assays in 96 well plates.

| | |
|---|---|
| G3P Assay Buffer<br>Catalog Number MAK207A | 25 mL |
| G3P Enzyme Mix<br>Catalog Number MAK207B | 1 vl |
| G3P Probe<br>Catalog Number MAK207C | 1 vl |
| G3P Standard<br>Catalog Number MAK207D | 1 vl |

### Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

### Precautions and Disclaimer

This product is 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.

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

G3P Assay Buffer – Warm buffer to room temperature before use.

G3P Enzyme Mix – Reconstitute with 220 µL of G3P Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice while in use.

G3P Probe – Reconstitute with 220 µL of water. Mix well by pipetting and store at –20 °C. Use within 2 months.

G3P Standard – Reconstitute with 100 µL of water to generate a 100 mM (100 nmole/µL) G3P Standard Solution. Store at –20 °C. Use within 2 months. Keep on ice while in use.

### Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

### Procedure

All samples and standards should be run in duplicate.

<u>G3P Standards for Colorimetric Detection</u>

Dilute 10 µL of the 100 mM G3P Standard with 990 µL of water and mix well to make a 1 mM (1 nmole/µL) G3P Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM (1 nmole/µL) G3P Standard Solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add G3P Assay Buffer to each well to bring the volume to 50 µL,.

Sample Preparation

Clear liquid samples may be assayed directly.

Tissue samples (10 mg) or cells ($1 \times 10^6$) can be homogenized in 100 µL of ice cold G3P Assay Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 12,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

Add 1–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using G3P Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, especially samples containing NADH, include a Sample Blank for each sample by omitting the G3P Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction

1. Set up Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

**Table 1.**
Reaction Mixes

| Reagent | Standards and samples | Sample Blank |
|---|---|---|
| G3P Assay Buffer | 46 µL | 48 µL |
| G3P Enzyme Mix | 2 µL | – |
| G3P Probe | 2 µL | 2 µL |

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 40 minutes at 37 °C.

4. Measure the absorbance ($A_{450}$) in a microplate reader.

**Results**

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) G3P standard from that of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate G3P Standards to plot a standard curve. Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the Sample value to obtain the corrected measurement. Using the corrected measurement, determine the amount of G3P (nmole/well) generated by the assay ($S_a$).

Concentration of G3P

$C = S_a/S_v$

where:
$S_a$ = Amount of G3P in unknown sample well (nmole) from standard curve
$S_v$ = Sample volume (μL) added into the well
C = Concentration of G3P in sample (nmole/μL)

Sample Calculation

Amount of G3P ($S_a$) = 5.841 nmole (from standard curve)

Sample volume ($S_v$) = 10 μL

Concentration of glycerol 3-phosphate in sample:

5.841 nmole/10 μL = 0.5841 nmole/μL

Molecular weight of G3P = 172.1 g/mole

0.5841 nmole/μL × 172.1 ng/nmole = 100.5 ng/μL

**References**


1. Crabtree, B., and Newsholme, E.A., The activities of phosphorylase, hexokinase, phospho-fructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. Biochem. J., **126**, 49–58 (1972).
2. Larsson, C. et al., The importance of the glycerol 3-phosphate shuttle during aerobic growth of *Saccharomyces cerevisiae*. Yeast, **14**, 347–357 (1998).
3. Kunst, L. et al., Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast Glycerol 3-phosphate acyltransferase activity. Proc. Natl. Acad. Sci. U. S. A., **85**, 4143–4147 (1988).

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay not working | Cold assay buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | For colorimetric assays, use clear plates |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Reaction Mixes before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the Reaction Mixes |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare Reaction Mixes whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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