Technical Bulletin

Glucose-6-Phosphate (G6P) Assay Kit

Catalogue number MAK548

Product Description

Glucose-6-phosphate (G6P) is a key intermediate for glucose transport into cells. G6P may also be converted to glycogen or starch for storage in the liver and muscles. G6P is utilized by glucose-6-phosphate dehydrogenase (G6PD) to generate the reducing equivalents in the form of NADPH. This is particularly important in red blood cells where G6PD deficiency leads to hemolytic anemia.

The Glucose-6-Phosphate Assay Kit provides a simple, sensitive, and rapid fluorescence-based method for detecting G6P in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the coupled enzyme assay, the G6P concentration is proportionally related to NADPH that is specifically monitored by a fluorogenic NADPH sensor. The detection limit of the G6P Assay Kit is 3 µM G6P in a 100 µL reaction volume.

Components

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

- Enzyme Probe 1 Vial
  Catalogue Number MAK548A
- Assay Buffer 10 mL
  Catalogue Number MAK548B
- NADP 1 Vial
  Catalogue Number MAK548C
- Glucose-6-Phosphate Standard 1 Vial
  Catalogue Number MAK548D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Black, 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 to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

NADP Stock Solution (100X): Add 100 µL of purified water into the vial of NADP to make 100X NADP stock solution.

G6P Standard Solution (100 mM): Add 100 µL of purified water or 1X PBS buffer into the vial of G6P Standard to make 100 mM G6P Standard Solution.

Preparation of G6P Standard Solution

1. Add 10 µL of 100 mM G6P Standard solution into 990 µL 1x PBS buffer to generate 1 mM G6P Standard solution.
2. Add 100 µL of 1 mM G6P Standard solution into 900 µL 1 x PBS buffer to make 100 µM G6P Standard Solution (G6P1).
3. Take 100 µM G6P Standard solution (G6P1) and perform 1:3 serial dilutions to get serially diluted G6P Standards (G6P2 - G6P7) with 1x PBS buffer per Table 1.

Note: Diluted G6P Standard Solution is unstable and should be used within 4 hours.

Table 1:

Serial dilution of Glucose 6 Phosphate (G6P) Standard

Dilution	G6P Std Vol (µL)	Serial Dilution Source	1X PBS Vol (µL)	Conc (µM)
G6P1	225	100 µM stock	0	100
G6P2	75	From G6P1	150	33.3
G6P3	75	From G6P2	150	11.1
G6P4	75	From G6P3	150	3.7
G6P5	75	From G6P4	150	1.2
G6P6	75	From G6P5	150	0.4
G6P7	75	From G6P6	150	0.14

Preparation of G6P Working Solution

1. Add 5 mL of Assay Buffer into a vial of Enzyme Probe and mix well.
2. Add 50 µL of 100X NADP stock solution into this vial and mix well to make the G6P working solution.

Note: This G6P working solution is enough for one 96-well plate.

Assay Reaction

1. Add 50 µL of each G6P Standards, blank (PBS), and test Samples into separate wells of a 96-well plate.
2. Add 50 µL of G6P working solution to each well of G6P Standard, blank, and test Sample to make the total G6P assay volume of 100 µL/well.
3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.

Measurement

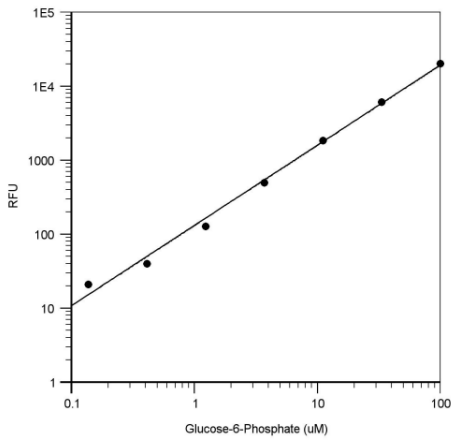
1. Read the fluorescence at λ_{Ex} = 530-570nm/λ_{Em} =590-600 nm. (Optimal $\lambda_{Ex}/\lambda_{Em}$ = 540/590 nm, cutoff = 570nm).

Results

1. The reading (RFU) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the standards readings to obtain the baseline corrected values.
3. Plot the standards to obtain a standard curve and equation.
4. This equation can be used to calculate Glucose-6-Phosphate samples.

Figure 1.

Typical Glucose 6 Phosphate Standard Curve



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