Technical Bulletin

Glucose-6-Phosphate Dehydrogenase Assay Kit

Catalog Number MAK451

Product Description

Glucose-6-Phosphate Dehydrogenase (G6PDH) is a cytosolic enzyme in the pentose phosphate pathway which supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). G6PDH reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate (G6P). Humans with a genetic deficiency of G6PDH are predisposed to non-immune hemolytic anemia.

The non-radioactive, colorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit is based on the reduction of the tetrazolium salt MTT in a NADPH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity. The linear detection range for the assay method is 0.2 to 100 U/L for a 15 minute reaction.

The kit is suitable for the quantitative determination of Glucose-6-Phosphate concentration and evaluation of drug effects on its metabolism in plasma, serum, tissue and culture media, etc.

Components

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

- Assay Buffer 10 mL
  Catalog Number MAK451A
- Diaphorase 120 µL
  Catalog Number MAK451B
- NADP/MTT 1 mL
  Catalog Number MAK451C
- Calibrator 1.5 mL
  Catalog Number MAK451D
- Substrate 1 mL
  Catalog Number MAK451E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 10,000 × *g*
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalog Number P0662 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. 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. Assays can be executed at any desired temperature (e.g., 25 °C or 37 °C). Equilibrate reagents to desired reaction temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and Plasma

Serum and plasma samples can be assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood.
2. Homogenize tissue (50 mg) in 200 µL of buffer containing 50 mM potassium phosphate, pH 7.5.
3. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
4. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate, pH 7.5.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

All Samples

1. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
2. All Samples can be stored at -20 to -80 °C for at least one month.
3. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.

Working Reagent

Mix enough reagents for the number of assays to be performed. For each Sample well, prepare 87 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	**Working Reagent**
Assay Buffer	70 µL
Substrate	8 µL
NAD/MTT	8 µL
Diaphorase	1 µL

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to Samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Assays can be executed at any desired temperature (e.g., 25 °C or 37 °C).

1. Transfer 100 µL of purified water (OD_{H2O}) and 100 µL of Calibrator (OD_{CAL}) into separate wells of a clear flat bottom 96-well plate.
2. Add 80 µL of Working Reagent to each Sample well. Do not add Working Reagent to Water or Calibrator wells.
3. Tap plate briefly to mix.

Measurement

Read optical density (OD) at 565 nm immediately (OD_0), and again after 15 minutes (OD_{15}) on a plate reader.

Results

1. Calculate the ΔOD_S by subtracting the OD_0 value from OD_{15} for each Sample.
2. Calculate G6PDH activity as follows:

G6PDH Activity (U/L) =

$$\frac{\Delta OD_S}{\varepsilon_{MTT} \times L} \times \frac{RV}{T \times SV} \times DF$$

=

$$\frac{\Delta OD_S}{OD_{CAL} - OD_{H2O}} \times \frac{273}{T} \times DF$$

where

ΔOD_S = Sample OD_{15} value minus OD_0 value

OD_{CAL} = OD value at 565 nm of Calibrator

OD_{H2O} = OD value at 565 nm of the Water blank

RV = Total reaction volume (100 µL)

T = Reaction time. The standard is 15 minutes but may be extended or shortened due to low or high enyzme activity.

SV = Sample volume used in reaction (20 µL)

ε_{MTT} = Molar absorption coefficient of reduced MTT

L = Light pathlength which is calculated from the calibrator

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

Note: If Sample G6PDH activity exceeds 100 U/L, repeat the assay and either use a shorter reaction time or dilute samples in purified water. For Samples with G6PDH activity < 1 U/L, the incubation time can be extended up to 2 hours.

Unit definition: 1 Unit (U) of G6PDH will catalyze the conversion of 1 µmole of NADP to NADPH per minute at pH 8.2.

Figure 1.
G6PDH titration curve, raw kinetic data.

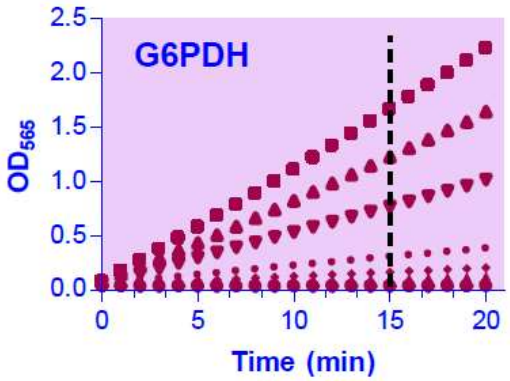
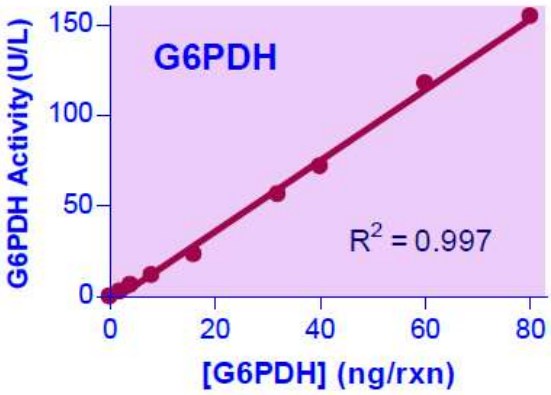


Figure 2.
G6PDH titration curve. G6PDH Activity (15 minutes at 25 °C).



References

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2. Kirman, H.N. and Hendrickson, E.M., Glucose 6-phosphate dehydrogenase from human erythrocytes. II. Subactive states of the enzyme from normal persons. *J. Biol. Chem*., **237**, 2371-6 (1962).
3. Tian, W-N, et. al., Importance of glucose-6-phosphate dehydrogenase activity for cell growth. *J. Biol. Chem*., **273**, 10609-17 (1998).

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