Product Manual

# Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Assay Kit

**Catalog Number**

**MET-5081** **100 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

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## Introduction

Glucose-6-phosphate dehydrogenase (G6PDH) is a key cytosolic enzyme, broadly distributed in many species (bacteria, humans, plants). Within the pentose phosphate pathway (PPP), G6PDH plays an important metabolic roll in generating pentose sugars, ribose 5-phosphate (nucleotide precursor), and NADPH. NADPH is critical during fatty acid synthesis and helps maintain glutathione levels in erythrocytes, protecting against oxidative damage. Additionally, glucose-6-phosphate dehydrogenase deficiency is a genetic disease causing acute hemolytic anemia in response to a number of triggers (certain foods, illness, or medication).

Cell Biolabs' Glucose-6-Phosphate Dehydrogenase Activity Kit measures G6PDH activity in cell and tissue samples. G6PDH-containing samples oxidize the kit's substrate in a 2-step, redox reaction; the resulting coenzyme product then reacts with the kit's Colorimetric Probe (absorbance maxima of 450 nm).

The G6PDH Activity Assay Kit is a simple, colorimetric assay that quantitatively measures the G6PDH activity in cell/tissue lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards, and unknown samples. The kit contains an enzyme standard and has a detection sensitivity limit of ~1 mUnits/mL.

## Related Products

1. MET-5019: Total Phosphatidic Acid Assay Kit (Fluorometric)
2. MET-5024: Phosphatidylglycerol/Cardiolipin Assay Kit (Fluorometric)
3. MET-5028: DAG (Diacylglycerol) Assay Kit (Fluorometric)
4. MET-5036: DAG Kinase Activity Assay Kit
5. MET-5078: Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit
6. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
7. STA-390: Total Cholesterol Assay Kit
8. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
9. STA-394: HDL Cholesterol Assay Kit
10. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
11. STA-398: Free Glycerol Assay Kit (Colorimetric)
12. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

## Kit Components

1. G6PDH Enzyme Standard (Part No. 50811D): One 1 mL vial containing 500 mUnits/mL of G6PDH.

   *Note: One unit corresponds to the amount of enzyme which will oxidize 1 µmole of D-glucose-6-phosphate to 6-phospho-D-gluconate per minute at pH 7.8 and 30ºC.*

2. 5X Lysis Buffer (Part No. 50782B): One 30 mL bottle.
3. Assay Buffer (Part No. 50783B): Two 1.5 mL vials.
4. 5X G6PDH Substrate (Part No. 50812D): Two 1 mL vials.
5. Colorimetric Probe (Part No. 50181C): One 1 mL amber vial.

## Materials Not Supplied

1. PBS
2. 96-well microtiter plate
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm

## Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

## Preparation of Reagents

- 1X Lysis Buffer: Dilute the 5X Lysis Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Assay Buffer and Colorimetric Probe: Thaw and maintain at room temperature during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- G6PDH Enzyme Standard and 5X G6PDH Substrate: Thaw and maintain at 4ºC during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

  *Note: 5X G6PDH Substrate is provided in multiple tubes to minimize multiple freeze/thaws.*

## Preparation of G6PDH Enzyme Standard Curve

- G6PDH Enzyme Standard should be thawed/maintained at 4ºC during assay preparation. For longer term storage, the G6PDH Enzyme Standard should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

- Freshly prepare a dilution series of G6PDH Enzyme Standard in the concentration range of 50 mUnits/mL – 0.78 mUnits/mL by diluting the enzyme stock solution (provided at 500 mUnits/mL) in 1X Lysis Buffer (see Table 1). Standards should be prepared fresh, vortexed well and used immediately. Do not store diluted standards.

| Standard Tubes | 500 mUnits/mL G6PDH Enzyme Standard (µL) | 1X Lysis Buffer (µL) | Final G6PDH Enzyme Standard (mUnits/mL) |
|---|---|---|---|
| 1 | 50 | 450 | 50 |
| 2 | 250 of Tube #1 | 250 | 25 |
| 3 | 250 of Tube #2 | 250 | 12.5 |
| 4 | 250 of Tube #3 | 250 | 6.25 |
| 5 | 250 of Tube #4 | 250 | 3.13 |
| 6 | 250 of Tube #5 | 250 | 1.56 |
| 7 | 250 of Tube #6 | 250 | 0.78 |
| 8 | 0 | 250 | 0 |

**Table 1. Preparation of G6PDH Enzyme Standard Curve**

## Preparation of Samples

- Plasma and serum: This kit is not recommended for these samples**.**
- Tissue Samples: Weigh out 100 mg of tissue and mince into small pieces. Rinse the tissue with cold PBS to remove red blood cells and clots. Homogenize the minced tissue in 1 mL cold 1X Lysis Buffer. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 1 month. Tissue homogenates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Suspension Cells: Collect 1 x $10^7$ cells by centrifugation at 1000 x g for 10 minutes. Carefully aspirate the culture media and wash once with cold PBS. Centrifuge at 1000 x g for 10 minutes at 4°C. Discard the supernatant and resuspend in 1 mL cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Adherent Cells: Carefully aspirate the culture media and wash once with the recommended volume of PBS (Table 2). Discard the supernatant and add the appropriate volume of cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Transfer the lysate to a microcentrifuge tube. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For

longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.

| Culture Dish | 96-well | 48-well | 24-well |
|---|---|---|---|
| PBS Wash Volume (µL/well) | 200 | 400 | 800 |
| 1X Lysis Buffer (µL/well) | 75 | 150 | 300 |

**Table 2: Dispensing Volumes of Different Plate Formats**

## Assay Protocol

***Important Note: Freshly prepare G6PDH Enzyme standards each time the assay is performed. Maintain the G6PDH Enzyme Standard and 5X G6PDH Substrate at 4ºC during assay preparation.***

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   *Note: Each unknown sample replicate requires two paired wells, one to be treated with 5X G6PDH Substrate (+Sub) and one without (-Sub) to determine background.*

2. Add 50 µL of the G6PDH Enzyme standards, samples or blanks to the 96-well microtiter plate.
3. Prepare the desired volume of Substrate Mixture (+Sub) according to Table 3 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
   a. In a tube, add the appropriate volume of Assay Buffer.
   b. Next, add the corresponding volume of 5X G6PDH Substrate.
   c. Finally, add the corresponding volume of Colorimetric Probe. Mix well and immediately use.

| Assay Buffer (mL) | 5X G6PDH Substrate (mL) | Colorimetric Probe (mL) | Total Volume of Substrate Mixture (mL) | **# of Tests in 96-well Plate (50 µL/well)** |
|---|---|---|---|---|
| 1 | 1 | 0.5 | 2.5 | **50** |
| 0.5 | 0.5 | 0.25 | 1.25 | **25** |
| 0.2 | 0.2 | 0.1 | 0.5 | **10** |

**Table 3. Preparation of Substrate Mixture (+Sub)**

4. Prepare the desired volume of Control Mixture (-Sub) according to Table 4 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
   d. In a tube, add the appropriate volume of Assay Buffer.
   e. Next, add the corresponding volume of deionized water.

f. Finally, add the corresponding volume of Colorimetric Probe. Mix well and immediately use.

| Assay Buffer (mL) | Deionized $H_2O$ (mL) | Colorimetric Probe (mL) | Total Volume of Substrate Mixture (mL) | **# of Tests in 96-well Plate (50 µL/well)** |
|---|---|---|---|---|
| 1 | 1 | 0.5 | 2.5 | **50** |
| 0.5 | 0.5 | 0.25 | 1.25 | **25** |
| 0.2 | 0.2 | 0.1 | 0.5 | **10** |

**Table 4. Preparation of Control Mixture (-Sub)**

5. Transfer 50 µL of the Substrate Mixture (+Sub) from step 3 above to all wells containing standards and to one half of the paired sample wells. Mix thoroughly.
6. Transfer 50 µL of the Control Mixture (-Sub) from step 4 above to the remaining half of the paired sample wells. Mix thoroughly.
7. Incubate at 37ºC for 15 minutes.
8. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.
9. Subtract the OD of the control wells (-Sub) from the OD of the substrate wells (+Sub) to obtain the Net OD for each sample replicate, before comparing to the standard curve.

## Example of Results

The following figures demonstrate typical G6PDH Activity Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

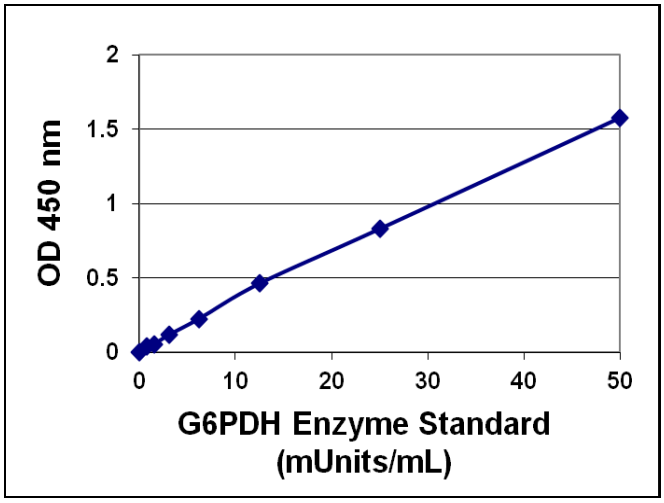


**Figure 1: G6PDH Enzyme Standard Curve.** G6PDH Enzyme standard curve was performed according to the Assay Protocol. Background has been subtracted.

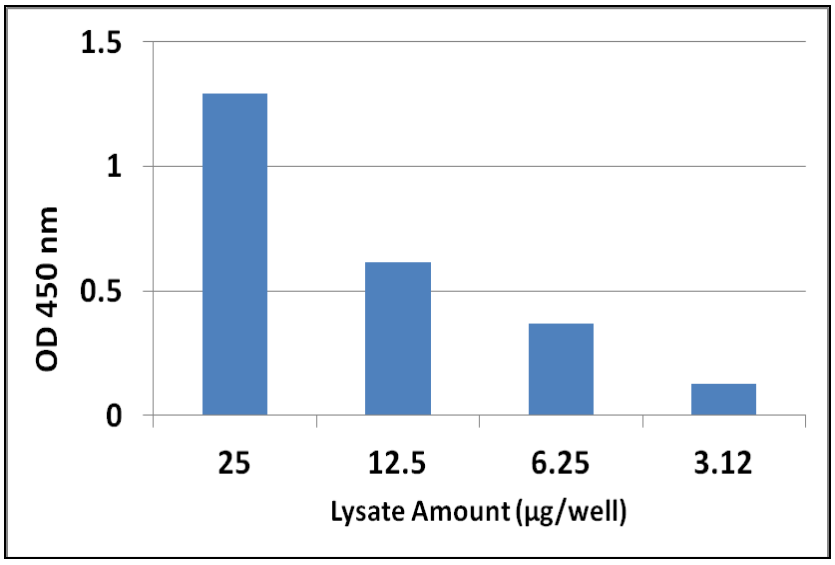


**Figure 2: G6PDH Activity of HEK-293 Lysate.** HEK-293 lysate was prepared according to the Assay Protocol (total protein concentration was also determined). Background has been subtracted.

## References

1. Lai, Y., Lai, N., and Lee, S. (2017) *Ann. Hematol.* **96**, 839-845.
2. Peters, A., van Noorden, C. (2017) *Methods Mol. Biol.* **1560**, 3-13.
3. Reclos, G., Hatzidakis, C., and Schulpis, K. (2000) *J. Med. Screen.* **7**, 46-51.

## Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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