QuantiChrom™ Glucose-6-Phosphate Dehydrogenase Inhibitor Screening Kit (IGPDH-100)

Quantitative Determination of Glucose-6-Phosphate Dehydrogenase Inhibitor Activity

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. Recently, studies have found G6PDH plays a critical role in survival, proliferation, and metastasis of cancer cells. Therefore, inhibitors of the enzyme are attractive candidates for new cancer therapeutics.

BioAssay Systems' non-radioactive, colorimetric G6PDH assay 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 percent inhibition of a test compound can be determined by comparing the activity of G6PDH treated with a test compound to the activity of untreated G6PDH.

KEY FEATURES

Safe. Non-radioactive assay.

High-throughput. Homogenous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling system.

Rapid and reliable. Can be completed in 30 minutes and no 37°C heater is needed.

APPLICATIONS

HTS for inhibitor screening and evaluation of glucose-6-phosphate dehydrogenase inhibitors.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**Diaphorase:**	120 µL
NADP/MTT:	1 mL	**10×Substrate:**	100 µL (450 mM G6P)

Storage conditions. The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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).

Sample Preparation:

The following protocol is optimized for *L. Mesenteroides* G6PDH. Dilute purified G6PDH to 0.0003 U/µL using dH_2O. If another species is being analyzed, we recommend that you experimentally determine the K_m and then adjust the volume of substrate in the Working reagent so that the final concentration of the substrate in the 100 µL reaction is near the K_m. Dissolve the test compounds in solvent of choice. If using DMSO or DMF, it is prudent to first test the tolerance of DMSO and DMF by the enzyme of choice. For G6PDH from *L. Mesenteroides*, the DMSO concentration of the 5 µL of test compounds added to the reaction should be 2 v/v% DMSO or less; while the DMF concentration of the 5 µL of test compounds added to the reaction should be 40 v/v% DMF or less.

G6PDH Reaction Preparation:

1. Transfer 20 µL of G6PDH into separate wells.
2. Reserve two wells with G6PDH for the Blank (no substrate) and Control (no inhibitor).
3. To the Control and Blank wells, add 5 µL of the solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 2 v/v% DMSO, add 5 µL 2 v/v% DMSO to these wells.
4. To the remainder of the wells containing G6PDH, add 5 µL of the test compounds. Tap plate and mix.
5. Incubate the plate for 15 minutes at 25°C.
6. Prepare enough 1× Substrate by diluting 10× Substrate 10-fold in dH_2O. (Each Reaction Well will need 8 µL of 1× Substrate)
7. Prepare sufficient Reaction Mix (RM) by mixing for each well (except the Blank well), 70 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase, and 8 µL 1× Substrate.

   Prepare Blank Reaction Mix (BRM) by mixing for each blank well, 70 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase, and 8 µL dH_2O *(No 1× Substrate)*.

   Add 75 µL BRM to the Blank well. Add 75 µL RM to the remaining wells. Tap plate to mix briefly and thoroughly.
8. Incubate the plate for 15 minutes at room temperature and read optical density at 565 nm.

CALCULATION

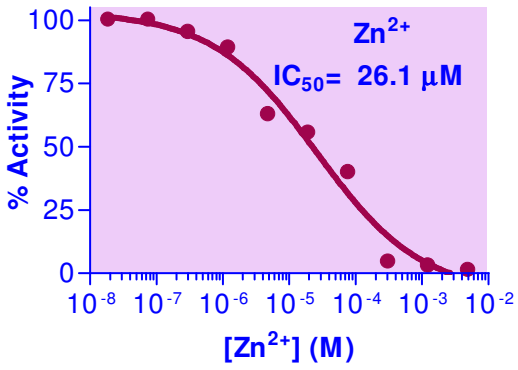
Glucose-6-Phosphate Dehydrogenase inhibition for a test compound is calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta OD_{\text{Test Cpd}}}{\Delta OD_{\text{No Inhibitor}}}\right) \times 100\%$$

Where $\Delta OD_{\text{Test Cpd}}$ is the OD_{565nm} value of a test compound minus the OD_{565nm} value of the Blank well at 15 min and $\Delta OD_{\text{No Inhibitor}}$ is the OD_{565nm} value of the Control minus the OD_{565nm} value of the Blank well at 15 min.

MATERIALS REQUIRED, BUT NOT PROVIDED

Purified G6PDH (e.g. Calzyme Cat# 078A0020) and if desired a control Zn^{2+} inhibitor (e.g. $Zn(NO_3)_2 \cdot 6H_2O$, Sigma Cat# 228737). Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



$Zn(NO_3)_2$ titration: G6PDH from *L. Mesenteroides* was incubated with various concentrations of $Zn(NO_3)_2$ in dH_20.

LITERATURE

1. Zhang, Zhaoyun, et al. (2010) High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and β-cell apoptosis. The FASEB Journal 24.5: 1497-1505.
2. Tian, Wang-Ni, et al. (1998) Importance of glucose-6-phosphate dehydrogenase activity for cell growth. Journal of Biological Chemistry 273.17:10609-10617.
3. Atamanalp, M., et al. (2016) Investigation of in vitro effects of some metal ions on glucose 6-phosphate dehydrogenase which purified from freshwater fish Capoeta umbla Kidney. HydroMediT 2016: 39.