G6PDH Inhibitor Screening Kit

G6PDH Inhibitor Screening Kit is a screening kit for inhibitor screening and evaluation of G6PDH inhibitors.

Catalog number: ARG82159

Package: 100 tests

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD or G6PDH) (EC 1.1.1.49) is a cytosolic enzyme that catalyzes the chemical reaction:

D-glucose 6-phosphate + $NADP^+$ + H_2O ⇌ 6-phospho-D-glucono-1, 5-lactone + NADPH + H^+

This enzyme participates in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage from compounds like hydrogen peroxide. Of greater quantitative importance is the production of NADPH for tissues involved in biosynthesis of fatty acids or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. G6PD reduces $NADP^+$ to NADPH while oxidizing glucose-6-phosphate. [Provide by Wikipedia: G6PDH]

PRINCIPLE OF THE ASSAY

This G6PDH Inhibitor Screening Kit is a simple colorimetric assay that measures the amount of G6PDH Inhibitor in samples. This 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 O.D. 565 nm. The increase in absorbance at O.D. 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.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
NADP / MTT	1 mL	-20°C
Diaphorase	120 µL	-20°C
10X Substrate (450 mM G6P)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Clear flat-bottom 96 well microplate
- Purified G6PDH (E.g., Calzyme Cat# 078A0020)
- Zn^{2+} inhibitor (E.g., Sigma Aldrich Cat# 228737)
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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).
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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.

REAGENT PREPARATION

- **Working G6PDH Solution:** This protocol is optimized for *L. Mesenteroides* G6PDH. Dilute purified G6PDH to 0.0003 U/µL using distilled water.
- **1X Substrate:** diluting 10X Substrate 10-fold in distilled water. (Each well need 8 µL of 1X Substrate)
- **Reaction Mix:** for each 96 well assay, mix 8 µL of 1X Substrate, 8 µL of MTT / NAD Solution, 1 µL of Diaphorase and 70 µL of Assay Buffer. Fresh preparation before assay.
- **Blank Reaction Mix:** for each 96 well assay, mix 8 µL of MTT / NAD Solution, 1 µL of Diaphorase and 70 µL of Assay Buffer. Fresh preparation before assay.

ASSAY PROCEDURE

Equilibrate reagents to desired reaction temperature (E.g., 25°C or 37°C). Briefly centrifuge tubes before use.

	Sample	Blank (No substrate)	Control (No inhibitor)
Working G6PDH Solution	20 µL	20 µL	20 µL
Solvent (test compounds are dissolved in)		5 µL	5 µL
Test compounds	5 µL		
Tap microplate to mix briefly and thoroughly. Incubate for **15 minutes** at **room temperature (25°C)**.			
Blank Reaction Mix		75 µL	
Reaction Mix	75 µL		75 µL
Tap microplate to mix briefly and thoroughly. Incubate for **15 minutes** at **room temperature (25°C)**. And read the absorbance at **O.D. 565 nm**.			

Note:

- This protocol is optimized for *L. Mesenteroides* G6PDH. Dilute purified G6PDH to 0.0003 U/µL using distilled water.
- If another species is being analyzed, we recommend that you experimentally determine the Km 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 Km.

CALCULATION OF RESULTS

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

   % Inhibition = $(1 - \Delta OD_{Test} / \Delta OD_{No\ inhibitor}) \times 100\%$

   Note:

   - ΔOD_{Test}: the $OD_{565\ nm}$ value of a test compound minus $OD_{565\ nm}$ value of the Blank well at 15 minutes.
   - $\Delta OD_{No\ inhibitor}$: the $OD_{565\ nm}$ value of the Control minus the $OD_{565\ nm}$ value of the Blank well at 15 minutes.

EXAMPLE OF TYPICAL STANDARD CURVE

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