Technical Bulletin

Glucose-6-Phosphate Dehydrogenase Inhibitor Screening Kit

Catalog Number MAK452

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. 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.

The Glucose-6-Phosphate Dehydrogenase Inhibitor Screening 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 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.

The kit is suitable for high throughput inhibitor screening and evaluation of glucose-6-phosphate dehydrogenase inhibitors.

Components

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

- Assay Buffer 10 mL
  Catalog Number MAK452A
- Diaphorase 120 µL
  Catalog Number MAK452B
- NADP/MTT 1 mL
  Catalog Number MAK452C
- 10x Substrate (450 mM G6P) 100 µL
  Catalog Number MAK452D

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.
- Glucose-6-phosphate Dehydrogenase from *Leuconostoc mesenteroides* (Catalog Number G5885 or equivalent)
- Zinc nitrate hexahydrate (optional) (Catalog Number 228737)

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. 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 should be run in duplicate.

Enzyme Preparation

The following procedure is optimized for *L. mesenteroides* G6PDH.

1. Dilute purified G6PDH to 0.0003 U/µL using purified water.
2. If G6PDH isolated from another species is being analyzed, 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.

Test Compound (Inhibitor) Preparation

Dissolve the Test Compounds in solvent of choice. If using DMSO or DMF, it is prudent to first test the tolerance by the enzyme of choice to DMSO and DMF. For G6PDH from *L. mesenteroides*, the DMSO concentration of the 5 µL of Test Compound solution added to the reaction should be ≤ 2% (v/v) DMSO, while the DMF concentration of the 5 µL of Test Compound solution added to the reaction should be ≤ 40% (v/v) DMF.

Assay Reaction Preparation

1. Transfer 20 µL of G6PDH solution 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 Compound is dissolved in. For example, if the Test Compound is dissolved in 2% (v/v) DMSO, add 5 µL of 2% (v/v) DMSO to each of these wells.
4. To the remainder of the wells containing G6PDH, add 5 µL of the Test Compound solution.
5. Tap plate and mix.
6. Incubate the plate for 15 minutes at 25 °C.

1× Substrate Preparation

Mix enough reagent for the number of assays to be performed. For each well (except the Blank well), prepare 8 µL of 1× Substrate. Prepare 1× Substrate by diluting 10x Substrate 10-fold in purified water.

Reaction Mix

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. Mix enough reagents for the number of assays to be performed. For each well (except the Blank well), prepare 87 µL of Reaction Mix. For the Blank well, prepare 87 µL of Blank Reaction Mix. Prepare Reaction Mixes according to Table 1.

Table 1.
Preparation of Reagent Mixes

Reagent	Rection Mix	Blank Rection Mix
Assay Buffer	70 µL	70 µL
NADP/MTT	8 µL	8 µL
Diaphorase	1 µL	1 µL
1× Substrate	8 µL	-
Purified Water	-	8 µL

2. Add 75 µL of Blank Reaction Mix to the Blank well.
3. Add 75 µL of Reaction Mix to the remaining wells.
4. Tap plate to mix briefly and thoroughly.

Measurement

Incubate the plate for 15 minutes at room temperature and then read the optical density at 565 nm (OD_{565}).

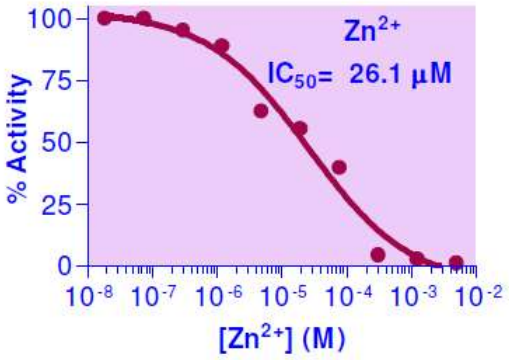
Results

1. Calculate $\Delta OD_{Test\ Cpd}$: $\Delta OD_{Test\ Cpd}$ is the OD_{565} value of the Test Compound well minus the OD_{565} value of the Blank well at 15 minutes.
2. Calculate $\Delta OD_{No\ Inhibitor}$: $\Delta OD_{No\ Inhibitor}$ is the OD_{565} value of the Control well minus the OD_{565} value of the Blank well at 15 minutes.
3. Calculate Glucose-6-Phosphate Dehydrogenase inhibition for the Test Compound using the following equation:

% Inhibition =

$$\left(1 - \frac{\Delta OD_{Test\ Cpd}}{\Delta OD_{No\ Inhibito}}\right) \times 100\%$$

Figure 1.
$Zn(NO_3)_2$ titration: G6PDH from *L. mesenteroides* was incubated with various concentrations of $Zn(NO_3)_2$ in purified water.



References

1. Zhang, Z., et al., High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and β-cell apoptosis. *FASEB J.*, **24(5)**, 1497-1505 (2010).
2. Tian, W.-N., et al., Importance of glucose-6-phosphate dehydrogenase activity for cell growth. *J. Biol. Chem.*, **273(17)**, 10609-17 (1998).

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