Glucose-6-Phosphate Dehydrogenase Inhibitor Screening Kit (Colorimetric)

(Catalog #BN00498; 100 assays; Store at -20°C)

I. Introduction:

Glucose-6-Phosphate Dehydrogenase (G6PDH) is a cytosolic enzyme which plays a key role in the Pentose Phosphate Pathway (PPP). The PPP pathway 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. Recently studies have found increased G6PDH activity in various human tumors and knockdown of this G6PDH decreases cell proliferation and enhances apoptosis *in vitro*. Therefore, targeting this key enzyme is essential for developing novel therapeutic approach for treatment/cure of cancer. In Assay Genie's G6PDH assay kit, Glucose-6-Phosphate is oxidized by glucose-6-phosphate dehydrogenase to generate 6-Phosphoglucono-δ-Lactone and form NADH, which reacts with a probe to produce a signal at OD 450 nm. In the presence of G6PDH inhibitor, these reactions are impeded, thus decreasing the rate and/or extent of generation of G6PDH-dependent absorbance. Thus, this kit provides a sensitive, quick, and easy method for screening potential inhibitors of G6PDH. G6PDH Inhibitor Control is included to compare the efficacy of test inhibitors. The assay is high-throughput adaptable and can be performed in less than 30 min.

II. Application:

- Screening/characterizing/studying potential inhibitors of Glucose-6-Phosphate Dehydrogenase.

III. Kit Contents:

Components	BN00498	Cap Color	Part Number
G6PDH Assay Buffer	25 ml	WM	BN00498-1
G6PDH Substrate	1 vial	Orange	BN00498-2
G6PDH Developer	1 vial	Red	BN00498-3
G6PDH Enzyme	1 vial	Green	BN00498-4
G6PDH Inhibitor Control	1 vial	Purple	BN00498-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **G6PDH Assay Buffer:** Bring to room temperature before use. Store at 4ºC or -20°C.
- **G6PDH Developer:** Reconstitute with 220 µl dH_2O.Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.
- **G6PDH Substrate:** Reconstitute with 220 µl G6PDH Assay Buffer. Store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **G6PDH Enzyme:** Reconstitute with 100 µl G6PDH Assay Buffer, mix thoroughly. Aliquot and store at -70°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **G6PDH Inhibitor Control:** Reconstitute with 200 µl dH_2O. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

VI. G6PDH Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at highest concentration to be tested. Dilute to 2X desired test concentration with G6PDH Assay Buffer. Add 50 µl diluted candidate inhibitor or G6PDH Assay Buffer into desired wells, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), add 50 µl of Inhibitor Control into desired well(s).

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control wells (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s).

2. **Background Control (BC):** Prepare a Background Control (BC) with 50 µl of G6PDH Assay Buffer alone.

3. **G6PDH Enzyme Preparation:** Dilute G6PDH Enzyme 1:20 with assay buffer. Add 5 µl diluted G6PDH Enzyme into Sample, Enzyme Control, Solvent Control and Inhibitor Control wells. Incubate for 15 min. at 25°C.

   Note: The diluted G6PDH Enzyme is unstable. Make fresh each time.

4. **Substrate Solution Preparation**: Make enough reagents for the number of assays to be performed. For each well, prepare 45 µl of Substrate solution containing:

G6PDH Assay Buffer	41 µl
G6PDH Substrate	2 µl
G6PDH Developer	2 µl

   Mix and add 45 µl of Substrate solution into each well. Mix well with gentle shaking.

5. **Measurement:** Measure OD_{450nm} in kinetic mode for 5-30 min at 37°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the OD_{450nm} (OD_1 & OD_2).
6. **Calculations:** Calculate the slope for all samples, including Enzyme Control (EC) as 100%, by dividing the net ΔOD (=OD_2-OD_1) value by the time ΔT (=T_2-T_1). Calculate % relative inhibition as follows:

$$\textbf{Relative Inhibitor } (\%) = \frac{(\textbf{Slope of EC} - \textbf{Slope of S})}{\textbf{Slope of EC}} \textbf{ X 100}$$

Notes:

- Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S).
- If Solvent control (SC) values are significantly different from the EC, use these values in the equation above.
- Irreversible inhibitors that inhibit the G6PDH activity completely at the tested concentration will have ΔOD_{450nm} = 0 and thus the % Inhibition will be 100%.

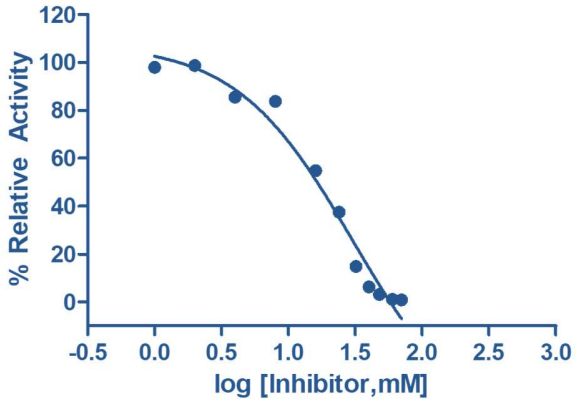

Figure: Inhibition of G6PDH activity by G6PDH Inhibitor Control. IC_{50} was determined as 30 mM. Assay was performed following the kit protocol.

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