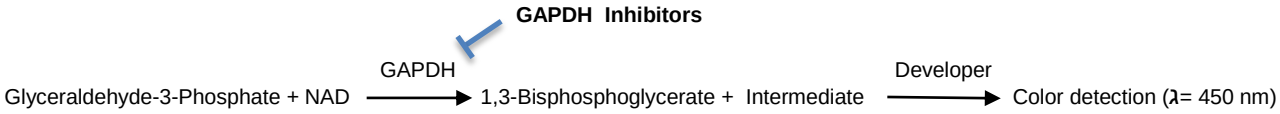
Human GAPDH Inhibitor Screening Kit (Colorimetric)

rev 06/20

(Catalog # K2043-100; 100 assays; Store at -20°C)

I. Introduction:

Glucose 3-Phosphate Dehydrogenase (GAPDH; EC 1.2.1.12) is a cytosolic enzyme, which catalyzes the conversion of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate (BPG) with the reduction of NAD to NADH in the glycolytic pathway. GAPDH is a multifunctional protein involved in many intracellular processes such as apoptosis, membrane trafficking, iron metabolism, nuclear translocation etc. Although GAPDH is considered as housekeeping gene, increased GAPDH activity has been detected in various human cancers. Additionally, knockdown of GAPDH has been shown to decrease cell proliferation, cell migration and metastasis in *in vitro* experiments. Therefore, targeting this key enzyme is essential for developing novel therapeutics for treating cancer. In our **GAPDH Inhibitor Screening Kit,** Glyceraldehyde 3-Phosphate is oxidized by GAPDH to generate 1,3-Bisphosphoglycerate and NADH, which reduces the probe thereby generating a strong absorbance at 450 nm. In the presence of GAPDH inhibitors, the reactions are impeded, thus decreasing the rate and/or extent of generation of GAPDH-dependent absorbance at OD 450 nm. This kit provides a sensitive, quick, and easy method for screening potential inhibitors of GAPDH. GAPDH Inhibitor Control is included in the kit 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 Human GAPDH

III. Kit Contents:

Components	K2043-100	Cap Color	Part Number
GAPDH Assay Buffer	25 ml	WM	K2043-100-1
GAPDH Substrate	1 ml	Blue	K2043-100-2
GAPDH Developer	1 vial	Red	K2043-100-3
Human GAPDH	1 vial	Brown	K2043-100-4
GAPDH Reconstitution Buffer	1.5 ml	White	K2043-100-5
GAPDH Inhibitor Control	1 vial	Purple	K2043-100-6

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- dH_2O

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.

- **GAPDH Assay Buffer & GAPDH Reconstitution Buffer:** Bring to room temperature (RT) before use. Store at -20°C.
- **GAPDH Substrate:** Reconstitute with 220 µl dH_2O. Divide into aliquots and store at -20°C. Avoid freeze-thaw cycles. Keep on ice while in use. Use within two months.
- **GAPDH Developer:** Reconstitute the vial with 220 µl GAPDH Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. Keep on ice while in use.
- **Human GAPDH:** Reconstitute the vial with 100 µl GAPDH Reconstitution Buffer. Vortex several times and put on ice for 5 min to completely dissolve the GAPDH. Divide into aliquots and store at -20°C. Avoid multiple freeze-thaw cycles. Keep on ice while in use. Use within two months.
- **GAPDH Inhibitor Control (in DMSO):** Bring to RT before use. Store at -20°C.

VI. GAPDH Inhibitor Screening Protocol:

1. **Screening compounds, Inhibitor Control, Enzyme Control, Solvent Control & Background Control Preparation:** Dissolve Test Compound(s) at 100X in appropriate solvent. Further dilute to 10X (the desired test concentration) with GAPDH Assay Buffer. Add 10 µl diluted Test compound(s) into designated well(s) of a 96-well clear plate designated as **Test Sample(s) [S].**

   Enzyme Control [EC]: Add 10 µl of GAPDH Assay Buffer into designated well(s) as **Enzyme Control [EC].**

   Inhibitor Control [IC]: Dilute the GAPDH Inhibitor Control by adding 5 µl of GAPDH Inhibitor Control into 45 µl GAPDH Assay Buffer. Mix well. Add 10 µl of diluted GAPDH Inhibitor Control into designated well(s) as **Inhibitor Control [IC]**.

   Solvent Control [SC] and **Background Control [BC]:** Add 5 µl DMSO into 45 µl GAPDH Assay Buffer. Mix well and add 10 µl diluted DMSO into wells designated as **Solvent Control [SC]** and **Background Control [BC].**

   Adjust the volume of all wells including **[S], [EC], [IC], [SC]** to 50 µl/well and to 55 µl/well of **[BC]** with GAPDH Assay Buffer.

   Notes:

   a. Do not store the diluted GAPDH Inhibitor Control.

   b. If your screening compounds are dissolved in a different solvent than DMSO, prepare **Solvent Control [SC]** and **Background Control [BC]** with the same final concentration of solvent as the one in your test wells.

2. **GAPDH Enzyme Solution Preparation:** Prepare 10-fold dilution of the reconstituted human GAPDH by adding 20 µl of reconstituted human GAPDH with 180 µl of GAPDH Reconstitution Buffer. Add 5 µl of diluted human GAPDH into all wells containing **[S]**, **[EC], [IC]** and **[SC]**. Do not add enzyme to the **[BC]** wells. Mix well with gentle shaking for 5 min.

   Note: Do not store the diluted human GAPDH solution.

3. **Reaction Mix Preparation**: Make enough reagents for the number of assays to be performed. For each well, prepare a total of 45 µl Reaction Mix containing:

	Reaction Mix
GAPDH Assay Buffer	41 µl
Reconstituted GAPDH Substrate	2 µl
Reconstituted GAPDH Developer	2 µl

   Mix well. Add 45 µl of Reaction Mix to all wells including **[S], [EC], [IC], [SC]**. Mix well with gentle shaking. ***Final Volume: 100 µl***

4. **Measurement:** Measure absorbance immediately at 450 nm in kinetic mode for 5-30 min at 37°C. Choose any two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

5. **Calculation:** Calculate the slope for all **Test Sample(s) [S], Enzyme Control [EC],** and **Solvent Control [SC]** by dividing the net ΔOD (OD_1 - OD_2) values with the time Δt (T_2 - T_1). Subtract the Slope of **[BC]** from **[S], [SC]** and **[EC]**. Calculate the % relative inhibition as follows:

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

Note: If SC slope is significantly different from EC slope, use SC slope instead of EC slope in the formula above.

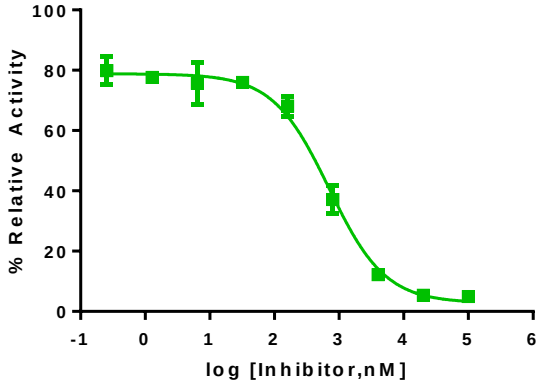


Figure: Inhibition of Human GAPDH activity by GAPDH Inhibitor. IC_{50} was determined as 698.7 nM. Assay was performed following the kit protocol.

VII. Related Products:

Glucose-6-Phosphate Dehydrogenase Inhibitor Screening Kit (K225)
GAPDH Activity Assay Kit (K680)
Glucose-1-Phosphate Colorimetric Assay Kit (K697)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K751)
Glyceraldehyde 3-Phosphate Assay Kit (K2018)

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