ab283369 – GAPDH Inhibitor Screening Kit (Colorimetric)

For the screening of GAPDH inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283369

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
GAPDH Assay Buffer	25 mL	-20°C
GAPDH Substrate	200 µL	-20°C
GAPDH Developer	1 vial	-20°C
Human GAPDH	1 vial	-20°C
GAPDH Reconstitution Buffer	1.5 mL	-20°C
GAPDH Inhibitor Control	20 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

GAPDH Assay Buffer and GAPDH Reconstitution Buffer: Warm to room temperature (RT) before use. Store at -20°C.

GAPDH Substrate: Reconstitute with 220 µl dH2O. Divide into aliquots and store at -20°C. Avoid freeze-thaw cycles. Keep on ice while in use. Use within 2 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 2 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 2 months.

GAPDH Inhibitor Control (in DMSO): Bring to RT before use. Store at -20°C.

Screening Protocol

Screening Compounds, Inhibitor Control, Enzyme Control, Solvent Control & Background Control preparations:

1. Dissolve Test Compound(s) at 100X in appropriate solvent.
2. Dilute to 10X (the desired test concentration) with GAPDH Assay Buffer.
3. Add 10 µl diluted Test compound(s) into designated well(s) of a 96-well clear plate designated as Test Sample(s) [S].
4. **For Enzyme Control [EC]:** Add 10 µl of GAPDH Assay Buffer into designated well(s) as Enzyme Control [EC].
5. **For 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].
6. **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].
7. 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.

Δ 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.

GAPDH Enzyme Solution Preparation:

1. Prepare 10-fold dilution of the reconstituted human GAPDH by adding 20 µl of reconstituted human GAPDH with 180 µl of GAPDH Reconstitution Buffer.
2. 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.

Δ Note: Do not store the diluted human GAPDH solution.

Reaction Mix Preparation:

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

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

2. 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.

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).

Calculation

1. 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).
2. Subtract the Slope of [BC] from [S], [SC] and [EC]. Calculate the % relative inhibition as follows:

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

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)