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IDH1 R132H Mutant Inhibitor Screening Kit (Colorimetric)

(Catalog # K493-100; 100 assays; Store at -20°C)

I. Introduction:

In eukaryotic cells, Isocitrate Dehydrogenase (IDH1, IDH2 and IDH3) is an enzyme that catalyzes the decarboxylation of Isocitrate producing α-Ketoglutarate and CO_2. Mutations of the different isoforms of IDH, IDH1 and IDH2, are commonly found in human cancers. Among these mutants, the cytosolic IDH1 mutant at the Arginine 132 position substitution with Histidine (IDH1 R132H) is the most commonly mutation found in primary human brain cancers. Isocitrate Dehydrogenase Mutant (IDH mutant) causes a "gain-of-function" mutation, which reduces its affinity for isocitrate and favors the conversion of α-ketoglutarate to D-2-Hydroxyglutarate (D2HG). High level of D2HG correlates with an increased risk for malignant brain tumors. Thus, the search for potential novel as well as specific inhibitors for this target has increased considerably in recent years. In BioVision's IDH1 R132H Mutant Inhibitor Screening Kit, IDH1 R132H Mutant oxidizes NADPH into NADP+, which decreases the absorbance at 340 nm. In the presence of IDH1 R132H Mutant inhibitor, the reaction is impeded. An IDH1 R132H Mutant Inhibitor Control is included to compare the efficacy of the sample inhibitors. The assay is high-throughput adaptable and can be completed in less than 1 hr.

$$\text{α-Ketoglutarate + NADPH} \xrightarrow{\text{IDH1 Mutant}} \text{D-2-Hydroxyglutarate + NADP}^+ \text{ (}\Delta\text{OD 340 nm decrease)}$$

$$\text{α- Ketoglutarate + NADPH} \xrightarrow[\text{IDH1 Mutant Inhibitor}]{\text{IDH1 Mutant}} \text{D-2-Hydroxyglutarate + NADP}^+ \text{ (No/Low }\Delta\text{OD 340 nm decrease)}$$

II. Applications:

- Screening/studying/characterizing potential Mutant IDH inhibitors

III. Kit Contents:

Components	K493-100	Cap Color	Part Number
IDH1 R132H Assay Buffer	25 ml	NM	K493-100-1
IDH1 R132H Substrate	1 vial	Red	K493-100-2
NADPH	1 vial	Purple	K493-100-3
IDH1 R132H Enzyme	1 vial	Orange	K493-100-4
IDH1 R132H Inhibitor (100X)	50 µl	Blue	K493-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Glycerol

V. Storage Conditions and Reagent Preparation:

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

- **IDH1 R132H Assay Buffer:** Bring to room temperature before use.
- **IDH1 R132H Substrate:** Reconstitute with 220 µl Mutant IDH Assay Buffer. Mix well to dissolve completely. Store at -20°C. Use within two months.
- **NADPH:** Reconstitute with 550 µl Mutant IDH Assay Buffer to generate 20 mM NADPH. Aliquot and store at -20°C. Avoid freeze and thaw. Keep on ice while in use. Use within two months.
- **IDH1 R132H Enzyme:** Reconstitute with 220 µl of a 30% Glycerol solution in dH_2O. Mix well to dissolve completely. Store at -20°C. Use within two months.
- **IDH1 R132H Inhibitor:** Prior to use, bring to room temperature. Store at -20°C.

VI. IDH1 R132H Mutant Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Blank Control Preparation:** Dissolve candidate inhibitors into appropriate solvent at 100X the highest final concentration to be tested. Dilute to 10X the desired test concentration with IDH1 R132H Assay Buffer. Add 10 µl diluted test inhibitor, or IDH1 R132H Assay Buffer into wells containing IDH1 R132H Enzyme, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor). For inhibitor control: dilute IDH1 R132H Inhibitor Control by adding 10 µl of IDH1 R132H Mutant Inhibitor to 90 µl of IDH1 R132H Assay Buffer. Add 10 µl of diluted Inhibitor into desired well(s). Incubate at room temperature for 10 minutes.

   Notes:

   a. Use diluted Mutant IDH Inhibitor within 4 hrs.

   b. High inhibitor solvent concentrations might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on IDH1 R132H enzyme activity. In case SC is significantly different from EC, use its value to determine the effect of tested compound(s).

2. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mutant IDH Enzyme Solution:

IDH1 R132R Assay Buffer	38 µl
IDH1 R132H Enzyme	2 µl

Mix and add 40 µl of the Mutant IDH Enzyme Solution into desired wells.

3. **IDH1 R132H Substrate Solution Preparation:** For each well, prepare 50 µl of substrate solution.

IDH1 R132H Substrate	2 µl
NADPH	4 µl
IDH1 R132H Assay Buffer	44 µl

Mix and add 50 µl of Substrate solution into each well.

4. **Measurement:** Measure absorbance at OD 340 nm at kinetic mode for 40 minutes at 37 °C, and choosing two time points (t_1 & t_2) in the linear range to calculate the IDH1 R132H Mutant activity of the Sample Screen, Enzyme Control, Solvent Control and Inhibitor Control.

5. **Calculation:** Choose the absolute value of slope, and set the slope of enzyme control (EC) as 100%, and calculate the relative % inhibition of test inhibitors as follows:

$$\% \textbf{ Inhibiton} = \frac{\textbf{Slope of EC} - \textbf{Slope of } S}{\textbf{Slope of EC}} \textbf{ X 100}$$

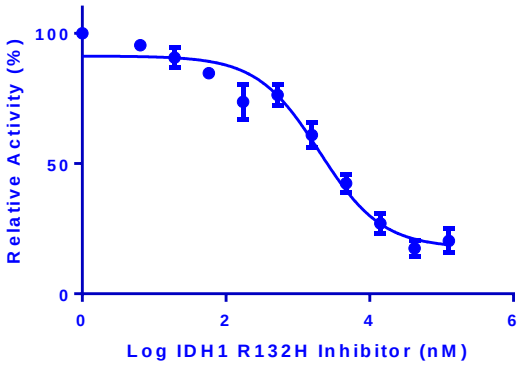

Figure: Inhibition of IDH1 R132H Mutant activity by IDH1 R132H Inhibitor, IC_{50} = 2.0 µM. Assays were performed following the kit protocol.

VII. Related Products:

PicoProbe D-2-Hydroxyglutarate Assay Kit (K970)
Mutant Isocitrate Dehydrogenase Activity kit (K985)
MPO Inhibitor Screening Kit (K746)
CEPT Activity Assay Kit II (K595)
Sphingomyelin Quantification Assay Kit (K600)
Sphingomyelinase Activity Fluorometric Assay Kit (K574)
CETP Blocking Peptide (3413BP)
AG-120 (Ivosidenib) (B1163)
AGI-5198 (2369)

PicoProbe D-2-HG Activity Assay kit (K248)
D-2 Hydroxyglutarate (D2HG) Assay Kit (K213)
MPO Peroxidation Assay Kit (K747)
Sphingomyelinase Activity Assay Kit (K599)
CETP Inhibitor Screening Kit II (K594)
Mutant IDH Activity Assay (K985)
Active Recombinant Human CETP (7606)
AG-221 (Enasidenib) (B1070)
AGI-6780 (9624)

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