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Data Sheet ***IDH1(R132H) Assay Kit*** **Catalog # 79376**

BACKGROUND : Isocitrate dehydrogenases are enzymes that catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. Mutations in isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) have been reported in a multitude of human cancers. While the wild type IDH1, produces α -ketoglutarate (α -KG) through the reduction of NADP⁺ to NADPH, the IDH1(R132H) mutant catalyzes conversion of α -ketoglutarate to 2-hydroxyglutarate (2-HG) by oxidizing NADPH to NADP⁺. Genomic studies have identified the IDH1(R132H) mutation in various cancer types, including glioma, chondrosarcoma, and AML as well as a small percentage of prostate, lung and colon cancers.

DESCRIPTION: The *IDH1(R132H) Assay Kit* is designed to measure IDH1(R132H) activity for screening and profiling applications by measuring NADPH consumption. The *IDH1(R132H) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant IDH1(R132H) enzyme, α -KG, NADPH, NADPH detection reagents, and assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71099	IDH1(R132H)	5 μ g	-80°C	Avoid multiple freeze/thaw cycles!
	1x IDH1 Assay Buffer	20 ml	-20°C	
	α -Ketoglutarate (40 mM)	1 ml	-20°C	
	NADPH (500 μ M)	2 x 100 μ l	-20°C	
	NADPH Detection Reagent A	2 x 1 mg	-20°C	
	NADPH Detection Reagent B	2 x 1 mg	-20°C	
79685	96-well plate, black	2	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

NADPH (optional)*
Microplate reader capable of reading Fluorescence
Adjustable micropipettor and sterile tips

**Note: NADPH is not stable for multiple freeze/thaw cycles. We have provided the reagent as 2 x 100 μ l to allow you to use only half the plate at a time. If you plan to use the same plate for additional experiments, please purchase additional NADPH and prepare a fresh 500 μ M stock in H₂O at the time of the assay.*

APPLICATIONS: Useful for screening small molecular inhibitors for drug discovery and HTS applications.

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STABILITY: Up to 6 months when stored as recommended but varies by components.

REFERENCE: Wang, F., *et. al. Science* **340**:622-626 (2013)

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **1x IDH1 Assay Buffer**, **α -Ketoglutarate (40 mM)**, and **NADPH (500 μ M)**.
- 2) Prepare the master mixture (70 μ l per well): N wells x (58 μ l **1x IDH1 Assay Buffer**, + 2 μ l **NADPH (500 μ M)**, and + 10 μ l **α -Ketoglutarate (40 mM)**). Add 70 μ l to every well.

	Positive Control	Test Inhibitor	Blank
1x IDH1 Assay buffer	58 μ l	58 μ l	58 μ l
NADPH (500 μ M)	2 μ l	2 μ l	2 μ l
α -Ketoglutarate (40 mM)	10 μ l	10 μ l	10 μ l
Test Inhibitor	-	10 μ l	-
Inhibitor Buffer (no inhibitor)	10 μ l	-	10 μ l
1x IDH1 Assay buffer	-	-	20 μ l
IDH1(R132H) enzyme; 2-2.5 ng/ μ l~2.5 ng/ μ l	20 μ l	20 μ l	-
Total	100 μl	100 μl	100 μl

- 3) Add 10 μ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 10 μ l of the same solution without inhibitor (Inhibitor buffer).

Note: Some inhibitors require preincubation with IDH1(R132H) to exhibit maximum effect. Also, depending on the mechanism of the inhibitor, the pre-incubation may require adding one or both substrates (α -Ketoglutarate and NADPH) separately (step 2). For example, for IDH-C100 inhibition, we preincubate with IDH1(R132H) enzyme for 90 min in the presence of NADPH, and then initiate the reaction by adding the α -Ketoglutarate.

- 4) To the wells designated as "Blank," add 20 μ l **1x IDH1 Assay buffer**.

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- 5) Thaw **IDH1(R132H)** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **IDH1(R132H)** required for the assay and dilute enzyme to 2~2.5 ng/ μ l for **IDH1(R132H)** with **1x IDH1 Assay Buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: IDH1(R132H) enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 6) Initiate reaction by adding 20 μ l of diluted **IDH1(R132H)** enzyme to the wells designated "Positive Control" and "Test Inhibitor." Incubate at room temperature for 1 hour.
- 7) Immediately before terminating the reaction, add 1 ml distilled water to **NADPH Detection Reagent A** and 0.4 ml water to **NADPH Detection Reagent B** and mix well to completely dissolve the reagents. Store the tubes on ice.
- 8) Prepare the NADPH detection reagent by adding 180 μ l of **NADPH Detection Reagent A** and 10 μ l of **NADPH Detection Reagent B** to 4,810 μ l of **1x IDH1 Assay Buffer**. *Note: NADPH detection reagents are not stable so it must be used immediately; discard any unused reagent. The kit provides enough reagents for two separate assays.*
- 9) Add 25 μ l of NADPH detection reagent to the wells of a new plate (provided). Using a multipipettor, remove 50 μ l of the **IDH1(R132H)** reaction mixture and add to the wells containing the NADPH detection reagent. Cover the plate with aluminum foil and incubate for 5 minutes at room temperature.
- 10) Read the fluorescence at Ex544 nm/Em600 nm using a microplate reader.

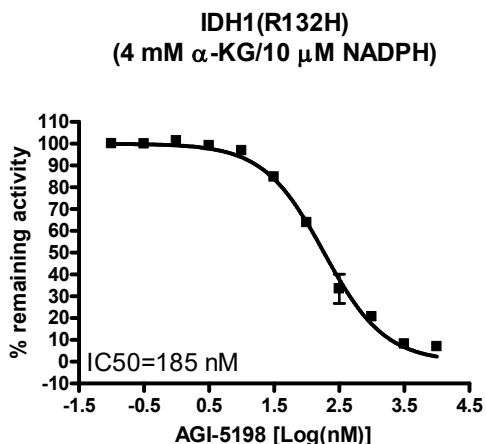
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Example of Assay Results:



Inhibition of IDH1(R132H) by AGI-5198, measured using the *IDH1(R132H)* assay kit (Cat. #79376). For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
IDH2, FLAG-tag	71074-1	50 μ g
IDH2, FLAG-tag	71074-2	100 μ g
IDH2 (R140Q), FLAG-tag	71100-1	50 μ g
IDH2 (R140Q), FLAG-tag	71100-2	100 μ g
IDH1, FLAG-tag	71075-1	50 μ g
IDH1, FLAG-tag	71075-2	100 μ g
IDH1 (R132H), FLAG-tag	71099-1	50 μ g
IDH1 (R132H), FLAG-tag	71099-2	100 μ g
IDH2(R140Q) Assay Kit	79309	96 rxns.

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