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Data Sheet

IDH2(R140Q) Assay Kit

Catalog # 79309

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 IDH2, produces α-ketoglutarate (α-KG) through the reduction of $NADP^+$ to NADPH, the IDH2(R140Q) mutant catalyzes conversion of α-ketoglutarate to 2-hydroxy-glutarate (2-HG) by oxidizing NADPH to $NADP^+$. Genomic studies have identified the IDH2(R140Q) mutation in various cancer types, including as glioma, chondrosarcoma, AML as well as small percentage of prostate, lung and colon cancers.

DESCRIPTION: The *IDH2(R140Q) Assay Kit* is designed to measure IDH2(R140Q) activity for screening and profiling applications by measuring NADPH consumption. The *IDH2(R140Q) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant IDH2(R140Q) enzyme, α-KG, NADPH, NADPH detection reagents, and assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71100	IDH2(R140Q)	3 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
	1x IDH2 Assay Buffer	20 ml	-20°C	
	α-Ketoglutarate (40 mM)	1 ml	-20°C	
	NADPH (500 µM)	2 x 100 µl	-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 µl 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 µM stock in H_2O 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 IDH2 Assay Buffer, α-Ketoglutarate, and NADPH**.

2) Prepare the master mixture (70 µl per well): N wells x (58 µl **1x IDH2 Assay Buffer** + 2 µl **NADPH (500 µM)** + 10 µl **α-Ketoglutarate**). Add 70 µl to every well.

	Positive Control	Test Inhibitor	Blank
1x IDH2 Assay Buffer	58 µl	58 µl	58 µl
NADPH (500 µM)	2 µl	2 µl	2 µl
α-Ketoglutarate	10 µl	10 µl	10 µl
Test Inhibitor	–	10 µl	–
Inhibitor Buffer (no inhibitor)	10 µl	–	10 µl
1x IDH2 Assay Buffer	–	–	20 µl
IDH2(R140Q) enzyme; 1-1.5 ng/µl~1.5 ng/µl	20 µl	20 µl	–
Total	100 µl	100 µl	100 µl

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

Note: Some inhibitors require preincubation with IDH2(R140Q) 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 IDH2(R140Q) 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 µl of **1x IDH2 Assay Buffer**.

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5) Thaw **IDH2(R140Q)** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **IDH2(R140Q)** required for the assay and dilute enzyme to 1~1.5 ng/µl for **IDH2(R140Q)** with **1x IDH2 Assay Buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: IDH2(R140Q) 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 µl of diluted **IDH2(R140Q)** 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 A** and 0.4 ml water to **NADPH detection B** and mix well to completely dissolve the reagents. Store the tubes on ice.

8) Prepare the NADPH detection reagent by adding 180 µl of **NADPH detection A** and 10 µl of **NADPH detection B** to 4,810 µl of **1x IDH2 Assay Buffer**. *Note: NADPH detection reagent is not stable so it must be used immediately; discard any unused reagent. The kit provides enough reagents for two separate assays.*

9) Add 25 µl of NADPH detection reagent to the wells of a new plate (provided). Using a multipipettor, remove 50 µl of the **IDH2(R140Q)** 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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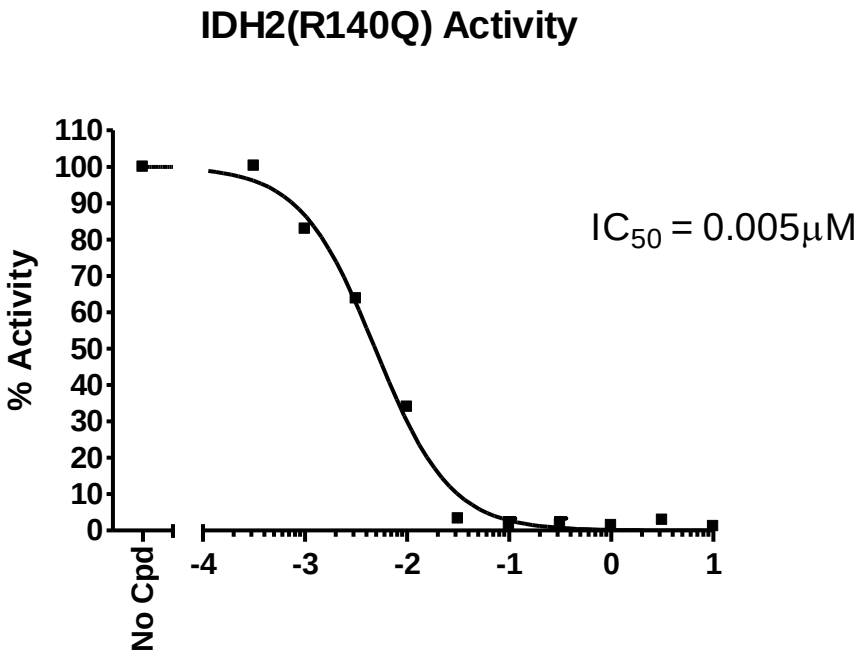
Example of Assay Results:



Inhibition of IDH2(R140Q) by IDH-C100, measured using the *IDH2(R140Q) assay kit* (Cat. #79309). *Data shown is lot-specific and IDH-C100 was preincubated with IDH2(R140Q) for 90 min in the presence of NADPH, and the enzymatic reaction was initiated by adding α-ketoglutarate. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
IDH2, FLAG-tag	71074-1	50 µg
IDH2, FLAG-tag	71074-2	100 µg
IDH2 (R140Q), FLAG-tag	71100-1	50 µg
IDH2 (R140Q), FLAG-tag	71100-2	100 µg
IDH1, FLAG-tag	71075-1	50 µg
IDH1, FLAG-tag	71075-2	100 µg
IDH1 (R132H), FLAG-tag	71099-1	50 µg
IDH1 (R132H), FLAG-tag	71099-2	100 µg

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