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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-hydroxyglutarate (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 μ g	-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 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 IDH2 Assay Buffer, α -Ketoglutarate, and NADPH.**
- 2) Prepare the master mixture (70 μ l per well): N wells x (58 μ l **1x IDH2 Assay Buffer** + 2 μ l **NADPH (500 μ M)** + 10 μ l **α -Ketoglutarate**). Add 70 μ l to every well.

	Positive Control	Test Inhibitor	Blank
1x IDH2 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	10 μ l	10 μ l	10 μ l
Test Inhibitor	-	10 μ l	-
10% DMSO in water (Inhibitor buffer)	10 μ l	-	10 μ l
1x IDH2 Assay Buffer	-	-	20 μ l
IDH2(R140Q) enzyme; 1-1.5 ng/ μ l~1.5 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 10% DMSO in water (Inhibitor buffer).

Note: Some inhibitors require preincubation with IDH2(R140Q) to exhibit maximum effect. Also, depending on the mechanism of the inhibitor, the preincubation 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 μ l 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/ μ l 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 μ l 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 μ l of **NADPH detection A** and 10 μ l of **NADPH detection B** to 4,810 μ l 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 μ l of NADPH detection reagent to the wells of a new plate (provided). Using a multipipettor, remove 50 μ l 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.

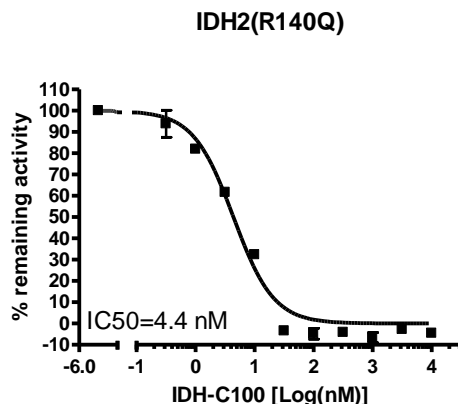
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Example of Assay Results:



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 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

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