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Mutant Isocitrate Dehydrogenase Activity Assay Kit (Colorimetric)

rev 01/21

(Catalog # K985-100; 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 in both isoforms of IDH (IDH1 and IDH2) are commonly found in human cancers. The Mutant Isocitrate Dehydrogenase (Mutant IDH) causes a "gain-of-function", which reduces its affinity for isocitrate and favors the conversion of α-ketoglutarate to D-2-Hydroxyglutarate. D-2-Hydroxyglutarate (D2HG) is present at low level in normal cells and tissues, but is significantly elevated in metabolic diseases and various cancers. D2HG functions as an "oncometabolite" promoting cellular transformation. Recent studies show that increased Mutant IDH activity is associated with various cancers; therefore, detection of Mutant IDH activity is important for diagnosis and developing therapeutic strategies (e.g. Mutant IDH inhibitors). BioVision's Mutant IDH assay kit provides a quick and simple assay to monitor the Mutant IDH activity in biological samples. In the assay, Mutant IDH oxidizes NADPH into an $NADP^+$, which decreases the absorbance at 340 nm. The assay is simple, sensitive, and can detect Mutant Isocitrate Dehydrogenase activity lower than 2 mU/ml in a variety of cancer samples.

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

II. Applications:

- Measurement of Mutant Isocitrate Dehydrogenase activity in various cancer tissues/cells.
- Analysis of cell signaling pathways.

III. Sample Types:

- Cancer Tissues: Colon, Lung, etc.
- Cancer Cell Cultures: Adherent or suspension cells
- Purified enzyme preparation

IV. Kit Contents:

Components	K985-100	Cap Code	Part Number
Mutant IDH Lysis Buffer	25 ml	WM	K985-100-1
Mutant IDH Assay Buffer	25 ml	NM	K985-100-2
Mutant IDH Substrate	1 vial	Red	K985-100-3
NADPH	1 vial	Purple	K985-100-4
Mutant IDH Positive Control	1 vial	Orange	K985-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- 4.1 M Saturated Ammonium Sulfate
- Multi-well spectrophotometer (ELISA reader)

VI. 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.

- **Mutant IDH Lysis Buffer and Mutant IDH Assay Buffer:** Warm to room temperature (RT) before use. Store at 4 ºC.
- **Mutant IDH Substrate:** Reconstitute the vial with 220 µl dH_2O. Store at -20 °C. Keep on ice while in use. Use within two months.
- **NADPH:** Reconstitute the vial with 500 µl dH_2O to generate 20 mM NADPH. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Mutant IDH Positive Control:** Reconstitute the vial with 44 µl dH_2O. Keep on ice while in use, store at -20 °C. Use within two months.

VII. Mutant IDH Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (50 mg) or cells (5~10 x 10^6) with 200 µl ice cold Mutant IDH Lysis Buffer, and keep samples on ice for 10 min. Centrifuge at 10,000 x g for 5 min at 4°C to remove cell debris, and transfer the supernatant to a fresh tube. Small molecules in samples may interfere with the assay. To remove small molecules, we suggest using the ammonium sulfate method. Pipette 50-100 µl of lysate into a fresh tube; add 2X volume of saturated ammonium sulfate (~ 4.1 M) [BV Cat. # 7096] (at RT) and then keep on ice for 20 min. Spin down at 10,000 X g for 5 min at 4°Ca and carefully remove and discard the supernatant. Resuspend the pellet to the original volume with ice-cold Mutant IDH Assay Buffer. Add 5-50 µl deproteinized, reconstituted sample per well & adjust the volume to 50 µl with Mutant IDH Assay Buffer. For the Mutant IDH Positive Control, take 4-12 µl of Mutant IDH Positive Control into desired well(s) and adjust the final volume to 50 µl with Mutant IDH Assay Buffer.

   Notes:

   For unknown samples, we suggest testing several doses to ensure the reading are within the Standard Curve range.

2. **NADPH Standard Curve:** Dilute NADPH by taking 50 µl of 20 mM NADPH into 50 µl Mutant IDH Assay Buffer to generate 10 mM NADPH Standard. Add 0, 2, 4, 6, 8 and 10 µl of 10 mM NADPH Standard into a series of wells in 96 well plate to generate 0, 20, 40, 60, 80 and 100 nmol/well of NADPH Standard. Adjust the volume to 100 µl/well with Mutant IDH Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	*Background Control Mix
Mutant IDH Assay Buffer	44 µl	46 µl
NADPH	4 µl	4 µl
Mutant IDH Substrate	2 µl	----

Mix and add 50 µl of the Reaction Mix to each well containing the Positive Control and test samples. Mix well. ***For** Background Control (BC), add 50 µl of Background Control Mix (without the substrate) to the sample background control well(s).

4. **Measurement:** Measure absorbance at 340 nm in kinetic mode for 60 min at 25 °C.

   Note: Incubation time depends on the Mutant Isocitrate Dehydrogenase activity in samples. We recommend measuring OD 340 nm in kinetic mode, and choosing two time points (t_1 & t_2) in the linear range to calculate the Mutant Isocitrate Dehydrogenase activity of the samples. The NADPH Standard Curve can be read in Endpoint mode (i.e. at the end of the incubation time).

5. **Calculation:** Subtract 0 Standard reading from all NADPH Standard readings. Plot the NADPH Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the Mutant Isocitrate Dehydrogenase activity of the test sample: $\Delta OD = A_1 - A_2$. Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample Mutant Isocitrate Dehydrogenase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = NADPH amount from Standard Curve (nmol).
Δt = reaction time (min.).
V = sample volume added into the reaction well (ml).
D = Dilution Factor

Unit Definition: One unit of Mutant Isocitrate Dehydrogenase is the amount of enzyme that oxidizes 1.0 µmol of NADPH per min. at pH 7.5 at 25 °C.

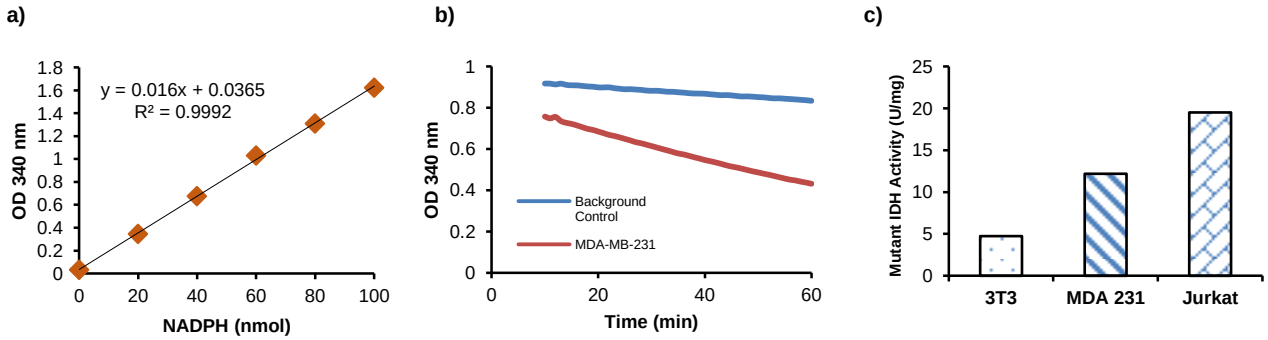

Figure: (a) NADPH Standard Curve. **(b)** Mutant Isocitrate Dehydrogenase activity in MDA-MB-231 cell extracts (25 µg), Background control (no Substrate). **(c)** Mutant Isocitrate Dehydrogenase specific activity was calculated in cell extracts from 3T3 (13 µg), MDA-MB-231(25 µg) and Jurkat (28 µg) cells. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

D-2-Hydroxyglutarate (D2HG) Assay (K213-100)
Malate Colorimetric Assay Kit (K637)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
α-Ketoglutarate Colorimetric Assay Kit (K677)
PicoProbe D-2-Hydroxyglutarate Dehydrogenase Assay (K248-100)
Fumarate Colorimetric Assay Kit (K633)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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