

## ab284568 – Mutant Isocitrate Dehydrogenase (Mutant IDH) Activity Assay Kit (Colorimetric)

For the measurement of Mutant IDH in various cancer samples.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

[www.abcam.com/ab284568](http://www.abcam.com/ab284568)

**Storage and Stability:** Store kit at -20°C immediately upon receipt. Protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

### Materials Supplied

Item	Quantity	Storage Condition
Mutant IDH Assay Buffer	25 mL	-20°C
Mutant IDH Lysis Buffer	25 mL	-20°C
Mutant IDH Positive Control	1 Vial	-20°C
Mutant IDH Substrate	1 Vial	-20°C
NADPH	1 Vial	-20°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

### Reagents Preparation

Mutant IDH Lysis Buffer and Mutant IDH Assay Buffer: Warm to room temperature (RT) before use.

Mutant IDH Substrate: Reconstitute the vial with 220 µL dH<sub>2</sub>O. Store at -20 °C. Keep on ice while in use. Use within two months.

NADPH: Reconstitute the vial with 500 µL dH<sub>2</sub>O 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<sub>2</sub>O. Keep on ice while in use, store at -20 °C. Use within two months.

### Mutant IDH Assay Protocol

#### Sample Preparation:

1. Rapidly homogenize tissue (50 mg) or cells (5 ~10 x 10<sup>6</sup>) with 200 µL ice cold Mutant IDH Lysis Buffer, and keep samples on ice for 10 min.
2. 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.
3. 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) (at RT) and then keep on ice for 20 min. Spin down at 10,000 X g for 5 min at 4°C and carefully remove and discard the supernatant.
4. Resuspend the pellet to the original volume with ice-cold Mutant IDH Assay Buffer.
5. Add 5-50 µL deproteinized, reconstituted sample per well & adjust the volume to 50 µL with Mutant IDH Assay Buffer.

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6. 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.

#### Δ Notes:

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

#### NADPH Standard curve:

1. Dilute NADPH by taking 50 µL of 20 mM NADPH into 50 µL Mutant IDH Assay Buffer to generate 10 mM NADPH Standard.
2. 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.
3. Adjust the volume to 100 µL/well with Mutant IDH Assay Buffer.

#### Reaction Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

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

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

### Measurement

Measure absorbance at 340 nm in kinetic mode for 60 min at 25 °C.

**Δ 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<sub>1</sub> & t<sub>2</sub>) 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).

### Calculation

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

$$\text{Sample Mutant Isocitrate Dehydrogenase Activity} = \frac{B}{(\Delta t \times V) \times D} = \text{nmol}/\mu\text{L}/\text{ml} = \text{mU}/\text{ml}$$

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 minute at pH 7.5 at 25 °C.

### **Technical Support**

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