Isocitrate Dehydrogenase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6767

Detection and Quantification of Isocitrate Dehydrogenase (IDH) Activity inTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Isocitrate Dehydrogenase (IDH)(EC 1.1.1.42) and (EC 1.1.1.41) is an enzyme which catalyzes the interconversion of isocitrate and α -ketoglutarate. There are three IDH isoforms: IDH3 uses the cofactor NAD+ and catalyzes the third step in the citric acid cycle, while IDH1 and IDH2 use the cof actor NADP+ and catalyze the same reaction outside the citric acid cycle. This kit measuresthe activity of the NADP+ isoforms. Mutations in IDH1 and IDH2 have been linked with various brain tumors and acute myeloid leukemia.

Isocitrate Dehydrogenase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining isocitrate dehydrogenase activity in various samples.Isocitrate dehydrogenase activity is determined by the product of α -ketoglutarate. The increase in absorbance at 410 nm is directly proportional to the enzyme activity.

2. Kit Components

Component	Volume	Storage
96-WellMicroplate	1 plate	-
Assay Buffer I	30 mlx 4	4 °C
Assay Buffer II	1.2 mlx 1	4 °C
Assay Buffer III	20 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Substrate Diluent	4 mlx 1	4 °C
Dye Reagent I	5 mlx 1	4 °C, keep in dark
Dye Reagent II	15 mL x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 410 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 20 mmol/ L of standardtop solution , store at 4°C for 1-2 weeks or -20°C for3month safter reconstitution. Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312mmol/L.

Substrate: Add 4 mL Substrate Diluent to dissolve before use.Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 mL Assay Buffer to dissolve before use, mix. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 mL Assay Buffer I and 10 μL Assay Buffer I I on ice, centrifuged at 600g 4°C for 5 minutes.T ake the supernatant into a new centrifuge tube , 11000 g 4°C for 10 minutes , discard the supernatant. Add 198 μL Assay Buffer I II and 2 μL Assay Buffer I I to the precipitation, shock , sonicate (with power 20 %, sonicate 3s, interval 10s,repeat 30 times). Centrifuged at 11000 g 4°C for 1 0minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 0.99ml Assay Buffer I and 10 μL Assay Buffer IIon ice, centrifuged at 600g 4°C for 5 minutes.T ake the supernatant into a new centrifuge tube , 11000 g 4°C for 10 minutes , discard the supernatant. Add 198 μL Assay Buffer I II and 2 μL Assay Buffer I I to the precipitation, shock ,sonicate (with power 20 %, sonicate 3s, interval 10s ,repeat 30 times). Centrifuged at 11000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples Detect directly.

6. Assay Procedure

Warm all thereagents to 37°C before use . Add following reagents in to the microplate :

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 μl	--	--	--	--
Standard	--	--	10 μl	--	--
Distilled water	--	10 μl	40 μl	50 μl	--
Positive Control	--	--	--	--	10 μl
Substrate	40 μl	40 μl	--	--	40 μl
Mix, incubate at 37°C for 30 minutes.					
Dye Reagent I	50 μl	50 μl	50 μl	50 μl	50 μl
Dye Reagent II	150 μl	150 μl	150 μl	150 μl	150 μl
Mix, stand at room temperature for 10 minutes, record absorbance measured at 410 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One Unit of IDHactivity is defined as the enzyme produces 1

μmolα -ketoglutarate per min at 37°C .

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

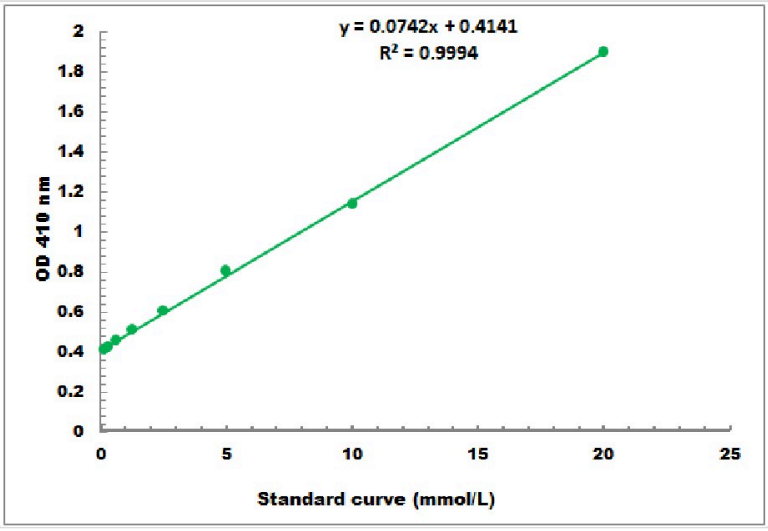
2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

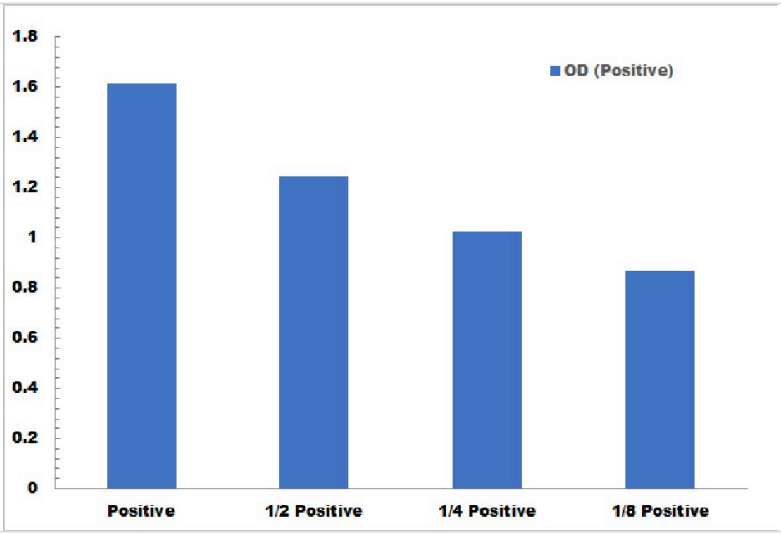
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, mmol/L = μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer I II and Assay Buffer I I in sample preparation, mL
T	the reaction time , minute

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 0.2 mmol/L - 20 mmol/L



Positive Control reaction in 96 -well plate assay with decreasing the concentration