Isocitrate Dehydrogenase

Microplate Assay Kit

User Manual

Catalog # CAK1173

(Version 1.3B)

Detection and Quantification of Isocitrate Dehydrogenase (IDH) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. 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 cofactor NADP+ and catalyze the same reaction outside the citric acid cycle. This kit measures the activity of the NADP+ isoforms. Mutations in IDH1 and IDH2 have been linked with various brain tumors and acute myeloid leukemia.

Isocitrate Dehydrogenase 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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Substrate Diluent	4 ml x 1	4 °C
Dye Reagent I	5 ml x 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
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Note:

Substrate: add 4 ml Substrate Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use. The concentration will be 20 mmol/L.

Positive Control: add 0.1 ml Assay Buffer to dissolve before use, mix.

III. 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

IV. 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 II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 4 °C for 10 minutes, 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 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm all the reagents to 37°C before use.

Add following reagents into 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One Unit of IDH activity is defined as the enzyme produces 1 μmol α-ketoglutarate per min at 37°C.

1. According to the volume of serum or plasma

$$IDH\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.67 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= 0.134 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 0.134 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of Standard, 20 mmol/L = 20 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

$V_{Standard}$: the volume of standard, 0.01 ml;

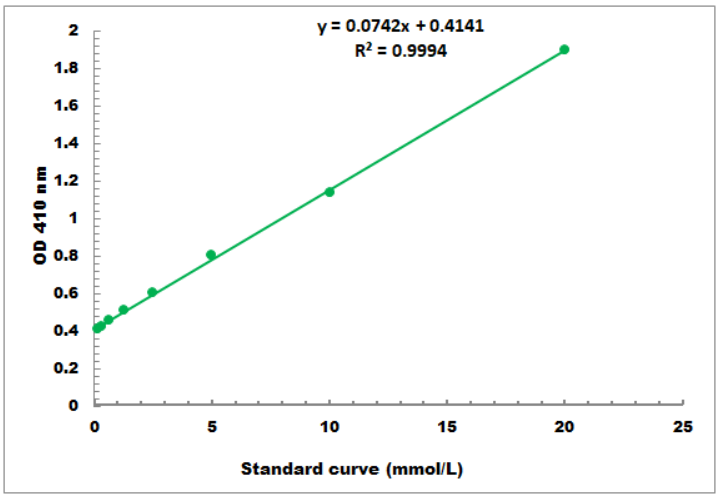
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 0.2 ml;

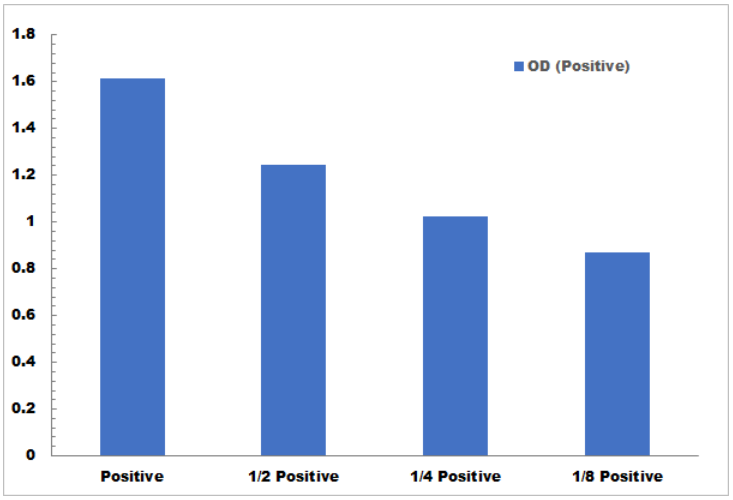
T: the reaction time, 30 min.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES