Isocitrate Dehydrogenase Assay Kit

Isocitrate Dehydrogenase Assay Kit is a detection kit for the quantification of Isocitrate Dehydrogenase Activity in tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82035

Package: 96 wells

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INTRODUCTION

Isocitrate dehydrogenase (IDH) (EC 1.1.1.42) and (EC 1.1.1.41) is an enzyme that catalyzes the oxidative decarboxylation of isocitrate, producing alpha-ketoglutarate (α-ketoglutarate) and CO2. This is a two-step process, which involves oxidation of isocitrate (a secondary alcohol) to oxalosuccinate (a ketone), followed by the decarboxylation of the carboxyl group beta to the ketone, forming alpha-ketoglutarate. In humans, IDH exists in three isoforms: IDH3 catalyzes the third step of the citric acid cycle while converting NAD^+ to NADH in the mitochondria. The isoforms IDH1 and IDH2 catalyze the same reaction outside the context of the citric acid cycle and use $NADP^+$ as a cofactor instead of NAD^+. They localize to the cytosol as well as the mitochondrion and peroxisome. [Provide by Wikipedia: Isocitrate Dehydrogenase]

PRINCIPLE OF THE ASSAY

This Isocitrate Dehydrogenase Assay Kit is a sensitive assay for determining Isocitrate Dehydrogenase activity in tissue extracts, cell lysate and cell culture supernatants. Isocitrate Dehydrogenase activity is determined by the product of α-ketoglutarate. The increase in absorbance at O.D. 410 nm is directly proportional to the enzyme activity. The concentration of Isocitrate Dehydrogenase in the samples is then determined by comparing the O.D. 410 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Assay Buffer I	30 mL x 4 (ready to use)	4°C
Assay Buffer II	1.2 mL (ready to use)	4°C
Assay Buffer III	20 mL (ready to use)	4°C
Substrate (powder)	1 vial	-20°C
Substrate Diluent	4 mL	4°C
Dye Reagent I	5 mL (ready to use)	4°C (protect from light)
Dye Reagent II	15 mL (ready to use)	4°C
Standards (powder)	1 vial	4°C
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 410 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria samples: Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Add 0.99 mL of Assay Buffer I and 10 µL of Assay Buffer II on ice, and centrifuged at 600 x g for 5 minutes at 4°C. Take the supernatant into a new centrifuge tube, centrifuged at 11,000 x g for 10 minutes at 4 °C. Discard the supernatant. Add 198 µL Assay Buffer III and 2 µL Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times). Centrifuged at 11,000 x g for 10 minutes at 4°C, and then take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 0.99 mL of Assay Buffer I and 10 µL of Assay Buffer II on ice. Centrifuged at 600 x g for 5 minutes at 4°C, and then take the supernatant into a new centrifuge tube. Centrifuged at 11,000 x g for 10 minutes at 4°C. After discard the supernatant, Add 198 µL of Assay Buffer III and 2 µL of Assay Buffer II to the precipitation. Shock, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times). Centrifuged at 11,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Cell culture medium and other biological fluids: Detect directly.

REAGENT PREPARATION

- Substrate: add 4 mL of Substrate Diluent to dissolve before use.
- Standards: add 1 mL of distilled water to dissolve before use, the concentration will be 20 mmol/L. Use the 20 mmol/L Standards to prepare a series of standards according to the Table below.

Standard tube	Final standard conc. (mmol/L)	Volume of distilled water (µL)	Volume of 20 mmol/L Standards (µL)
S1	20	0	500
S2	10	250	250 of S1
S3	5	250	250 of S2
S4	2.5	250	250 of S3
S5	1.25	250	250 of S4
S6	0.625	250	250 of S5
S7	0.313	250	250 of S6
S8	0.156	250	250 of S7

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Reagent	Sample	Control	Standard	Blank
Sample	10 µL			
Standard			10 µL	
Distilled water		10 µL	40 µL	50 µL
Substrate	40 µL	40 µL		
Mix well and incubate for **30 minutes** at **37°C**.				
Dye Reagent I	50 µL	50 µL	50 µL	50 µL
Dye Reagent II	150 µL	150 µL	150 µL	150 µL
Mix well and incubate for **10 minutes** at **room temperature**, and then read the absorbance at **O.D. 410 nm**.				

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank, Control and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One Unit of IDH activity is defined as the enzyme produces

1 μmol α-ketoglutarate per min at 37°C.

6. According to the weight of sample:

IDH (U/g)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})\} / T$

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

7. According to the quantity of cells or bacteria:

IDH ($U/10^4$)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / (N \times V_{Sample} / V_{Assay})\} / T$

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

8. According to the volume of sample

IDH (U/mL)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / V_{Sample}\} / T$

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

Note:

W: the weight of sample, g;

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

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

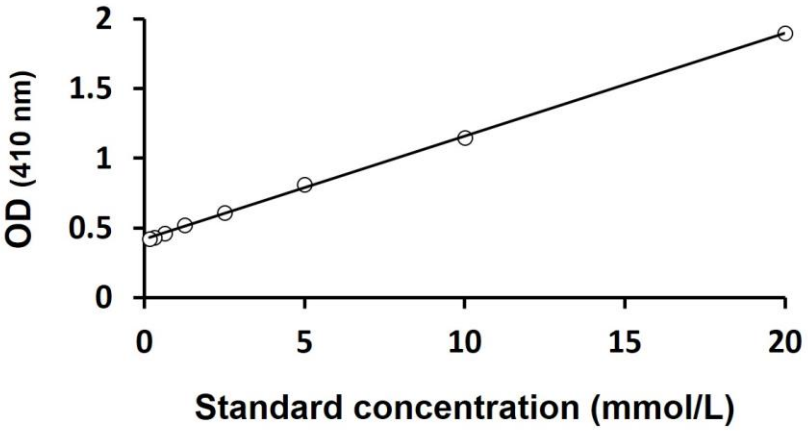
V_{Sample}: the volume of sample, 0.01 mL;

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

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Isocitrate Dehydrogenase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.2 mmol/L