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Isocitrate dehydrogenase

Kimberly Chapman[1], Parith Wongkittichote[2]

[1]Children's National Rare Disease Institute,

[2]Children's National, Department of Pediatrics, St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO, USA, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400, Thailand

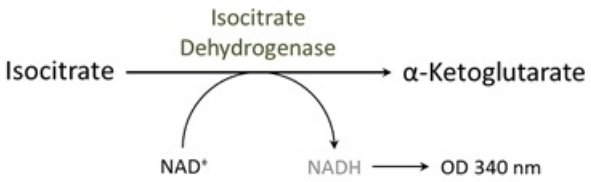
1 Works for me dx.doi.org/10.17504/protocols.io.bfisjkee

Kimberly Chapman
Children's National Rare Disease Institute

ABSTRACT

Isocitrate dehydrogenase is an enzyme which converts isocitrate to 2-oxoglutarate in the TCA cycle. IDH2 uses NADP and IDH3 uses NAD.

Our IDH2/3 assay examines accumulation of NADH/NADPH over time.



Reaction for IDH3, IDH2 uses NADP as its substrate and detected at OD 340 nm as well

MATERIALS TEXT

- 0.5 M Potassium Phosphate Buffer pH 7.4 (room temp)
- 1 M $MgCl_2$ (room temp)
- 100 mM NADP (-20) (made from β-Nicotinamide Adenine Dinucleotide Phosphate (CAS 53-59-8, kept at -20); dissolved in dW)
- 1 M Isocitrate (-20) (made from DL-isocitric acid (CAS 1637-73-6, kept at room temp); dissolved in dW)

BEFORE STARTING

Make sure have adequate amount of IDH buffer and that exactly pH 7.4 (best if prepared fresh with each assay)
Make sure that adequte mitochondrial lysate
Make sure spectrophotometer is set, wells labeled and plate ready to be read immediately

1 Prepare the IDH buffer new (Place on ice)

1.1 To make 10 mL IDH buffer
10 mM potassium phosphtate pH 7.4 (200 µL 0.5 M potasium phosphate pH 7.4),
2 mM $MgCl_2$ (20µL 1M $MgCl_2$),
1mM NADP (100 µL 100mM NADP)
5mM isocitrate (50 µL 1M isocitrate)
Add water to make 10 mL after pH ing to 7.4

2 Prepare mitochondrial lysates per protocol (will need 15-30 µg in 20 µL)

Citation: Kimberly Chapman, Parith Wongkittichote (04/27/2020). Isocitrate dehydrogenase. https://dx.doi.org/10.17504/protocols.io.bfisjkee

3 Load 20 µL into 96 well plate in triplicate

4 Set spectrophotometer to Kinetic, dual wavelength 340, 380 nm, 30 reads, interval 30 sec at 30°C

5 Add 200 µL of IDH buffer to each well of plate (use a multi-chamber pipetter) sitting on the holder for the spectrometer and mix by pipetting in and out 2x and start spectrophotometer

6 Calculate relative activity from the values of OD_{340}

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