NADP-DEPENDENT ISOCITRATE DEHYDROGENASE

[Summary and Explanation]

Isocitrate dehydrogenase (IDH=EC:1.1.1.42) is an enzyme that converts isocitrate to alpha-ketoglutarate (α-KG) and carbondioxide(CO_2). There are three isozymes of IDH (IDH1, IDH2 and IDH3), the IDH1 and IDH2 are NADP-dependent and the IDH3 utilize NAD as cofactor. NAD-dependent IDH3 is located in mitochondria and is well known for its central role for energy production in the Krebs cycle. NADP-dependent IDHs (EC 1.1.1.42) are primarily located either in cytoplasm (IDH1) or mitochondria(IDH2). Both IDH1 and IDH2 play important roles in a number of cellular metabolic functions, including glucose sensing, glutamine metabolism, lipogenesis, and regulation of cellular redox status(1, 2).

IDH activity significantly increases in human serum in parenchymal diseases of liver(3). Although activity of mutant IDH is elevated in cancers like glioblastomas(GBM)(4), acute myeloid leukemia(AML)(5), intrahepatic cholangiocarcinoma(6); particularly in the early phases of cancer, an increase is also observed in normal type of IDH enzyme activity.

[Principle of Assay]

The Isocitrate Dehydrogenase Activity Assay kit provides a simple and direct procedure for measuring $NADP^+$-dependent IDH activity in serum. The activity is determined using isocitrate as the substrate in IDH enzyme reaction. As a result of this reaction a change of absorbance at 340 nm is observed, proportional to the enzymatic activity of IDH. One unit of IDH is the amount of enzyme that will generate 1.0 µmole of NADPH per minute at pH 8.0 and 37 °C.

$$Izositrat + NAD^+ + 2H^+ \rightarrow 2-oksoglutarat + NADH + H^+ + CO_2$$

[Reagent Composition]

Reagent:	Buffer Solution
	Cofactors
	Coenzymes
Surfactants	
	Substrate
	Preservatives

[Intended Use]

This product can be used for quantitative *in vitro* determination of total NADP-dependent IDH activity from human serum and plasma.

[Stability and Preparation of Reagents]

Reagent:

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

CAL

The assay can be calibrated with deionized water. Daily calibration is recommended.

The procedure is standardized by means of the molar absorptivity of NADPH taken as 6300 at 340nm under the test conditions described (Opthical path: 1cm)

Calculation

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions (Opthical path: 1cm)

IDH (U/L) = ΔAbs/min. x 4290

If your opthical path is different from 1 cm, the factor (4290) must be divided into opthical path.

[Performance Characteristics]

Low linearity: 0 IU - *High linearity:*1200 IU

If the results obtained are greater than linearity limit, dilute the sample 1/5 with deionized water and multiply the result by 5.

[Precision]

Intra-assay CV was determined with 30 replicates of 20 samples. The %CV result was lower than 1 and inter-assay %CV result was 1.5.

[Normal range]

Adult Serum: ≤12 IU

Each laboratory is recommended to establish their own reference values.

Serum samples collected after at least 8-10 hours of fasting must be used for the test.

[Components]

All reagents are ready to use

Content		Amount
Reagent		60 ml
Level 1	90 IU (72-108)	3 ml
Level 2	300 IU (240-360)	3 ml

[Storage and Validity Date]

Storage: This kit should be stored at 2-8°C.

Validity date: The validity of reagent is indicated on the bottle and on the kit box.

(This kit is stable for up to expiry date when stored at 2-8°Cafter opened.)

[Sample]

Blood serum, plasma and urine could be used as sample.

Serum samples are stable up to 1 week stored at 4˚C, 2 months at -20˚C and 6 months at -80˚C.

Collect samples in collection tubes produced for serum and plasma.

Serum samples collected after at least 8-10 hours of fasting must be used for the test.

[Procedure and Calculation]

Serum and plasma application

Assay type	Rate Up
Wavelength	340
Opthical path	1cm
	R1
Reagent pipetting	164 µL
Sample	6.3 µL

[Interferences]

No interferences were observed with bilirubin up to 18 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

In order to obtain best results, pipettes and cuvettes used for the assay must be cleaned thoroughly. Make sure that no wash solution is left on pipettes and in cuvettes.

[Recovery]

Serum pool was prepared and NADP-dependent IDH activity was measured. Then enzyme activity were re-measured following addition of 1200 IU/mL NADP-dependent IDH enzyme solution.in ratios of 1/10 and 1/100 into the same serum pool. As a result, recovery was found in between %95 and %105range.

[Safety precautions and warning]

1. For *in vitro* diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the routine precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.
9. The Reagent contains sodium azide (0.01%) as a preservative. Do not ingest

References

1. Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, *et al.* Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. J Biol Chem. 2004;279(32):33946-57.
2. Ying W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid Redox Signal. 2008;10(2):179-206.
3. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostic. 3 ed. London: Elsevier Health Sciences; 1999.
4. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, *et al.* An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321(5897):1807-12.
5. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, *et al.* The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell. 2010;17(3):225-34.
6. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, *et al.* Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist. 2012;17(1):72-9.