MALATE DEHYDROGENASE

[Summary and Explanation]

Malate dehydrogenase (MDH=EC: 1.1.1.37) is an enzyme that converts oxaloacetate to L-malate. MDH is primarily located either in cytoplasm and mitochondria (1). MDH enzyme is one of the citric acid cycle enzymes used to obtain energy in mitochondria. Since the citric acid cycle is the center of energy production, it has an important role in maintaining cell homeostasis. Based on this, it has been observed that defects of the citric acid cycle cause a variety of pathologies ranging from cancer to neurological and metabolic disorders (2-5).

[Principle of Assay]

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

$$Oxaloacetate + NADH^+ + H^+ \rightarrow L-malate + NAD^+$$

[Reagent Composition]

Reagent 1:	Buffer Solution
	Cofactors
	Coenzyme
	Surfactants
	Preservatives
Reagent 2:	Buffer Solution
	Substrate
	Preservatives

[Intended Use]

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

[Stability and Preparation of Reagents]

Reagents:

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 NADH taken as 6.22 X 10^3 M^{-1} cm^{-1} at 340 nm 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)

MDH (U/L) = ΔAbs/min. x 6592

If your opthical path is different from 1 cm, the factor (6592) must be divided into opthical path.

[Performance Characteristics]

Low linearity: 0 IU - *High linearity:* 1000 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: ≤45 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 1	40 mL
Reagent 2	8 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°C after 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 Down
Wavelength	340/380
Opthical path	1cm

	Volume
Reagent 1	170 µL
Reagent 2	30 µL
Sample	5 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 MDH activity was measured. Then enzyme activity were re-measured following addition of 1000 IU/mL MDH 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* research 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. Minarik P, Tomaskova N, Kollarova M, Antalik M. Malate dehydrogenases-structure and function. General physiology and biophysics. 2002;21(3):257-66.
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3. Zelewski M, Swierczyński J. Malic enzyme in human liver. Intracellular distribution, purification and properties of cytosolic isozyme. European journal of biochemistry. 1991;201(2):339-45.
4. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008;245(3):194-205.
5. Hanse EA, Ruan C, Kachman M, Wang D, Lowman XH, Kelekar A. Cytosolic malate dehydrogenase activity helps support glycolysis in actively proliferating cells and cancer. Oncogene. 2017;36(27):3915-24.