Malondialdehyde(MDA) Colorimetric Assay Kit

Cat.No: Otto1001

Summary & Explanation

Chemically, MDA is a small and reactive organic molecule that occurs ubiquitously among eukaryotes, formed by three carbon molecules with two aldehyde groups at the carbon 1 and carbon 3 positions. MDA exists in different forms in aqueous solutions due to its pH-dependent tautomeric chemical property. At higher pH than its pKa of 4.46, the dominate form is the enolic anion, which displays low chemical reactivity. However, at lower pH (expected under oxidative stress conditions), MDA appears in equilibrium between its protonated enol (α-β-unsaturated carbonyl) aldehyde and the dialdehyde form. Oxidative stress has been related to the etiopathogenesis of several chronic diseases and plays a paramount role in the aging process. Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. These products are mainly aldehydes, with the ability to exacerbate oxidative damage. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. Malondialdehyde (MDA) is the organic compound with the formula CH2(CHO)2. The structure of this species is more complex than this formula suggests. This reactive species occurs naturally and is a marker for oxidative stress.

Test Principle

Colorimetric method assay
Detects Malondialdehyde concentration in serum ,tissue, plasma, biological fluid or food samples.
Sample + TCA → protein precipitation
Supernatan + TBA (90-100 °C ink.)→ Pink pigment read 532 nm.
The MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90–100_C. In the TBA test reaction, MDA or MDA-like substances and TBA react with the production of a pink pigment with a maximum absorption at 532 nm. The reaction was performed at pH 2–3 at 90_C for 15 min The sample was mixed with two volumes of cold 10% (w/v) richloroacetic acid for the precipitation of protein. The precipitate was pelleted by centrifugation, and an aliquot of he supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm.

Kit Companents

Content	Explanation	Shelf life
Reagent-1(TCA)	1 gr(lyophilized)	12 months
Reagent-2 (TBA)	1 gr(lyophilized)	12 months
Standard	1x3ml (Liquid)	12 months

Available in various sizes.

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Reagent preparation

1. Dissolve a vial of reagent 1(0,25 gr) with 100 ml of double distilled water fully. **The prepared solution can be stored at 4°C for 1 months.(The reagent should be kept away from moisture and kept closed).**

2. Dissolve a vial of reagent 2(1 gr) with 100 ml of double distilled water fully. **The reagent should be heated gradually(37°C-55°C), mixed and melted. Prepare the fresh solution before use.**

Standard Curve preparation : **1,1,3,3 tetraethoxypropane (TEP) in 100 ml of water to give a 1 mM stock solution**. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2 h at room temperature. The resulting MDA standard of 20 nmol/ml was further diluted with 1% sulfuric acid to yield the final concentration of 100, 50, 25, 12.5 and 6.25 nmol/ml to get the standard curve for the estimation of total MDA.

Reactivity

Universal

Specimen

Serum, Plasma (Edta or Heparin)
Tissue homogenate
Biological fluid
Food samples
Stability: +4°C 6 months

Assay Range

0.1 nmol/ml – 120.0 nmol/ml

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 2.6
Intra Assay Coefficent of Variation (CV) % 2.7

Procedure

Wavelength	532nm (±10nm)
Sample or Standard	250 µl
Reagent-1	250 µl
Reagent-2	250 µl

Reagent-1 and sample or standard, mix and wait 15 minutes, at room temperature, and than 4000 rpm 10 min centrifuged. Take 250 µl supernatant, add reagent-2 mix fully. bath for 90 min. After cooling.
Read absorbance 532 nm.

Calculation

Result (nmol/L): Create standard curves with standard concentrations on the x-axis and OD values on the y-axis.(Linear or polinom graph.)

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

References

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