BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Lipid Peroxidation (MDA) Assay Kit (Cat #: A-102)

COMPONENTS: TBA- 0.75 g, store at room temperature (RT)
MDA Assay Solution- 100 ml, store at room temperature (RT; for ~500 samples)

PRODUCT DESCRIPTION: Cells exposed to oxidative stress accumulate a complex profile of aldehydes which are derived from the decomposition of lipid hydroperoxides. Malondialdehyde (MDA) and MDA-like substances are by far the most abundant lipid peroxidation products. The analysis of MDA by the classic 2-thiobarbituric acid (TBA) test remains a simple and reliable method. The kit is stable for several years if stored and handled properly.

PROTOCOL:

Preparation of working solution

Prepared a fresh working solution by dissolving 6.5 mg TBA per ml of MDA Assay Solution prior to assay. Estimate the amount of working solution required for each assay (0.2 ml per sample). Vortex working solution vigorously to dissolve TBA. Several minutes may be required for complete dissolution of TBA.

Sample preparation

Cell sample: Pellet at least ~10^6 normal saline-washed cells in a microtube. Remove saline completely. Add 0.1 ml ice-cold 10% trichloroacetic acid (TCA) solution (not included) to cell pellet. Disrupt the cell pellet by vigorous trituration and vortexing. Mechanical homogenization can facilitate this step. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Tissue sample: Tissue sample should be washed with normal saline to remove blood residues. Tissue (~50 mg) is mechanically homogenized in 0.2 ml of ice-cold 10% TCA. Agitate extract on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Serum/plasma sample: Serum/plasma should be deproteinized by TCA precipitation. Mix sample with an equal volume of ice-cold 10% TCA and incubate on ice for 5 min. Clarify sample by centrifugation at ~13,000 rpm for 5 min. Collect supernatant for the assay.

Assay protocol

1. Mix 0.2 ml TCA-treated sample and 0.2 ml of freshly prepared working solution in a 1.5-ml microtube and secure the cap.
2. Heat samples at ~95°C for 30 min. Allow tube to cool down to room temperature. Spin tube briefly to deposit content before opening.
3. Add 0.3 ml n-butanol (not included) to each tube. Agitate tubes for ~1 min. Centrifuge tube in a microfuge at maximal speed (~13,000 rpm) for 3 min to separate the aqueous phase (bottom layer) and n-butanol phase (top layer).
4. Carefully transfer n-butanol (top layer) from each tube to a plain (uncoated) 96-well plate for absorbance (O.D.) measurement.
5. Measure O.D. at 532 nm using an equal volume of n-butanol as blank. Positive sample reactivity generates a pale pinkish color. Subtract blank reading from sample reading to generate Δ**O.D.** for each sample.
6. Sample MDA concentration is calculated using a molar extinction coefficient of 1.56 x 10^5 $cm^{-1}M^{-1}$.
   ΔO.D.= extinction coefficient x light path length in well x concentration = 1.56 x 10^5 x 0.7 x [μM MDA].
   MDA concentration in μM = **9.157 x O.D.**

Additional information:

- Trichloroacetic acid (TCA) is corrosive and should be handled with caution. n-Butanol is flammable. MDA Assay Solution contains sodium hydroxide. Avoid contact with skin. Please contact us or visit the product webpage for SDS information.