GENTAUR

DATA SHEET

NWLSS™ Malondialdehyde Assay

Product NWK-MDA01

For Research Use Only

Simple assay kit for quantitative measurement of lipid peroxidation as Thiobarbituric Acid Reactive Substances (TBARS) or more specifically, as malondialdehyde (MDA) when employing extraction or advanced data reduction techniques.

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Introduction:

Polyunsaturated fatty acids (PUFA) that contain 2 or more double bonds are particularly susceptible to oxidation by free radicals and other highly reactive species. In brief, an allylic hydrogen is abstracted by a reactive species, such as the hydroxyl radical (HO•), resulting in the formation of lipid peroxyl radicals (LOO•). This radical can then react with a second PUFA, forming a lipid hydoperoxide (LOOH) and a second LOO•, resulting in the propagation of the lipid oxidation. Alternatively, LOO• can attack an intramolecular double bond and form a cyclic endoperoxide which decomposes to malondialdehyde.[1,2]

Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid hydoperoxide decomposition and is the most often measured as an index of lipid peroxidation.[3] However, the use a MDA as a marker for lipid peroxidation is controversial. MDA can be formed during eicosanoid metabolism and the analytical methods for measuring MDA are prone to artifactual errors.[4] The NWLSS™ MDA assay conditions have been optimized to minimize errors in the determination of MDA concentration.

Intended Use:

The NWLSS™ Malondialdehyde Assay is used to quantify lipid peroxidation as the concentration of MDA in a variety of animal and plant samples.

Test Principle:

The NWK-MDA01 assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA_2 adduct that absorbs strongly at 532 nm.

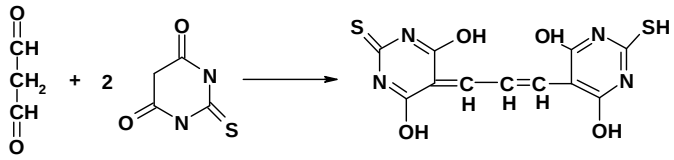

Figure 1.
Reaction between MDA and TBA.

Butylated hydroxytoluene (BHT) and EDTA are added tom the sample and reaction mixture to minimize oxidation of lipids that contribute artifactually during sample processing and the TBA reaction.[5,6] The temperature of reaction mixtures has also been reduced to minimize decomposition of lipid hydroperoxides. Because much of the MDA is protein bound, mostly as a Schiff base, the reaction pH has been optimized to facilitate hydrolysis of MDA.[7] Additionally, the reaction mixture can be extracted to further clarify or subjected to advanced data reduction techniques to reduce the effects of non-linear baseline prevalent with more complex sample types such as plasma and tissue (see www.nwlifescience.com/tba-mdaanalysis.html).

General Specifications:

Format:	Spectrophotometric
Number of tests:	200
Specificity:	Malondialdehyde
Sensitivity:	Processed Sample = 0.08 µM
	Reaction Mixture = 0.03 µM

Kit Contents:

TBA Reagent	2-Thiobarbituric acid, dry powder, 5 bottles
BHT Reagent	Butylated hydroxytoluene in ethanol, 2.0 mL vial
Acid Reagent	1M Phosphoric acid, 5 x 10 mL bottles
Assay Buffer	Phosphate buffer, pH 7.0 with EDTA, 125 mL bottle
Calibrators:	Tetramethoxypropane in a stabilizing buffer at 4,3,2,1 and 0 µM MDA equivalents; 2.5 mL each

Required Materials Not Provided:

Adjustable pipettes capable of 10 and 250 µL volumes
Pipette tips for 10 and 250 µL volumes
Stir bar, 1/8 inch (0.3 cm) or similar
Polypropylene microcentrifuge tubes
Semi-micro cuvettes (plastic glass or quartz)
Deionized water

Required Instrumentation:

Spectrophotometer
Vortex mixer
Magnetic stirrer (recommended)
Dry heating block or water bath set to 60°C
Microcentrifuge

Warnings & Precautions:

2-Thiobarbituric Acid (TBA, CAS 504-17-6) has a strong mercaptan odor. Do not breathe dust. Avoid contact with skin and eyes.

Phosphoric Acid (CAS 7664-38-2) is corrosive and can cause burns. In case of contact with eyes, rinse immediately with copious amounts of water and seek medical advice. Wear suitable protective clothing.

Butylated hydroxytoluene (2,6-di-tert-butyl-4-methylphenol, BHT, CAS 128-37-0) is harmful if swallowed and irritating to the eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with copious amounts of water and seek medical advice. Wear protective clothing.

Procedure Checklist:

___Process the sample and place on ice

___Preheat heat block or water bath to 60°C

___Reconstitute TBA Reagent with 10.5 mL deionized water

___Add 10 µL BHT Reagent to microcentrifuge vial

___Add 250 µL Calibrator or sample to microcentrifuge vial

___Add 250 µL Phosphoric Acid Reagent to microcentrifuge vial

___Add 250 µL TBA reagent to microcentrifuge vial

___Cap and vortex (to 5 count)

___Place at 60°C for 60 minutes

___Setup spectrophotometer

___Centrifuge at 10,000 x*g* for 2-3 minutes

___Analyze data

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

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Performance Details (continued)

Stability

All unopened reagents are stable until the expiration date stated on the package label when stored at 2-8°C.

Things to Notes:

Optimizing Analysis

The optimum analytical method for analyzing the $TBA_{2\text{-}MDA}$ reaction mixture is dependent on the sample type. The MDA concentration of samples such as buffer solutions, culture supernatants, tissue culture cell homogenates and low protein solutions can be determined by simple absorption at 532 nm. For complex samples such as plasma, high protein samples, tissue homogenates, small to moderate hemolysis and urine, the investigator is invited to visit the MDA Analysis web site at www.nwlifescience.com/tba-mdaanalysis.html or contact Northwest Life Science Specialties directly for additional info.

Alternative Analysis: The reaction mixture has also been analyzed using difference absorption spectroscopy, fluorescence spectroscopy or HPLC.[8,9,10]

TBA Reagent Precipitaion: If the TBA Reagent is refrigerated, the TBA will precipitate. One can re-dissolve the TBA with gentle warming. If the precipitate does not re-dissolve, discard the reagent.

References:

1. Halliwell, B.; Gutteridge, J.M.C. Free Radicals in Biology and Medicine. Oxford University.
2. Esterbauer, H., et.al., Chemistry and Biochemistry of 4-Hydroxynonenal, malondialdehyde and Related Aldehydes, *Free Rad. Biol. Med*. **11**:81-128; 1991).
3. de Zwart, L.L., et.al., Biomarkers of Free radical Damage Applications in Experimental Animals and Humans, *Free Rad. Biol. Med*. **26**:202-226; 1999.
4. Janero, D.R. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury, *Free Rad. Biol. Med.* **9**:515-540; 1990.
5. Botsoglou, N.A., Rapid, Sensitive, and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in animal Tissue, Food and Feedstuff Samples, *J. Agric. Food Chem.* **42**:1931-1937; 1994.
6. Jentzsch, A. M., et.al., Improved Analysis of Malondialdehyde in Human Body Fluids, *Free Rad. Biol. Med*. **20**:251-256; 1996.
7. Gerard-Monnier, D., et.al., Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxylalkenals. Analytical applications to a colorimetric assay of lipid peroxidation, *Chem. Res. Toxicol*. **11**; 1184-1194; 1997.
8. Carbonneau, M.A., et.al. Free and Bound Malondialdehyde Measured as Thiobarbituric Acid Adduct by HPLC in Serum and Plasma, *Clin. Chem.* 37:1423-1429; 1991.
9. Lykkesfeldt, J., Determination of Malondialdehyde as Dithiobarbituric Acid Adduct in Biological Samples by HPLC with Fluorescence Detection: Comparison with Ultraviolet-Visible Spectrophotometry, *Clin. Chem.* 47:1725-1727; 2001.
10. Yagi, K., Simple Procedure for Specific Assay of Lipid Hydroperoxides in Serum or Plasma, *Free Radical and Antioxidant Prot.* 108:101-106; 1998.

Storage Instructions:

Store all reagents at 2-8°C.

Reconstituted TBA Reagent is stable for at least 1 week when stored at room temperature. **Do not refrigerate.**

Assay Preparation

Spectrophotometer

- Wavelength: 400-700 nm
- Spectral bandwidth: ≤ 2 nm
- Resolution: ≤ 1 nm

Heat block or water bath preheated to 60°C

Reagent Preparation:

TBA Reagent

Add 10.5 mL deionized water to the TBA bottle. Insert magnetic stir bar and mix until TBA has dissolved; approximately 10 minutes. Store at room temperature, do not refrigerate.

Other Reagents

The Acid Reagent, BHT Reagent, Assay Buffer and Calibrators are supplied ready-to-use.

Sample Handling/Preparation:

The multi-disciplinary interest in measuring MDA has resulted in a myriad of sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail. However, general guidelines are provided below for representative sample types. Please contact NWLSS to discuss the particular sample under investigation.

Tissue

Hemoglobin interferes with the assay; therefore, if practical, blood should be removed by perfusion with an appropriate buffer, such as phosphate buffered saline containing heparin. Prepare a 10% w/v homogenate in cold Assay Buffer or other buffer. Clarify the homogenate by centrifugation and store the supernatant on ice. The MDA concentration can be normalized to the wet weight of the tissue sample or to the protein concentration of the homogenate.

Plasma

Blood, using EDTA, heparin or other anticoagulant, should be kept cold and the plasma separated as soon as possible after being drawn. It is important to minimize hemolysis. Plasma can be assayed without further processing. Note that the MDA concentration in normal plasma is at the limits of sensitivity for the assay.

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Sample Handling/Preparation (continued):

Urine

Urine samples should be stored at -20°C (-70°C long term storage). Upon thawing, the sample must be clarified by centrifugation prior to assay. The MDA concentration can be normalized to creatinine concentration or other appropriate markers.

Assay Protocol:

1. Add 10 µL BHT Reagent to microcentrifuge vial
2. Add 250 µL Calibrator or Sample to vial
3. Add 250 µL Acid Reagent to vial
4. Add 250 µL TBA Reagent to vial
5. Vortex vigorously (5-count)
6. Incubate 60 minutes at 60°C
7. Centrifuge at 10,000 x*g* for 2-3 minutes
8. Transfer reaction mixture to cuvette
9. Proceed to Data Analysis below.

Data Analysis:

The optimum analytical method for analyzing the TBA_2-MDA reaction mixture is dependent on the sample type. The MDA concentration of samples such as buffer solutions, culture supernatants, tissue culture cell homogenates and low protein solutions can be determined by simple absorption at 532 nm.

A typical MDA calibration curve generated using the standard method is shown in Figure 2

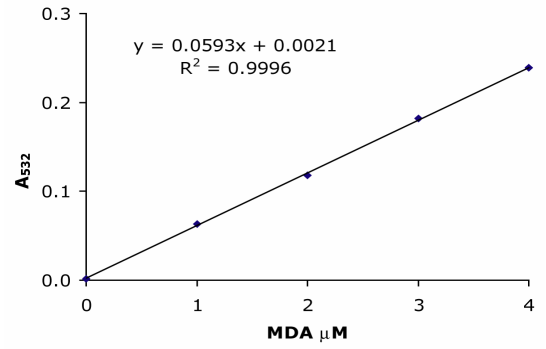

Figure 2.
Calibration curve for the NWLSS MDA01 Assay using the standard method.

For complex samples such as plasma, high protein samples, tissue homogenates, small to moderate hemolysis and urine, the investigator is invited to contact NWLSS or to visit the MDA Analysis web site at www.nwlifescience.com/tbaanalysis.html

Performance Details:

Sensitivity

The sensitivity of the NWK-MDA01 assay is defined as the MDA concentration obtained from 2-times the standard error of the calibration curve or 0.08 µM in the prepared sample or 0.03 µM in the reaction mixture.

Accuracy

Recovery: The recovery of MDA (hydrolyzed TMOP) and added to a muscle homogenate is shown in Table 1.

Table 1. Recovery of MDA added to a muscle homogenate and Assay Buffer.

		Initial	Observed	Added	Recovery
Buffer	MDA	0	1.83	1.99	92%
Muscle	MDA	0.50	1.81	1.83	71%

The recovery of less than 100% can be attributed to the tendency of MDA to undergo several reactions in a complex sample that are not reversible by acid hydrolysis.[1]

Dynamic Range:

As can be seen in Figure 3, the calibration curve is linear to 11 µM MDA. The standard method can measure up to 50 µM MDA but those concentrations will not be found in most biological samples. Therefore, the useful range of the NWLSS MDA assay is 0.1 to 10 µM

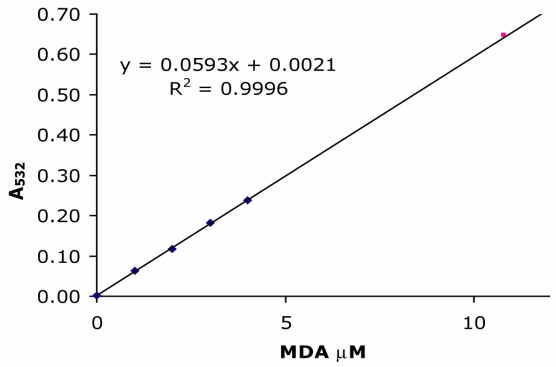

Figure 3
The 532 nm absorbance of a 10.8 µM MDA sample (■) was determined using the standard method and plotted against the calibration curve (♦).