QuantiChrom™ TBARS Assay Kit (DTBA-100)

Quantitative Determination of Thiobarbituric Acid Reactive Substances

DESCRIPTION

Oxidative attack of essential cell components by reactive oxygen species has been associated with several human diseases, such as atherosclerosis, cardiovascular diseases, diabetes, liver disorders, and inflammatory rheumatic diseases. *THIOBARBITURIC ACID REACTIVE SUBSTANCES* (TBARS) are low-molecular-weight end products (mainly malondialdehyde, MDA) that are formed during the decomposition of lipid peroxidation products. Increased levels of TBARS have been demonstrated in these diseases. Simple, direct and accurate assays for TBARS find wide applications in research and drug discovery. BioAssay Systems' TBARS assay is based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink colored product. The color intensity at 535nm or fluorescence intensity at ($\lambda_{ex}/_{em}$ = 560nm/585nm) is directly proportional to TBARS concentration in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range: colorimetric assay 1 - 30 µM, fluorometric assay 0.1 - 1.5 µM MDA.

APPLICATIONS

Direct Assays: serum, plasma, urine, saliva and other biological samples.
Drug Discovery/Pharmacology: effects of drugs on TBARS.

KIT CONTENTS

TBA Reagent: 25 mL **Standard:** 50 µL 15 mM MDA
10% Trichloroacetic acid (TCA): 25 mL

Storage conditions. The kit is shipped at room temperature. Store all components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Samples can be kept frozen at -80°C (stable for one month) if not assayed immediately. Urine and saliva samples can be assayed directly (n = 1). The following samples need to be deproteinated prior to assay:

1. For serum and plasma, transfer 100 µL of each sample into a labeled 1.5-mL tube. For tissue samples, weigh ~20 mg into 200 µL ice-cold phosphate buffered saline (PBS). Homogenize tissue by brief sonication (e.g. 20 seconds) on ice. If desired, remove 20 µL aliquot for protein analysis. Place 100 µL tissue lysate into a labeled 1.5 mL micro-centrifuge tube. For cells, harvest 5 x 10^6 cells in 200 µL ice-cold PBS and sonicate to disrupt cells. If desired, remove 20 µL aliquot for protein analysis. Place 100 µL cell lysate into a labeled 1.5mL micro-centrifuge tube.

2. Add 200 µL ice cold 10% TCA to the 100 µL of each sample. Incubate for 5 minutes on ice.

3. Centrifuge 5 min at 14,000 rpm in an Eppendorf Centrifuge. Transfer 200 µL of each clear supernatant into a new labeled tube. Dilution factor for these pretreated samples is n = 3.

COLORIMETRIC ASSAY PROCEDURE

Set up water bath or heat block and adjust the temperature to 100°C. Equilibrate all components to room temperature. Add 450 µL dH_2O to the 15 mM Standard tube and mix (final 1.5 mM MDA). Store unused Standard at -20°C for future use.

1. *Standards*. Mix 15 µL of the 1.5 mM MDA with 735 µL dH_2O (final 30 µM MDA). Dilute standards as shown in the Table. Transfer 200 µL standards into labeled 1.5-mL screw cap tubes.

No	30 µM MDA + H_2O	Vol (µL)	MDA (µM)
1	300µL + 0µL	300	30.0
2	180µL + 120µL	300	18.0
3	90µL + 210µL	300	9.0
4	0µL + 300µL	300	0.0

Samples. Transfer 200 µL of each sample into separate tubes.

2. *Color reaction*. To each of the standards and samples, add 200 µL TBA Reagent. Vortex tubes to mix and incubate at 100°C for 60 min. Cool down tubes to room temperature. Vortex and briefly centrifuge tubes.

3. Load 100 µL in duplicate from each tube to wells of a clear flat-bottom 96-well plate. Read OD at 535nm (525 to 545nm).

FLUORIMETRIC ASSAY PROCEDURE

The fluorescence assay is 20 times more sensitive than the colorimetric assay.

1. Prepare the standards as described in the Colorimetric Assay Procedure. Transfer 10 µL of each Standard into labeled tubes. Add 190 µL dH_2O (final concentrations 0, 0.45, 0.90, 1.50 µM MDA) .

Samples. In separate tubes, add 200 µL of treated samples.

2. For color reaction, add 200 µL TBA Reagent. Vortex tubes to mix and incubate at 100°C for 60 min. Cool down tubes to room temperature. Vortex and briefly centrifuge tubes.

3. Load 100 µL in duplicate from each tube to wells of a black flat-bottom 96-well plate. Read fluorescence intensity ($\lambda_{ex}/_{em}$ = 560nm/585nm) on a plate reader.

CALCULATION

Subtract blank OD or fluorescence intensity value (#4) from all standard and sample values. Plot the ΔOD_{535nm} or ΔF against standard concentrations and determine the slope of the standard curve. Calculate the TBARS concentration of Sample,

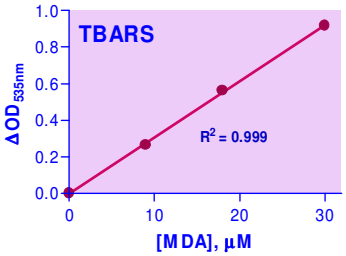
$$[\text{TBARS}] = \frac{R_{Sample} - R_{Blank}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M \text{ MDA equivalents})$$

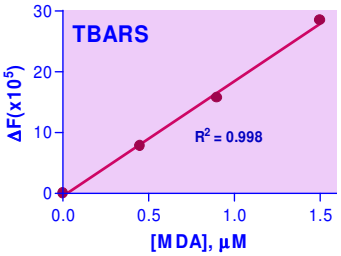
R_{SAMPLE} and R_{BLANK} are the OD_{535nm} or fluorescence intensity values of the sample and H_2O blank (standard #4). n is the sample dilution factor (n = 3 for deproteinated samples).

Note: if calculated TBARS concentration is higher than 30 µM MDA (colorimetric assay) or 1.5µM MDA (fluorometric assay) , dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, centrifuge, clear flat-bottom uncoated 96-well plates, optical density or fluorescence plate readers, sonicator, water-bath or heat block.





LITERATURE

1. Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Res. 15:212-216.

2. Satoh, K. (1978). Serum lipid peroxide in cerebrovascular disorder determined by a new colorimetric method. Clin. Chim. Acts 90:37-43.

3. Okawa H. et al (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.