

## 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 535 nm or fluorescence intensity at ( $\lambda_{ex/em} = 530 \text{ nm} / 550 \text{ nm}$ ) is directly proportional to TBARS concentration in the sample.

#### KEY FEATURES

**Sensitive and accurate.** Linear detection range: colorimetric assay 1 - 30  $\mu\text{M}$ , fluorometric assay 0.1 - 1.5  $\mu\text{M}$  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  $\mu\text{L}$  6 M MDA

**10% Trichloroacetic acid (TCA):** 25 mL

**Storage conditions:** The kit is shipped at room temperature. Store all components at  $-20^\circ\text{C}$ . 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  $-80^\circ\text{C}$  (stable for one month) if not assayed immediately. Urine samples are stable at  $25^\circ\text{C}$  or below for at least 3 days. Urine and saliva samples can be assayed directly ( $n = 1$ ). The following samples need to be deproteinated prior to assay:

- For serum and plasma, transfer 100  $\mu\text{L}$  of each sample into a labeled 1.5-mL tube. For tissue samples, weigh  $\sim 20 \text{ mg}$  into 200  $\mu\text{L}$  ice-cold phosphate buffered saline (PBS). Homogenize tissue by brief sonication (e.g. 20 seconds) on ice. If desired, remove 20  $\mu\text{L}$  aliquot for protein analysis. Place 100  $\mu\text{L}$  tissue lysate into a labeled 1.5 mL micro-centrifuge tube. For cells, harvest  $5 \times 10^6$  cells in 200  $\mu\text{L}$  ice-cold PBS and sonicate to disrupt cells. If desired, remove 20  $\mu\text{L}$  aliquot for protein analysis. Place 100  $\mu\text{L}$  cell lysate into a labeled 1.5 mL micro-centrifuge tube.
- Add 200  $\mu\text{L}$  ice cold 10% TCA to the 100  $\mu\text{L}$  of each sample. Incubate for 5 minutes on ice.
- Centrifuge 5 min at 14,000 rpm in an Eppendorf Centrifuge. Transfer 200  $\mu\text{L}$  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  $100^\circ\text{C}$ . Equilibrate all components to room temperature.

- Standards.** First, briefly centrifuge the Standard tube to pellet any MDA that may be stuck in the cap or on the sides of the tube. Then mix 4  $\mu\text{L}$  of the 6 M MDA with 2396  $\mu\text{L}$   $\text{dH}_2\text{O}$  (final 10 mM MDA). Next, prepare 30  $\mu\text{M}$  MDA by mixing 3  $\mu\text{L}$  of the 10 mM MDA with 997  $\mu\text{L}$   $\text{dH}_2\text{O}$ . Dilute standards as shown in the Table.

No	30 $\mu\text{M}$ MDA + $\text{H}_2\text{O}$	Vol ( $\mu\text{L}$ )	MDA ( $\mu\text{M}$ )
1	300 $\mu\text{L}$ + 0 $\mu\text{L}$	300	30.0
2	180 $\mu\text{L}$ + 120 $\mu\text{L}$	300	18.0
3	90 $\mu\text{L}$ + 210 $\mu\text{L}$	300	9.0
4	0 $\mu\text{L}$ + 300 $\mu\text{L}$	300	0.0

Transfer 200  $\mu\text{L}$  of each standard into separate, labeled 1.5-mL screw cap tubes.

**Samples.** Transfer 200  $\mu\text{L}$  of each sample into separate tubes.

- Color reaction.** To each of the standards and samples, add 200  $\mu\text{L}$  TBA Reagent. Vortex tubes to mix and incubate at  $100^\circ\text{C}$  for 60 min. Cool down tubes to room temperature. Vortex and briefly centrifuge tubes.
- Load 100  $\mu\text{L}$  in duplicate from each tube to wells of a clear flat-bottom 96-well plate. Read OD at 535 nm (525 to 545 nm).

#### FLUORIMETRIC ASSAY PROCEDURE

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

- Prepare the standards as described in the Colorimetric Assay Procedure. Transfer 10  $\mu\text{L}$  of each Standard into labeled tubes. Add 190  $\mu\text{L}$   $\text{dH}_2\text{O}$  (final concentrations 0, 0.45, 0.90, 1.50  $\mu\text{M}$  MDA) .  
**Samples.** In separate tubes, add 200  $\mu\text{L}$  of treated samples.
- Add 200  $\mu\text{L}$  TBA Reagent. Vortex tubes to mix and incubate at  $100^\circ\text{C}$  for 60 min. Cool down tubes to room temperature. Vortex and briefly centrifuge tubes.
- Load 100  $\mu\text{L}$  in duplicate from each tube to wells of a black flat-bottom 96-well plate. Read fluorescence intensity ( $\lambda_{ex/em} = 530 \text{ nm}/550 \text{ nm}$ ) on a plate reader.

#### CALCULATION

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

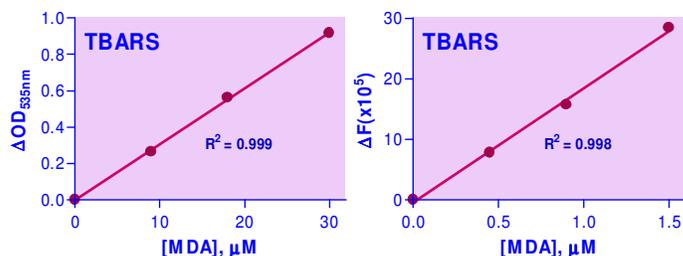
$$[\text{TBARS}] = \frac{R_{\text{Sample}} - R_{\text{Blank}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M MDA equivalents})$$

$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are the  $\text{OD}_{535\text{nm}}$  or fluorescence intensity values of the sample and  $\text{H}_2\text{O}$  blank (standard #4).  $n$  is the sample dilution factor ( $n = 3$  for deproteinated samples).

**Note:** if calculated TBARS concentration is higher than 30  $\mu\text{M}$  MDA (colorimetric assay) or 1.5  $\mu\text{M}$  MDA (fluorometric assay), dilute sample in  $\text{dH}_2\text{O}$  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.



#### PUBLICATIONS

- Paul, S., et al. (2021). D4F prophylaxis enables redox and energy homeostasis while preventing inflammation during hypoxia exposure. *Biomedicine & Pharmacotherapy*. 133: 111083.
- Liang, K., et al. (2020). Effect of storage on n-3 PUFA-enriched eggs. *CyTA - Journal of Food*. 18(1): 102-107.
- Nawab, A., et al. (2019). Effect of dietary curcumin on the antioxidant status of laying hens under high- temperature condition. *Journal of Thermal Biology*. 86: 102449.

