**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 thio-barbituric acid (TBA) to form a pink colored product. The color intensity at 535 nm or fluorescence intensity at (*λ*<sub>ex/em</sub> = 530 nm / 550 nm) 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:** 6 M 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 samples are stable at 25°C or below for at least 3 days. Urine and saliva samples can be assayed directly (*n* = 1). The following samples need to be deproteinized 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 microcentrifuge tube. For cells, harvest 5 × 10⁶ 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 1.5 mL 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 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.

1. **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 μL of the 6 M MDA with 2396 μL dH₂O (final 10 mM MDA). Next, prepare 30 μM MDA by mixing 3 μL of the 10 mM MDA with 997 μL dH₂O. Dilute standards as shown in the Table.

<table>
<thead>
<tr>
<th>No.</th>
<th>30 μM MDA + H₂O</th>
<th>Vol (μL)</th>
<th>MDA (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300 μL + 0 μL</td>
<td>300</td>
<td>30.0</td>
</tr>
<tr>
<td>2</td>
<td>180 μL + 120 μL</td>
<td>300</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>90 μL + 210 μL</td>
<td>300</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>0 μL + 300 μL</td>
<td>300</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Transfer 200 μL of each standard into separate, labeled 1.5-mL screw cap tubes.

**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 535 nm (525 to 545 nm).

**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 dH₂O (final concentrations 0, 0.45, 0.90, 1.50 μM MDA).
2. Samples. In separate tubes, add 200 μL of treated samples.
3. 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.

Load 100 μL in duplicate from each tube to wells of a black flat-bottom 96-well plate. Read fluorescence intensity (*λ*<sub>ex/em</sub> = 530 nm/550 nm) on a plate reader.

**CALCULATION**

Subtract blank OD or fluorescence intensity value (#4) from all standard and sample values. Plot the ∆OD₅₃₅ₙ₅ₓ₅₅₀ or ∆F against standard concentrations and determine the slope of the standard curve. Calculate the TBARS concentration of Sample,

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

Where: *R<sub>Sample</sub>* and *R<sub>Blank</sub>* are the OD₅₃₅ₙ₅₅₀ or fluorescence intensity values of the sample and H₂O blank (standard #4). *n* is the sample dilution factor (*n* = 3 for deproteinated samples).

**PUBLICATIONS**