Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit

Catalog No.: abx097981

Size: 96 tests

Range: 2.6 µmol/L– 100 µmol/L

Sensitivity: 0.85 µmol/L

Storage: Store all components at 4°C

Application: For detection and quantification of Thiobarbituric Acid Reactive Substances (TBARS) concentration in serum, plasma, tissue and other biological fluids.

Introduction

Thiobarbituric Acid Reactive Substances (TBARS) are low-molecular-weight end products (mainly malondialdehyde, MDA) that are formed during the decomposition of lipid peroxidation products. Malondialdehyde is a biomarker of oxidative stress within the cell, which has been associated with development of several human diseases, such as atherosclerosis, cardiovascular diseases, diabetes, liver disorders, and inflammatory rheumatic diseases.

Abbexa's Thiobarbituric Acid Reactive Substances (TBARS) is a quick, convenient, and sensitive method for measuring and calculating TBARS concentration. Malondialdehyde and TBA can react under high temperature and acidic conditions, forming a pink compound. The intensity of the color is proportional to the TBARS content, which can then be calculated by measuring the absorbance at 532 nm.

Kit components

1. 96-well microplate
2. Clarificant: 12 ml
3. Acid Reagent: 12 ml
4. TBA Reagent: 1 vial
5. Standard: 1 vial (200 µmol/L)
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (532 nm)
2. Double distilled water
3. Acetic acid
4. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vials/tubes
7. Centrifuge
8. Water bath
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube in undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 min at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge at 700-1000 x g for 10 mins at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1,500 x g at 4°C for 10 min. Collect the supernatant and assay immediately or store at -80°C for up to 1 month. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Rat Serum	1
10% Mouse Brain Tissue Homogenate	1
Human Plasma	1
Rat Plasma	1
10% Rat Liver Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Clarificant:** Clarificant will solidify when stored at 4 °C. Incubate in a water bath at 37 °C until transparent, use immediately.
- **Acid Reagent working solution:** Add 1.2 ml of Acid reagent to 34 ml of Double Distilled Water. The working solution can then be stored at 4°C for up to 1 day.
- **TBA Reagent working solution:** Dissolve TBA Reagent in 60 ml of double distilled water at 90-100 °C. Mix thoroughly. Add 60 ml of self-prepared acetic acid, mix thoroughly and allow to cool to room temperature. The working solution can then be stored at 4°C in the dark for up to 1 month.
- **Chromogenic Reagent working solution:** Add the Acid Reagent working solution to the TBA Reagent working solution in the following ratio: 3:1 (i.e add 3 ml Acid Reagent working solution and 1 ml of TBA Reagent working solution to prepare 4 ml of Chromogenic Reagent working solution).

B. Assay Procedure

1. Label 8 tubes, and prepare dilutions of the standard (200 µmol/L) using double distilled water: 100, 80, 60, 40, 20, 10, 5 µmol/L. The double distilled water itself serves as the 0 µmol/L (blank) standard.
2. Set sample and standard tubes. We recommend setting each sample and standard in duplicate.
3. Add 100 µl of the prepared standard to the standard tubes.
4. Add 100 µl of each sample to the sample tubes.
5. Add 100 µl of Clarificant to each standard and sample tube.
6. Add 4 ml of Chromogenic Reagent working solution to each standard and sample tube.
7. Incubate the tubes in a water bath at 100 °C for 60 minutes.
8. Cool the tubes with running water, then centrifuge at 1600 x g for 10 minutes.
9. Take 250 µl of the supernatant from each tube and add to the 96-well plate.
10. Measure the OD of each well with a microplate reader at 532 nm.

B. Calculation of Results

The standard curve can be plotted as the absolute OD_{532} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The TBARS concentration of the samples can be interpolated from the standard curve.

1. Serum, plasma and other biological fluids:

$$\text{TBARS}\ (\mu\text{mol/L}) = \frac{\Delta A_{532} - b}{a \times f}$$

2. Tissue samples:

$$\text{TBARS}\ (\mu\text{mol/gprot}) = \frac{\Delta A_{532} - b}{a} \times \frac{f}{C_P}$$

where:

TBARS	Thiobarbituric Acid Reactive Substances concentration
ΔA_{532}	OD value of the sample ($\mathrm{OD}_{\mathrm{Sample}} - \mathrm{OD}_{\mathrm{Blank}}$)
a	slope of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
C_{P}	concentration of protein in sample (gprot/L)