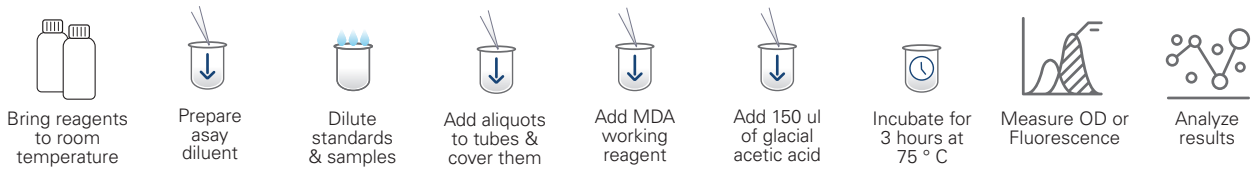
TBARS MALONDIALDEHYDE OXIDATIVE STRESS



DESCRIPTION

TBARS Assay for Oxidative Stress: An assay to measure thiobarbituric acid reactive substances in biological specimens.

Oxidative Stress is a consequence of free radical species mediated alteration of molecules, and is associated with a number of conditions and diseases. It can cause lipid peroxidation which can affect biological membranes by altering structure, permeability and function. Lipid peroxidation is implicated in pathogenesis for a number of disease states: premature birth disorders, diabetes, adult respiratory distress syndrome, shock, Parkinson's Disease, Alzheimer's Disease, chronic inflammatory conditions, and ischemia-reperfusion mediated injury to kidney, heart, brain and intestine.

The breakdown of lipid peroxidation products produces a number of secondary messengers including malondialdehyde (MDA), 4-hydroxynonenal and others. This assay is designed to measure thiobarbituric acid (TBA) reactive substances which include numerous lipid hydroperoxides and lipid aldehydes that produce products similar to those formed by MDA (2). The assay uses MDA as a standard, and results are expressed as MDA equivalents in biological samples.

The TBARS Assay has been tested with serum and urine. Suitability for other samples including plasma, tissue culture supernatant or tissue extracts must be determined by the user.

ASSAY PRINCIPLE

The TBARS Assay is a quantitative assay tool for determining TBA reacting substances in biological specimens. Dilutions of standard (MDA) and samples are reacted with TBA in 50% acetic acid at 75o C for 3 hours. MDA (and similar compounds) produce a colored-fluorescent product that may be measured spectrophotometrically or fluorometrically. A standard curve is generated, and sample lipid peroxidation product concentrations are estimated from that curve.

Kit Contents

1. 1 Microtiter plate that may be used to measure either optical density or determine relative fluorescence.
2. 1 vial of MDA Standard: Supplied at 300 uM MDA.
3. 1 Bottle of Assay Diluent: This must be reconstituted with 100 mL of purified water prior to use.
4. 1 Bottle of TBA Reaction Buffer: This is 50% Acetic Acid.
5. 1 bottle of TBA Reagent: 2.5 mL of this reagent is added to the bottle of TBA Reaction Buffer, and mixed together to make the TBA Working Reagent.

Assay Considerations and Requirements

The reaction details are determined by the equipment complement available in the laboratory.

- Optical Density is measured at 532 nm: Therefore, a UV-Visible Spectrophotometer or microtiter plate reader working at this wavelength is required.

- Relative Fluorescence: A fluorometer or microtiter plate reader run in fluorescence mode capable of excitation at 532 nm emission at 552 nm is required.

Equipment limitations will determine the reaction volume.

Reaction Volume

The assay is run by reacting one volume of sample with 4 volumes of TBA Working Reagent. The kit is supplied with enough reagent to complete the assay in different ways.

It may be run in tubes in 2.5 mL volumes with subsequent determination of optical density or fluorescence using standard cuvettes.

For 2.5 mL volumes, 16 x 120 mm glass screw-cap tubes work well. Suitable tube supports are required. They may be heated in a water bath.

Alternatively, it may be run in 0.5 mL reaction volumes. Microfuge tubes with 1.7 mL capacity work well in this format. Aliquots are then transferred to the microplate for analysis. For this reaction size a dry bath with blocks to accept 1.7 mL microfuge tubes works well.

In either case, the tubes must be tightly capped and heated at 75 ˚ C for three hours.

Pipets

Pipets are required to do the assay, but the size of the reaction volume dictates what the size of the pipets should be. For 2.5 mL reaction volumes, disposable pipets capable of measuring 0.5, 2 and 2.5 mL are required.

For the 0.5 mL reaction volume, micropipettes capable of delivering 100, 200 and 400 uL are required.

Sample Considerations

Serum samples and urine specimens may be run in the assay. They should be clarified prior to analysis, and should be diluted at least 1:4 in Assay Diluent.

Treatment for other sample types may require further processing. The intent is to eliminate turbidity before beginning the assay (or indeed, after the assay has been completed.)

Procedure

Place all reagents on the lab bench and allow them to come to room temperature.

1. Add 100 mL of purified water the Assay Diluent Bottle to reconstitute this buffer. It will be 1X PBS.
2. Prepare all dilutions in Assay Buffer. For standards, complete the serial dilution of MDA in tubes leaving behind the required assay volume (this is addressed further below).
3. Dilute the samples in Assay Diluent as well.

Completing the assay using 2.5 mL Volumes destined for standard cuvettes.

1. Set up a water bath at 75 ˚ C
2. Secure a supply of 16 x 120 mm glass screw cap tubes.
3. Label 11 tubes as follows: C (control), 1-10.
4. Transfer 0.5 mL of Assay Diluent into each tube.
5. Place the C tube in another tube support.
6. Vortex the MDA Standard briefly.
7. Using a 1.0 mL pipet, transfer 0.5 mL of standard to tube 1.
8. Mix by pipetting up and down 5 times.
9. Using the same pipet; transfer 0.5 mL from tube 1 to tube 2 (move tube 1 to the other support with the C tube).
10. Mix as before.
11. Continue to serially dilute the standard through tube 10.(moving tubes to the other support).
12. emove and discard 0.5 mL from the last dilution in tube 10.
13. Now there is a control and standard diluted from 150 uM to 0.293 uM MDA; each tube should have 0.5 mL.
14. Label new tubes appropriately, and dilute samples in a similar manner. It is wise to run a few dilutions of each sample in the assay.
15. Be sure to leave 0.5 mL in each tube.

Prepare the TBA working solution

Be sure that the TBA reagent is in liquid form. If necessary, warm it slightly to achieve solution.

1. Use a 5 mL disposable pipet to transfer 2.5 mL of TBA Reagent to the TBA Reaction Buffer bottle.
2. Replace and secure the cap.
3. Mix the contents of the bottle thoroughly.

Add the TBA Working Solution

1. Using a 5 mL disposable pipet, add 2.0 mL of TBA Working Reagent to each reaction tube.
2. Install and secure the screw-caps.
3. Vortex each tube briefly.

Incubate the tubes at 75 ˚ C

1. Place the support containing the tubes directly into the water bath.
2. Be sure the temperature is at least 75 ˚ C (slightly higher temperature is fine, up to 80 ˚ C).
3. Leave in place for at least 3 hours.
4. Remove from water bath and allow to cool to RT.
5. Vortex each tube briefly.

Measuring the OD: Using standard sized disposable cuvettes:

1. Use a 2 mL pipet to transfer 2.0 mL from C tube to a cuvette.
2. Use a fresh 2 mL pipet, and transfer 2 mL for tube 1 to a second cuvette.
3. Continue preparing cuvettes with the diluted standard and samples.
4. Set up Spectrophotometer for 532 nm.
5. Use a purified water as a reference (in dual beam instruments), and “blank” the spectrophotometer against the “C” tube cuvette.
6. Then determine the ODs for the remaining samples.

Completing the assay using 0.5 mL volumes

Use 1.7 mL microcentrifuge tubes.

1. Set-up dry bath/heating block to 75 ° C.
2. Prepare tubes labeling "C" and 1-10 as above.
3. Transfer 0.1 mL of Assay Diluent into each tube using a pipettor set to 100 uL.
4. Serially dilute the standard as above, but use 100 uL transfers.
5. Leave 100 uL in each tube.
6. Dilute samples again leaving 100 uL in each tube.

Prepare the TBA Working Solution as described above.

Add TBA Working Reagent

1. Add 400 uL of TBA Working Reagent to each tube.
2. Close the caps of the tubes.
3. Vortex briefly.

Place in heating block

1. Incubate at 75 ° C for three hours.
2. Remove tubes and allow to cool to RT.
3. Vortex briefly.

Transfer to microplate

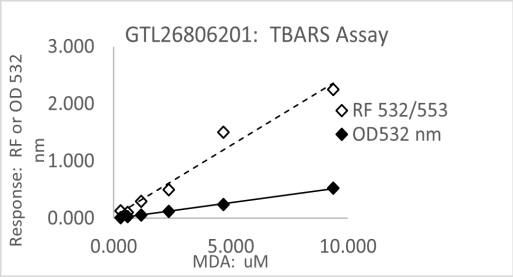
1. Prepare a plate map.
2. Transfer 200 uL of the "C" tube to the "blank" well.
3. Transfer 200 uL aliquots to the plate from the other tubes.

Determine OD or RF using a plate reader.

DATA ANALYSIS

OD (RF) show linear response to concentration within the usable portion of the standard curve.

The data may be analyzed using a linear model, and unknowns may be determined accordingly. The calculations are easily completed with a spreadsheet program such as Excel.



LIMITATIONS

1. Hemolyzed, icteric or grossly lipemic samples may not be suitable for analysis in this assay
2. Turbid samples should be clarified before analysis
3. If turbidity occurs after the incubation; try clarifying by centrifugation before determining the OD or RF.
4. This assay is run in 50% Acetic Acid. The TBA reaction occurs with numerous substances, and efforts to make it specific to MDA include protein removal, attention to pH and extraction of the TBA-MDA adduct. Users should review the references to determine suitability.

REFERENCES

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PRODUCT INFORMATION

Cat. #	Description
1020	TBARS Malondialdehyde Oxidative Stress Assay

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