2-Thiobarbituric Acid Reactive Substances (TBARS)

Catalog Number: 21030
Spectrophotometric Assay (100 tests)
For Research Use Only. Not For Use In Diagnostic Procedures.
Store at 2 to 8°C.

INTRODUCTION

2-**T**hio**B**arbituric **A**cid **R**eactive **S**ubstances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress.[1,2] TBARS assay values are usually reported in malonaldehyde (malondialdehyde, MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides. The TBARS assay is a well-recognized, established method for quantifying these lipid peroxides, although it has been criticized for its reactivity towards other compounds other than MDA.[3] This kit contains modifications from the traditional TBARS assay to reduce background and the generation of TBA reactive substances to give a straightforward, reproducible and consistent method for analyzing biological samples for lipid peroxidation products. This assay can be run colorimetrically at 532 nm or fluorometrically at 585 nm.

PRINCIPLES OF PROCEDURE

This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid *via* a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm.

MATERIALS PROVIDED

Component	Description	Amount	Storage
Indicator	2-Thiobarbituric Acid	4 x 1.5 g	4°C
Acid Reagent	10% Acid Solution in Dimethylsulfoxide	125 mL	4°C
MDA Standard	10 mM Malonaldehyde Tetrabutylammonium Salt	100 µL	4°C

MATERIALS NEEDED BUT NOT PROVIDED

1. Spectrophotometer
2. Glass test tubes
3. Deionized Water (dH_2O)
4. Adjustable micropipettes (10 – 1,000 µL) and tips
5. Spectrophotometric cuvettes (0.5 mL volume)

STORAGE

1. The reagents are stable until the indicated kit expiration date if handled and stored properly.
2. When not in use, store the kit at 4°C for up to one year.

3. MDA standards should be used within 24 hours of preparation.
4. The Indicator Solution (combined 2-TBA and Acid Reagent) can be stored at 4°C for one week.

WARNINGS AND PRECAUTIONS

1. Use aseptic techniques when opening and dispensing reagents.
2. Wear gloves and safety glasses when performing this assay, as the acid used is corrosive.
3. In case of accidental exposure to 2-TBA or Acid Reagent, thoroughly wash the exposed area with soap and water.
4. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. Do not leave the reagent bottles open. Replace the caps as soon as the desired volume is removed.
2. To minimize error due to handling, wipe the exterior of the cuvettes with a lint-free paper towel.
3. **Sample Blanks:** It is HIGHLY recommended that, for each sample analyzed, a sample blank is also performed to account for background interferences. While Oxford's TBARS method has the lowest background of all commercial assays, some background interferences are inherent in biological samples and cannot be avoided. The sample blank should contain the sample plus the Acid Reagent alone without TBA indicator. Following this procedure will result in more accurate, precise and reliable TBARS measurements.
4. There are sufficient reagents for 100 tests.

REAGENT PREPARATION

1. **Acid Reagent:** This solution may need to be thawed upon removal from 4°C. Allow to sit at room temperature for one hour. It is ready to use once thawed.
2. **Indicator Solution:** Add 30 mL of the Acid Reagent to the powdered contents of one bottle of Indicator and shake until completely dissolved. One bottle is sufficient for 25 tests.
3. **20 µM MDA Standard Stock (Colorimetric):** Dilute the 10 mM MDA Standard 1:500 in dH_2O by adding 20 µL of 10 mM MDA to 9.98 mL dH_2O. Prepare immediately prior to use.
4. **20 nM MDA Standard Stock (Fluorometric):** Further dilute the 20 µM MDA Standard Stock solution from above 1:1,000 in dH_2O by adding 10 µL to 9.99 mL dH_2O. Prepare immediately prior to use.

SAMPLE STABILITY

Studies at OBR show that this kit provides optimum results with urine that is less than 48 hours old, and is preferably run immediately after sample collection. If this is not possible, samples should be frozen at -70°C to prevent loss of MDA and HAE_4 and sample oxidation. Samples should **not** be stored at -20°C. Samples should not be refrozen and should be protected from light to avoid photooxidation.

SAMPLE PREPARATION

When working with plasma or serum, the sample should be deproteinated with an acid. Centrifuge and use the supernatant to perform the assay. This solution may appear cloudy after the reaction, and can be clarified by passing through a 0.2 µ syringe filter.

When working with urine, colored compounds contribute to the signal measured at 532 nm. This interference can be removed by running a sample blank with each sample.

Urine

1. Urine samples can be used directly and should be assayed immediately. If the assay is to be performed on a different day, the sample should be stored at -70°C.

Plasma and Serum

1. Collect blood samples and process immediately per the collection tube instructions.
2. Prepare a saturated solution of ammonium sulfate.

3. Add 200 uL of saturated ammonium sulfate to 1.0 mL of serum or plasma in a test tube or microcentrifuge tube.
4. Add 75 mg TCA (trichloroacetic acid) to each sample and vortex. A cloudy precipitate should form.
5. Centrifuge the tubes and transfer the supernatant to a clean tube. Plasma and serum samples can be run without dilution. Samples are now ready to assay.

STANDARD CURVE PREPARATION

Malondialdehyde is provided as a solution of the malondialdehyde tetrabutylammonium (MDA-TBA) salt in a slightly basic buffer because MDA itself is not stable. When mixed with the acidic Indicator Solution, the MDA-TBA molecule is acidified and generates MDA quantitatively. Please see the **Reagent Preparation** section for preparing the 20 µM or 20 nM MDA Standard Stocks.

Table 1: Colorimetric Standard Curve Preparation

Standard	MDA Conc. (µM)	Vol. of dH_2O (µL)	Vol. of 20 µM MDA
S0	0	1000	-
S1	0.5	975	25
S2	1.0	950	50
S3	2.5	875	125
S4	5.0	750	250
S5	10.0	500	500
S6	15.0	250	750
S7	20.0	-	1000

Table 2: Fluorometric Standard Curve Preparation

S0	0	1000	-
S1	0.5	975	25
S2	1.0	950	50
S3	2.5	875	125
S4	5.0	750	250
S5	10.0	500	500
S6	15.0	250	750
S7	20.0	-	1000

ASSAY PROCEDURE

NOTE: The Procedure is the same for both the Colorimetric and Fluorometric Assays.

Free MDA

1. Preparation of Standards and Samples: Add each of the following reagents into glass test tubes and mix well.
- **Standards**: 0.3 mL of standard and 0.3 mL of Indicator Solution.
- **Samples**: 0.3 mL of sample and 0.3 mL of Indicator Solution.
- **Blanks**: 0.3 mL of sample and 0.3 mL of Acid Reagent.

2. After the standards, samples and blanks have been mixed; allow them to react for 45 minutes at room temperature.
3. For **Colorimetric** reading, transfer 0.5 mL of each solution to cuvettes and read at 532 nm. The pink color is stable for several hours at room temperature.
4. For **Fluorometric** reading, transfer 0.5 mL of each solution to cuvettes and excite the sample at

532 nm and read at 585 nm.

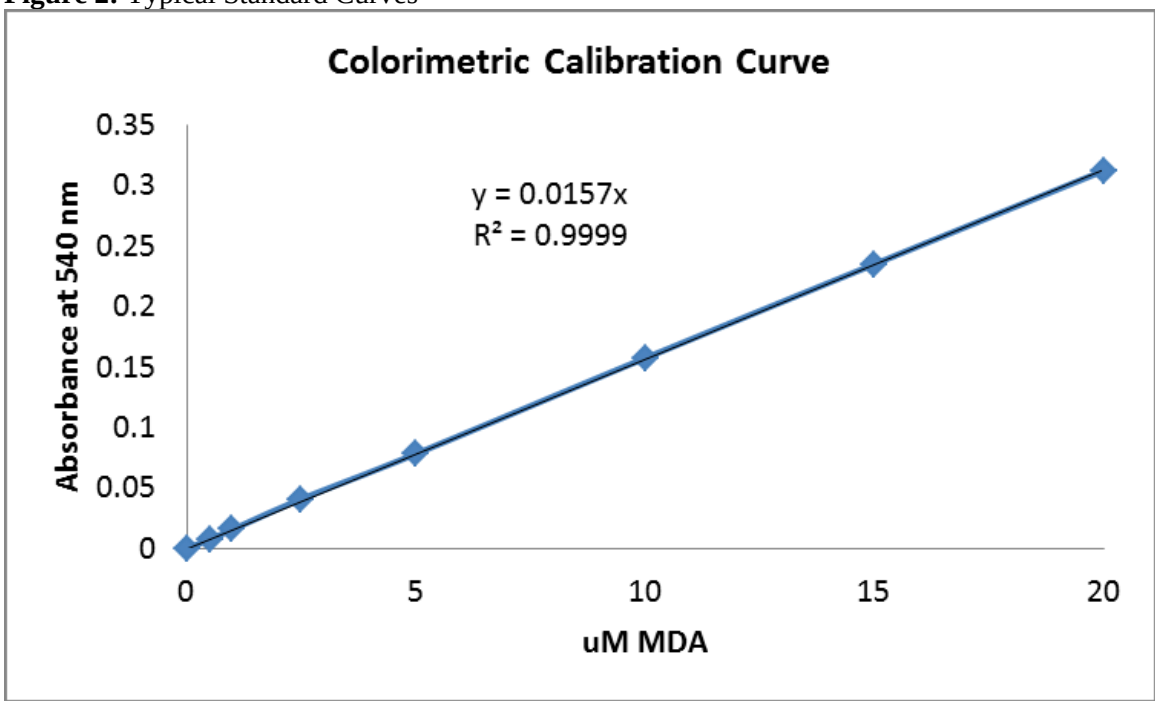
Total MDA

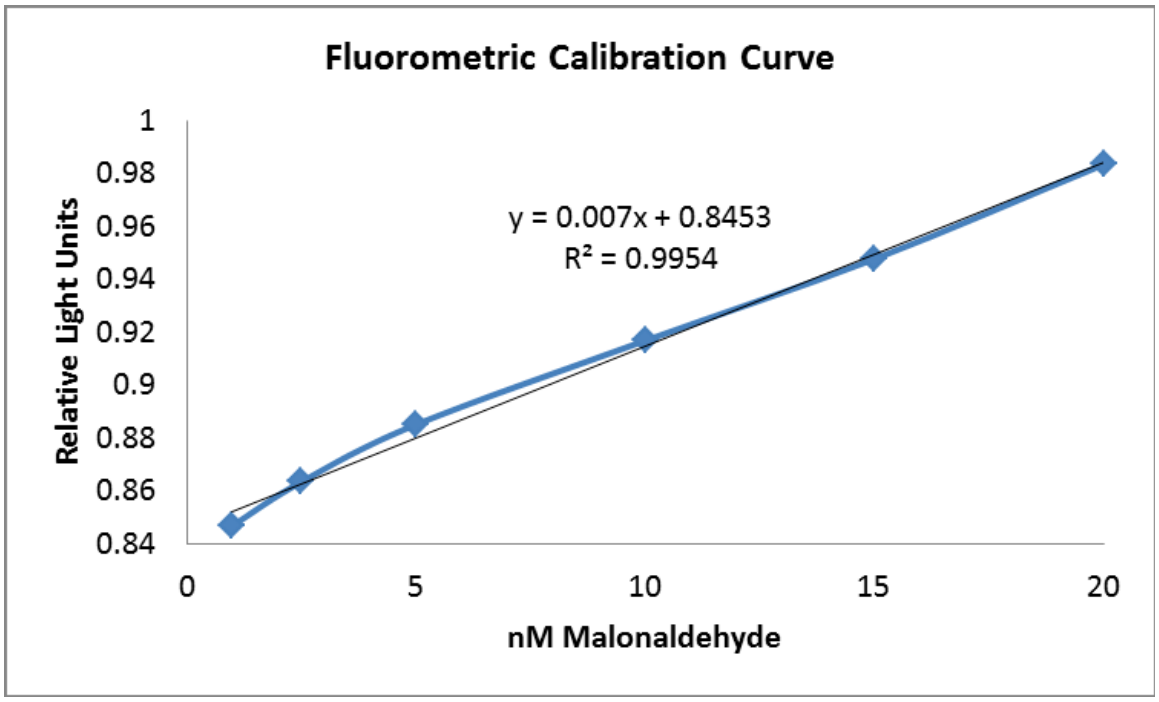
1. Prepare samples and standards exactly as above, but heat sample at 65°C for 45 minutes, then follow step 3 as above.

CALCULATIONS

1. Plot a standard curve using the A_{532} OD value for each Standard versus the MDA concentration for each Standard. The equation of the line can be found using a linear fit method.
2. Subtract the OD (A_{532}) for each Sample Blank from the Sample OD (A_{532}) to obtain a Net OD (A_{532}).
3. Calculate the MDA concentration for each Sample using the Net OD (A_{532}) value and the equation generated by the Standard Curve. If the Samples were diluted, the result must be multiplied by the dilution factor. If you are testing serum or plasma samples and have followed the deproteinization step above, your dilution factor is 1.2.

Figure 2: Typical Standard Curves





LIMITS OF DETECTION

The limits of detection have been determined to be 1.0 µM and 0.5 nM for the colorimetric and fluorometric assays, respectively.

PERFORMANCE LIMITATIONS

1. Although the standards in this assay will usually appear water clear, the samples may become colored. This is due to the formation of additional chromophores that absorb at various wavelengths other than 532 nm and will usually not interfere with the A_{532} signal.
2. In setting up this assay for the first time on a particular biological sample, the kinetics of color development in the samples should be followed in comparison with those of the MDA standards. The A_{532} of the standards should reach a plateau after approximately 15 minutes and then remain. If the A_{532} signal continues to increase after the standards have achieved a stable color, the researcher should be concerned that interfering non-TBARS related reactions are occurring in the sample.

REFERENCES

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