
# TBARS Food and Beverage Assay

Catalog Number: TBR96-k01

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 2 (13 NOV 24)*

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

## INTENDED USE

The Eagle Biosciences TBARS Assay for Food and Beverages has been designed detect malonaldehyde in food and beverage samples. The Eagle Biosciences TBARS Assay for Food and Beverages is for research use only and not to be used in diagnostic procedures.

*For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.*

## INTRODUCTION

Lipid peroxidation and its byproducts are commonly associated with off-flavor development. Fats, oils, and other lipids react with oxygen to form peroxides, which then further decompose to give aldehydes, including malonaldehyde (MDA) and hexanal - both of which are associated with deterioration in meats. Factors affecting the formation of lipid peroxidation products include how the product was stored, cooked, processed, and the addition of preservatives.

## PRINCIPLE OF THE ASSAY

Malondialdehyde is provided as a solution of the malondialdehyde tetrabutylammonium salt in a slightly basic buffer because MDA itself is not stable. When mixed with the acidic Indicator Solution, the MDA salt is acidified and generates MDA quantitatively. The traditional method used for detecting malonaldehyde is the 2-Thiobarbituric Acid Reactive Substances (TBARS) assay. In the reaction, shown below in Figure 1, one molecule of malonaldehyde is condensed with two molecules of 2-thiobarbituric acid under heated acidic conditions to form a pink chromogen. This assay has been modified many times since its discovery in the early 1960's and has always required heating to approximately 100°C for longer than 30 minutes with a strong acid. Heating biological samples in an acidic solution generates colors that interfere with the TBARS assay and generates artificially high MDA values.

The OBR Malonaldehyde Quantitation Kit is the first rapid TBARS assay that does not require heating. This feature makes the test more specific for malonaldehyde by greatly reducing the interferences generated by heating. The reaction is complete in 60 minutes at room temperature, and requires no preliminary extraction or distillation.

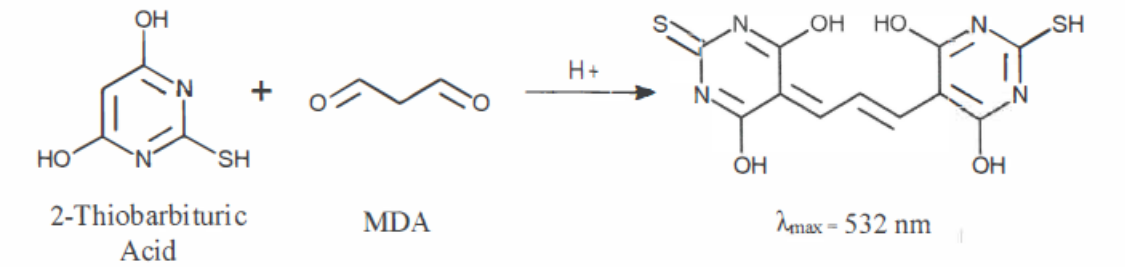



**Figure 1:** Reaction between 2 -thiobarbituric acid and malonaldehyde

## MATERIALS PROVIDED

| Component | Contents | Quantity | Storage |
|---|---|---|---|
| TBA REAGENT | 2-thiobarbituric acid | 2.5g | 4ºC |
| Acid Solution | Proprietary acid catalyst dissolved in DMSO | 2 x 50mL | 4ºC |
| Standard Solution | Malonaldehyde tetrabuty lammonium salt 150mg/L | 2 x 1mL | 4ºC |

This assay is sufficient for assaying 50 samples/standards and 50 sample blanks

## MATERIALS NEEDED BUT NOT PROVIDED

1. A spectrophotometer capable of measuring absorbance at 532 nm
2. An adjustable pipette (150-1,000µL) and disposable tips
3. Doubly-distilled, HPLC grade, or deionized water at room temperature
4. 15 or 25mL centrifuge tubes
5. Centrifuge with speeds up to 15,000 x g
6. Tissue homogenizer
7. Table-top vortex mixer
8. Spectrophotometer cuvettes

## STORAGE CONDITIONS

1. Store the MDA standard in the kit at 4°C upon receiving and until use.
2. The rest of the kit components can be stored at room temperature indefinitely

## WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. 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.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

## PROCEDURAL NOTES

### Interferences

1. Protein: Some references state that protein is an interfering compound in the TBARS assay. MDA reacts with primary amines on amino acids, forming Schiff bases (imines) which can be hydrolyzed to form free MDA during the TBARS assay. This is not classified as an interference, but the total MDA concentration of the sample.
2. Color: The color of a sample can cause significant interference in the TBARS assay. A sample that appears pink, purple or red may absorb in the 530 nm to 540 nm region. If the concentration of malonaldehyde in the sample is low (ca. 500 nM) and the absorbance of the sample at 532 nm is high, then the spectrophotometer could give a very high signal and may not provide accurate data. Diluting the sample with deionized water and back-calculating to the original concentration can overcome this problem, but may dilute MDA concentration to a point where it is too low to be measured. Also, when the Blank Solution is added to a sample, the sample becomes highly acidic and can cause a color change. The only way to ensure that the color of a sample does not interfere with the assay is to run a Sample Blank for each assay performed.
    a. EXAMPLE: A raspberry juice sample was measured at 532 nm to have an absorbance of 0.4 AU. When the assay was run, the Sample Blank gave an absorbance of 0.274 AU at 532 nm, while the sample had an absorbance of 0.278, or an effective MDA concentration of 4.7 µg/L MDA. Without the Sample Blank subtraction, the sample would have given a false reading of 333.0 µg/L MDA. This example illustrates how the color of a sample needs to be subtracted from the assay in order to obtain a true MDA concentration.
3. Reducing Sugars: These compounds contain aldehydes that react with 2-thiobarbituric acid which form adducts with an absorbance in the 450 nm region. Reducing sugars will only interfere with the 532 nm reading if they are in very high concentration (mM range), and include glucose, glyceraldehyde, galactose, lactose and maltose.

**Mixing samples and standards:** It is very important that the standards and samples are mixed with the Indicator Solution at the same time. Do not run the Standard Curve and Samples separately.

## SAMPLE COLLECTION AND PREPARATION

There are many different sample types which can be analyzed using this kit. The scientists at OBR have run analyses on a variety of foods, with a summary shown below. Please note that running samples in triplicate is highly recommended.

**PROTEIN CONTAINING SAMPLES** (meats, fish, nuts)

1. Add 5.0g of the sample to 5.0mL of deionized water in a 15mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.
3. Add enough water to bring the volume to exactly 10mL.
4. The sample has a solids concentration of 500g/L and a dilution factor of TWO.

**OILS AND OILY EMULSIONS** (frying oil, mayonnaise, sauces)

1. Add 1.0mL of the sample to 1.0mL of deionized water in a 15mL centrifuge tube.
2. Since DMSO is not miscible with oils, there is no dilution factor as the MDA is extracted into the water/DMSO layer when the assay is performed.

**SOLID FATS** (shortening, lard, cheese)

1. Add 5.0g of the sample to 5.0mL of deionized water in a 15mL centrifuge tube.
2. Homogenize the sample to a smooth emulsion.
3. Add enough water to bring the volume to exactly 10mL.
4. The sample has a solids concentration of 500 g/L and a dilution factor of TWO.

**DRY INGREDIENTS** (cereals, grains, dehydrated products)

1. Add 5.0g of the sample to 10.0mL of deionized water in a 25mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.
3. Add enough water to bring the volume to exactly 15mL.
4. This sample has a solids concentration of 333.3g/L and a dilution factor of THREE.

**WATER-BASED LIQUIDS** (juices, beverages, extracts, milk)

- These samples are run as-is

**HEMOGLOBIN-CONTAINING SAMPLES** (blood, bone marrow, liver)

1. Add 5.0g of the sample to 10.0mL of deionized water in a 25mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.
3. Add enough water to bring the volume to exactly 15mL.
4. Add 1.0mL of the suspension to 1.0mL of saturated salt water and 1.0mL of concentrated acetic acid; mix well.
5. The homogenized and salt water/acid treated sample has a solids concentration of 111.1 g/L and a dilution factor of NINE.

## REAGENT PREPARATION

Several minutes prior to running the assay, the following solutions should be prepared:

1. **Acid Solution**: Without TBA Reagent added, this is the Blank Solution and arrives ready for use.
    a. Please note that the DMSO solution will solidify slightly below room temperature and should be thawed completely before use.
2. **Indicator Solution:** Add the 2.5g bottle of TBA Reagent to one bottle of Acid Solution and mix well.
    a. This is enough solution to perform fifty tests using 1.0mL of solution for each sample/standard*.
    *If only a few samples are to be analyzed, then 0.5g of TBA Reagent can be added to 10mL of the Acid Solution
    b. This solution is stable for one week at 4°C and for one day at room temperature. The solution should appear near colorless when freshly prepared and will slowly tum yellow as it ages.

3. **150 mg/L MDA Standard:** Dilute 1:10 in deionized water by adding 1.0 mL of the 150.0mg/L MDA Standard Solution to 9.0mL of deionized water to make a 15.0mg/mL working solution. Prepare immediately prior to use.

## STANDARD PREPARATION

Begin set up for the standard curve preparation by labeling dilution tubes and dispensing the indicated volume of deionized water according to Table 1 below. Follow by transferring the indicated amount of Standard also as indicated in Table 1. Be sure to mix well after adding the transfer source to each standard.

**Table 1: Preparation of Standard Curve**

| Standard | MDA Concentration (mg/L) | Deionized water volume (mL) | Transfer Volume (mL) | Transfer Source | Final Volume (mL) |
|---|---|---|---|---|---|
| S7 | 15 | 0 | 2 | 1:10 Stock | 1 |
| S6 | 7.5 | 1 | 1 | S7 | 1 |
| S5 | 3.75 | 1 | 1 | S6 | 1 |
| S4 | 1.875 | 1 | 1 | S5 | 1 |
| S3 | 0.938 | 1 | 1 | S4 | 1 |
| S2 | 0.469 | 1 | 1 | S3 | 1 |
| S1 | 0.234 | 1 | 1 | S2 | 2* |
| S0 | 0 | 1 | --- | --- | 1 |

*Remove 1 mL from S1 prior to adding indicator solution. All standards should be 1mL prior to adding indicator solution.

## ASSAY PROCEDURE

Turn on and set up the spectrophotometer to read absorbances at 532 nm

### Standards:

1. Add 1.0mL of the Indicator Solution to each tube and mix well. Tube S7 should become noticeably pink within one minute of mixing.
2. Allow to react for 60 minutes.
3. Use a cuvette containing only 1mL of deionized water as a blank.
4. Transfer 1.0mL of the solution into a corresponding labeled cuvette and read each at 532 nm.

### Samples

Protein-Containing Samples (meats, fish)

1. *Sample:* Add 1.0mL of the **Indicator Solution** to 1.0mL of the emulsion.
   *Blank:* Add 1.0mL of the **Blank Solution** to 1.0mL of the emulsion.
2. Vigorously agitate the sample for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25°C for 5 minutes.
5. Transfer solution to a cuvette by carefully removing the lower aqueous layer without removing any solids which may skew the data.
6. Measure the absorbance at 532 nm.

Oils and Oily Emulsions (frying oil, mayonnaise, sauces)

1. *Sample:* Add 1.0mL of **Indicator Solution** to the tube containing 1.0mL each of oil and water.
   *Blank:* Add 1.0mL of the **Blank Solution** to the second tube containing 1.0mL each of oil and water.
2. Vigorously agitate the mixture for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25° C for 5 minutes.

5. Transfer solution to a cuvette by carefully removing the lower aqueous layer without removing any oil or getting oil droplets on the side which may skew the data.
6. Measure the absorbance at 532 nm.

Solid Fats (shortening, lard, cheese)

1. *Sample:* Add 1.0mL of the **Indicator Solution** to 1.0mL of the emulsion.
   *Blank:* Add 1.0mL of the **Blank Solution** to 1.0mL of the emulsion.
2. Vigorously agitate the sample for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25ºC for 5 minutes
5. Transfer solution to a cuvette by carefully removing the lower aqueous layer without removing any solids which may skew the data
6. Measure the absorbance at 532 nm.

Dry Ingredients (cereals, grains, dehydrated products)

1. *Sample:* Add 1.0mL of the **Indicator Solution** to 1.0mL of the suspension.
   *Blank:* Add 1.0mL of the **Blank Solution** to 1.0mL of the suspension.
2. Vigorously agitate the sample for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25ºC for 5 minutes
5. Transfer solution to a cuvette by carefully removing the lower aqueous layer without removing any solids which may skew the data.
6. Measure the absorbance at 532 nm.

Water-Based Liquids (juices, beverages, extracts, milk)

1. *Sample:* Add 1.0mL of the **Indicator Solution** to 1.0mL of the sample
   *Blank:* Add 1.0mL of the **Blank Solution** to 1.0mL of the sample
2. Vigorously agitate the mixture for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25ºC for 5 minutes
5. If the sample remains cloudy, filter through a 0.2- or 0.45-micron syringe filter directly into the cuvette.
6. Measure the absorbance at 532 nm.

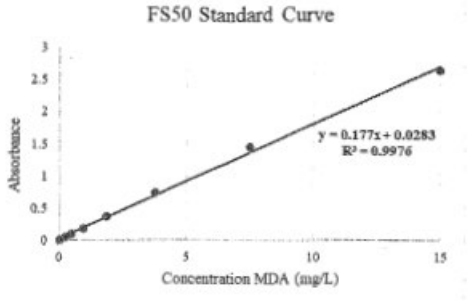
Hemoglobin-Containing Samples (blood, bone marrow, liver)

1. *Sample:* Add 1.0mL of the **Indicator Solution** to 1.0mL of the sample
   *Blank:* Add 1.0mL of the **Blank Solution** to 1.0mL of the sample
2. Vigorously agitate the mixture for one minute with a vortex mixer.
3. Allow the sample to react for 60 minutes at room temperature.
4. Centrifuge the tube at 15,000 x g and 22-25ºC for 5 minutes
5. If the sample remains cloudy, filter through a 0.2- or 0.45-micron syringe filter directly into the cuvette.
6. Measure the absorbance at 532 nm.

## CALCULATIONS

1. Subtract the Sample Blank absorbance from the Sample absorbance to obtain a Net absorbance for the sample. If no Sample Blank was nm, skip this step.
2. Plot a standard curve using the absorbance value for each Standard versus the MDA concentration for each Standard. The equation of the line can be found using a linear fit method shown in Figure 2.
3. Calculate the MDA concentration for each Sample using the Net absorbance value and the equation generated by the Standard Curve.

**Figure 2:** MDA Calibration Curve:




EXAMPLE: Using the equation from the calibration curve shown in Figure 2.
Y = 0.177x
or Absorbance = 0.177x
which gives: x = Absorbance/0.177
Where (x) equals mg/L of MDA
So, a fish sample that gives a net absorbance of 1.5 at 532 nm has a value of 8.475mg/L of MDA.

4. Most samples are run as a homogenate, so the result must be multiplied by the dilution factor specified under the Sample Preparation section. The fish sample was prepared as described under PROTEIN-CONTAING SAMPLES, so this MDA value should be multiplied by a factor of TWO to give a final value of 3.6mg MDA/kg of fish.
5. The value obtained will be in mg MDA per liter of sample. To convert to micromolar (µM) MDA divide the mg/L value by 0.072.

## REFERENCES


1. Morrow, J., et. Hammond, E.G.; White, P.J. A Brief History of Lipid Oxidation. J. Am. Oil Chem. Soc. 2011, 88, 891-897.
2. Papastergiadis, A; Mubiru, E.; Van Langenhove, H; De Meulenaer, B. Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric Thiobarbituric acid reactive substances (TEARS) test in various foods. J. Agric. Food Chem. 2012, 60, 1931-1937.
3. Ingle, J.D.Jr.; Crouch, S.R. Spectrochemical Analysis; Prentice-Hall: New Jersey, 1988; 5-6.


## WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*