TBARS Assay for Food and Beverages

Catalog Number:
TBR96-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 3.1 (08.31.2023)

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.

INTRODUCTION

Off-flavor odor development in lipids and lipid--containing foods is commonly attributed to the by-products of lipid peroxidation. 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[1]. Factors affecting the formation of lipid peroxidation products include how the product was stored, cooked, processed, and the addition of preservatives[2].

The traditional method used for detecting malonaldehyde is the 2-Thiobarbituric Acid Reactive Substances (TBARS) assay. In this 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 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.

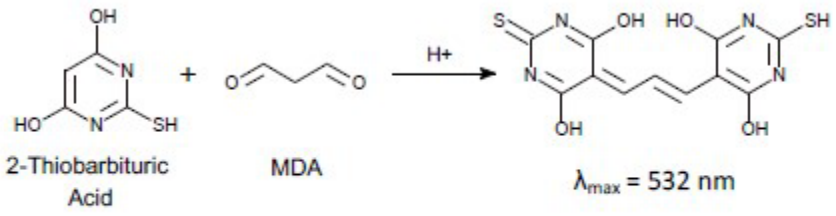

Figure 1: Reaction between 2-thiobarbituric acid and malonaldehyde.

PRECAUTIONS AND STORAGE

Carefully read and understand these instructions before beginning any testing. This kit is for research use only, and is not for diagnostic use or for use in humans.

Wearing appropriate personal protective equipment such as gloves, lab coat and eye protection is highly recommended. Contacting the dimethylsulfoxide (DMSO) acid solutions in this kit may cause irritation and burning. In case of contact with skin or eyes, immediately rinse with plenty of water for 15 minutes and consult a physician.

This kit will perform as specified if the standard is stored at 4˚C until the date printed on the outside of the box.

MATERIALS NEEDED BUT NOT PROVIDED

1. A spectrophotometer capable of measuring absorbance at 532nm
2. A 1.0 mL pipette
3. Doubly-distilled, HPLC grade, or deionized water at room temperature
4. 15 or 25 mL centrifuge tubes
5. Centrifuge with speeds up to 15,000 x g
6. Tissue homogenizer

Optional:

1. Saturated salt water solultion
2. Glacial (concentrated) acetic acid

KIT COMPONENTS

Acidic Solution:	Two bottles containing 50-ML of a proprietary acid catalyst dissolved in dimethylsulfoxide
TBA Reagent:	One bottle containing 2.5 g of 2-thiobarbituric acid
Standard Solution:	Two vials containing 1.0 mL of malonaldehyde tetrabutylammonium salt, 30.0 mg/L MDA in buffer, sufficient for two standard curves.

REAGENT PREPARATION

Except for the 30.0 mg/L MDA standard which should be stored at 4˚C upon receipt, the kit is shipped ready-to-use and can be stored at room temperature. Several minutes prior to running the assay, the following solutions should be prepared:

1. Add the 2.5 g bottle of **TBA reagent** to one bottle of **Acid Solution** and mix well to make the **Indicator Solution*.** This is enough solution to perform fifty tests using 1.0 mL of solution for each sample/standard. 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 turn yellow as it ages. Please note that the DMSO solution will solidify slightly below room temperature and should be thawed completely before use.
2. The **Acid Solution** is ready for use as supplied. Without **TBA Reagent** added, this is the **Blank Solution**.
3. **3.0 mg/L MDA Standard Stock**: Dilute the 30.0 mg/L MDA Standard 1:10 in deionized water by adding 1.0 mL of the 30.0 mg/L MDA **Standard Solution** to 9.0 mL of deionized water. Prepare immediately prior to use.

*If only a few samples are to be analyzed, then 0.5 g of 2-thiobarbituric acid can be added to 10 mL of the **Acid Solution**.

A WORD ON SAMPLE BLANKS

The reason that a Sample Blank is used in this kit can be summarized as follows: "The ideal blank contains all the sample constituents except the analyte. In practice, the blank is treated as identically to the sample as possible"[3].

It is up to the user to decide if a sample blank is necessary. If a sample absorbs at 532 nm before running the TBARS reaction, a sample blank should be run (please read "Interferences" under Technical Notes at the end of this insert). This can usually be determined by simply looking at the sample and seeing if it appears to be red or pink in color. If in doubt, prepare a solution by centrifuging a homogenate of the sample in water and find its absorbance at 532 nm.

Some samples have very little, if any, absorbance at 532 nm, such as cooking oils. It is possible to run the TBARS assay on this type of sample without running a sample blank.

SAMPLE AND STANDARD PREPARATION

SAMPLE PREPARATION

There are many different sample types which can be analyzed using this kit. Scientists 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 5.0 g of the sample to 5.0 mL of deionized water in a 15 mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.
3. Add enough water to bring the volume to exactly 10 mL.
4. The sample has a solids concentration of 500 g/L and a dilution factor of TWO.

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

1. Add 1.0 mL of the sample to 1.0 mL of deionized water in a 15 mL 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 5.0 g of the sample to 5.0 mL of deionized water in a 15 mL centrifuge tube.
2. Homogenize the sample to a smooth emulsion.
3. Add enough water to bring the volume to exactly 10 mL.
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 5.0 g of the sample to 10.0 mL of deionized water in a 25 mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.
3. Add enough water to bring the volume to exactly 15 mL.
4. This sample has a solids concentration of 333.3 g/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 5.0 g of the sample to 10 mL of deionized water in a 25 mL centrifuge tube.
2. Homogenize the sample to a smooth suspension.

3. Add enough water to bring the volume to exactly 15 mL.
4. Add 1.0 mL of the suspension to 1.0 mL of saturated salt water and 1.0 mL 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.

STANDARD CURVE

STANDARD PREPARATION

After dilution, the standard solutions should be used within one day and then discarded, as they are sensitive to oxygen. 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 acidic Indicator Solution, the MDA salt is acidified and generates MDA quantitatively. Table 1, below, shows the standards and their respective concentrations.

Standard	MDA Concentration, mg/L
S_0	0
S_1	0.047
S_2	0.094
S_3	0.188
S_4	0.375
S_5	0.75
S_6	1.5
S_7	3.0

Table 1. Standard Concentrations

Procedure:

1. Label eight test tubes S_0 to S_7.
2. Into tube S_7, add 1.0 mL of the 3.0 mg/L MDA Stock Solution and set aside.
3. Into tubes S_6 thru S_0, add 1.0 mL of deionized water and set tube S_0 aside.
4. Take 1.0 mL of the stock 3.0 mg/L MDA Stock Solution, add it to tube S_6 and mix.
5. Remove 1.0 mL of S_6, add it to tube S_5 and mix.
6. Remove 1.0 mL of S_5, add it to tube S_4 and mix.
7. Remove 1.0 mL of S_4, add it to tube S_3 and mix.
8. Remove 1.0 mL of S_3, add it to tube S_2 and mix.
9. Remove 1.0 mL of S_2, add it to tube S_1 and mix.
10. Remove 1.0 mL of this solution, leaving 1.0 mL in the tube and discard the excess.

RUNNING SAMPLES AND STANDARDS

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.

STANDARDS

1. Add 1.0 mL of the Indicator Solution to each tube and mix well. Tube S_7 should become noticeably pink within one minute of mixing.
2. Allow to react for 60 minutes.
3. Blank the S_0 standard to set it to zero.
4. Transfer enough of the solution (ca. 1.0 mL) into a cuvette and read at 532 nm.

SAMPLES

Protein-Containing Samples (meats, fish)

1. Sample: Add 1.0 mL of the Indicator Solution to 1.0 mL of the emulsion.
   Blank: Add 1.0 mL of the Blank Solution to 1.0 mL 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 × g and 22-25˚C for 5 minutes. Carefully remove the lower aqueous layer being careful not to remove any solids which may skew the data.
5. Transfer solution to a cuvette and measure the absorbance at 532 nm.

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

1. Sample: Add 1.0 mL of Indicator Solution to the tube containing 1.0 mL each of oil and water.
   Blank: Add 1.0 mL of the Blank Solution to the 1.0 mL 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. Carefully remove the lower aqueous layer from beneath the top oily layer and place into a cuvette, taking care not to get oil droplets on the sides of the cuvette which may skew the data.
6. Transfer solution to a cuvette and measure the absorbance at 532 nm.

Solid Fats (shortening, lard, cheeses)

1. Sample: Add 1.0 mL of Indicator Solution to 1.0 mL of the emulsion.
   Blank: Add 1.0 mL of the Blank Solution to 1.0 mL 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. Carefully remove the bottom aqueous layer being careful not to remove any solids which may skew the data.
5. Transfer solution to a cuvette and measure the absorbance at 532 nm.

Dry Ingredients (cereals, grains, dehydrated products)

1. Sample: Add 1.0 mL of Indicator Solution to 1.0 mL of the suspension.
   Blank: Add 1.0 mL of the Blank Solution to 1.0 mL 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 22-25 ˚C for 5 minutes. Carefully remove the top aqueous layer being careful not to remove any solids which may skew the data.
5. Transfer solution to a cuvette and measure the absorbance at 532 nm.

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

1. Sample: Add 1.0 mL of Indicator Solution to 1.0 mL of the sample.
   Blank: Add 1.0 mL of the Blank Solution to 1.0 mL 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 22-25 ˚C for 5 minutes. If the sample remains cloudy, filter through a 0.2 or 0.45 micron syringe filter directly into the cuvette and measure the absorbance at 532 nm.

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

1. Sample: Add 1.0 mL of Indicator Solution to 1.0 mL of the sample.
   Blank: Add 1.0 mL of the Blank Solution to 1.0 mL 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 22-25 ˚C for 5 minutes. If the sample remains cloudy, filter through a 0.2 or 0.45 micron syringe filter directly into the cuvette and measure absorbance at 532 nm.

CALCULATING SAMPLE MDA CONCENTRATIONS

1. Subtract the Sample Blank absorbance from the Sample absorbance to obtain a Net absorbance for the sample. If no Sample Blank was run, 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, below:

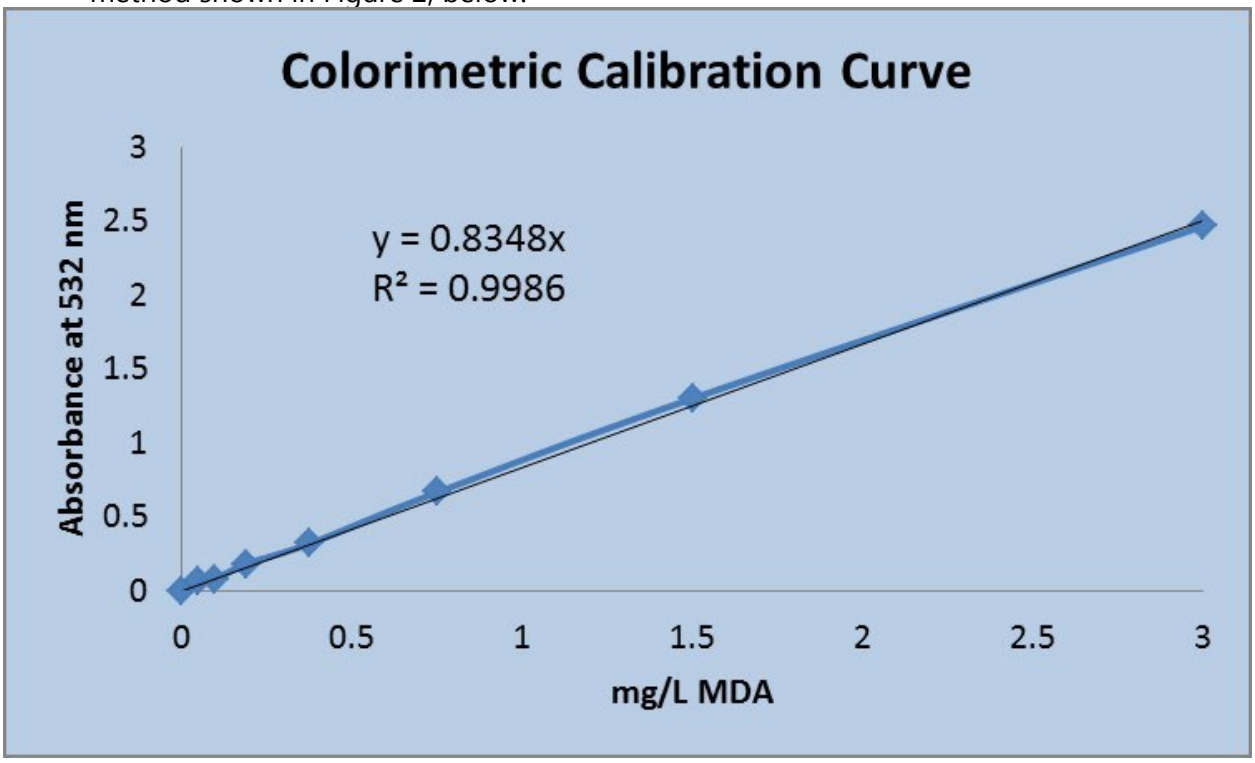

Figure 2: MDA Calibration Curve

3. Calculate the MDA concentration for each Sample using the Net absorbance value and the equation generated by the Standard Curve.

   Example: Using the equation from the calibration curve shown in **Figure 2**.

   Y=0.8348x
   Or Absorbance = 0.8348x
   which gives:
   x = Absorbance/0.8348
   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 1.79 mg/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-CONTAINING SAMPLES so this MDA value should be multiplied by a factor of TWO to give a final value of 3.6 mg 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.

TECHNICAL NOTES

Interferences

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.

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 insure that the color of a sample does not interfere with the assay is to run a Sample Blank for each assay performed.

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.

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.

REFERENCES

1. 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.; VanLangenhove, H.; De Meulenaer, B. Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) 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.