ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

TBARS/MDA Universal
Colorimetric Detection Kit

2 Plate Kit Catalog Number K077-H1

Species Independent

Sample Types Validated:

Serum, Plasma, Tissue, Cell and Food Extracts, Urine, and Buffers

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

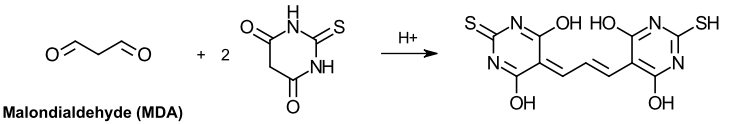
TABLE OF CONTENTS

BACKGROUND

Malondialdehyde (MDA) is a 3-carbon dialdehyde formed by lipid peroxidation of polyunsaturated fatty acids by reactive oxygen species and other oxidative mechanisms[1,2]. Lipid peroxidation is a well-established mechanism of cellular injury in plants and animals and is used as an indicator of oxidative stress in cells and tissues[3,4]. Lipid peroxidation products derived from polyunsaturated fatty acids decompose to form a diverse mixture of compounds including MDA.

Malondialdehyde

MDA is conveniently measured by the reaction of thiobarbituric acid in an acid environment according to the following reaction.



The MDA-TBA adduct formed in this reaction is pink-colored and can be read at ƛ = 532 nm. Measurement between 530-545 nm is acceptable.

In addition to MDA, other reactive aldehydes that react with TBA to generate color can be formed by these oxidative mechanisms, including 2-alkenals and 2,4-alkedienals. The combined total of MDA plus other reactive substances are termed TBARS for Thiobarbituric Acid Reactive Substances. Modifications of the TBARS assay have been used to evaluate several types of samples, including mammalian tissues, serum, plasma and urine along with food samples. There is some ambiguity surrounding the use of TBARS in different sample types under different oxidative stress because of the reactivity of acidified TBA toward reactive aldehydes, however the assay is used extensively to determine lipid peroxidation. In general lipids with greater unsaturation will yield higher TBARS values.

1. Yagi, K. (1998). Simple assay for the level of total lipid peroxides in serum or plasma. *Free Radical and Antioxidant Protocols*, 101–106.
2. Armstrong, D. & Browne, R. (1994). The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Free Radicals in Diagnostic Medicine*, 43–58.
3. Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.
4. Dawn-Linsley, M., et. al. (2005). Monitoring thiobarbituric acid-reactive substances (TBARs) as an assay for oxidative damage in neuronal cultures and central nervous system. *Journal of Neuroscience Methods, 141(2)*, 219–222.

ASSAY PRINCIPLE

The DetectX® TBARS/MDA Universal Colorimetric Detection Kit is designed to quantitatively measure malondialdehyde (MDA) in a variety of samples. Please read the complete kit insert before performing this assay. A MDA standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are diluted in Sample Diluent and mixed with the Substrate. The reaction is incubated at 37°C for one hour. The MDA reacts with the substrate in acid to convert the colorless substrate into a colored product. The pink product is read at 535 nm. Increasing levels of MDA or other reactive aldehydes cause a linear increase in color.

RELATED PRODUCTS

Kits	Catalog No.
Catalase Fluorescent Activity Kit	**K033-F1**
Hydrogen Peroxide Colorimetric Detection Kit	**K034-H1**
Glutathione Fluorescent Detection Kits	**K006-F1/F5**
Superoxide Dismutase (SOD) Activity Kit	**K028-H1**
FRAP™ (Ferric Reducing Antioxidant Power) Detction Kit	**K043-H1**
DNA Damage ELISA Kit	**K059-H1/H5**

Also see: https://www.arborassays.com/products/by-research-area/oxidative-stress/

SUPPLIED COMPONENTS

Clear Half Area 96 well Plates

Corning Costar Plate 3695.

2 Plates — Catalog Number X018-2EA

MDA Standard

1,1,3,3-Tetraethoxypropane at 2,000 µM in propanol. **CAUTION: Volatile liquid; keep capped.**

90 µL — Catalog Number C279-90UL

Sample Diluent

Acidic solution containing dye, detergents and stabilizers. **CAUTION: Acidic solution.**

100 mL — Catalog Number X145-100ML

TBA Substrate

Thiobarbituric acid solution in a special stabilizing buffer. **CAUTION: Basic solution.**

11 mL — Catalog Number C280-11ML

Plate Sealer

2 Each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Concentrated hydrochloric acid for samples. High quality 35–38% concentrated HCl.

Cell lysis buffer for cells and tissues. Suitable buffers are Arbor Assays Cell Lysis Buffer, Catalog Number X050-100ML, or ThermoFisher RIPA Buffer, Catalog Number 89900.

Repeater pipet with disposable tips capable of dispensing 50 µL.

A plate shaker capable of heating to 37°C. Alternatively an orbital shaker at 37°C with shaking speed set to mix contents of the plate.

96 well plate reader capable of reading at 535 nm (Acceptable Range 530-545 nm.). Set plate parameters for a 96-well Corning Costar 3695 plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

Software for converting colorimetric intensity readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

MDA Standard is supplied in propanol. Propanol is flammable and care should be taken to avoid sparks or flames.

Sample Diluent is dilute hydrochloric acid. Care should be taken in use.

TBA Substrate is supplied in dilute base. Care should be taken in use.

SAMPLE TYPES

Samples that need to be stored after collection should be stored at -70°C or lower, preferably after being frozen in liquid nitrogen. This assay has been validated for serum, plasma, urine and cell lysates as well as buffer and media samples. See below for sample specific information.

SAMPLE PREPARATION

All samples must be acidic prior to being added to the plate well. We suggest using high quality concentrated hydrochloric acid (35–38%) such as Sigma-Aldrich, catalog number 320331 or similar. Acidification of the sample allows MDA detection to be measured. Methods for treating each sample type are listed below. The hydrochloric acid acidification of 10 uL concentrated HCl per 200 uL of sample results in a 1.05 dilution.

Serum and Plasma

Collect blood using a serum separator tube or ETDA or heparin plasma tubes. Let sample clot for 30 minutes at room temperature. Centrifuge at 1,000 x g for 15 minutes. Remove serum or plasma, aliquot and freeze at ≤ -70°C if samples are not to be analyzed immediately. Samples that are hemolyzed cannot be used in this kit. Collect fresh samples.

Take 200 µL of serum or plasma in a microfuge tube and add 10 µL of concentrated hydrochloric acid (12.1 M). Vortex and centrifuge at 14,000 rpm for 10 minutes to remove precipitated proteins and debris. Reserve supernatant and dilute ≥ 1:10 with Sample Diluent.

Urine

Collect mid-stream urine samples in a sterile container. Aliquot and freeze at ≤ -70˚C is samples are not to be analyzed immediately.

Take 200 µL of urine in a microfuge tube and add 10 µL of concentrated hydrochloric acid (12.1 M). Vortex and centrifuge at 14,000 rpm for 10 minutes to remove precipitated proteins and debris. Reserve supernatant and dilute ≥ 1:5 with Sample Diluent. Normal urine levels of MDA are approximately 1 µM.

Cell and Tissue Lysates

Cells and tissues should be frozen in liquid nitrogen and stored at -70°C or lower.

Wash cells or tissue by suspension in cold PBS followed by centrifugation at 5,000 rpm in a microcentrifuge tube. Repeat. Take 1 by 106 cells or 25 mg of tissue and add 1 mL of Arbor Assays Cell Lysis Buffer, Catalog Number X050-100ML (sold separately), or Thermo Scientific RIPA Buffer, Catalog Number 89900. Vortex vigorously and freeze at ≤ -70°C for 10 minutes. Thaw and repeat freeze-thaw cycle. For hardy cells, sonication in a sonicator bath for 10 minutes may be needed. Centrifuge at 1,600 x g for 10 minutes at 4°C to remove debris. Use the supernatant immediately or freeze at ≤ -70°C if samples are not to be analyzed immediately.

For every 200 µL of supernatant placed in a microfuge tube, add 10 µL of concentrated hydrochloric acid (12.1 M). Vortex and centrifuge at 14,000 rpm for 10 minutes to remove precipitated proteins and debris. Starting volume of supernatant may need to be adjusted if resulting volume is not sufficient to run replicates in assay.

Food Samples

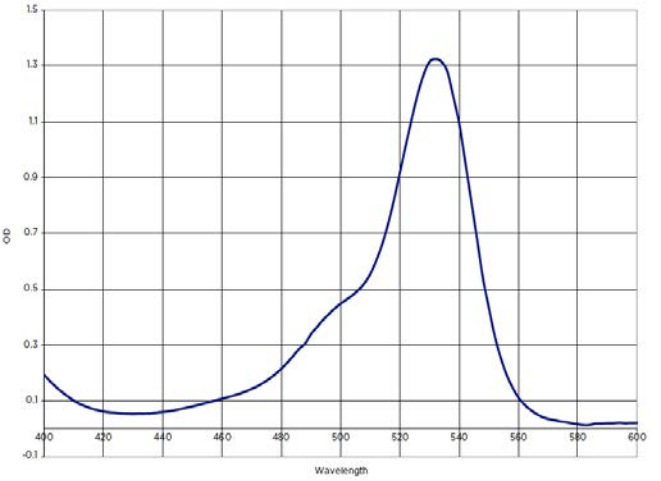
We recommend following the protocols laid out in the web pages of D. J. McClements from the Department of Food Science, University of Massachusetts, at https://people.umass.edu/~mcclemen/581Lipids.html. The sample should either be extracted and /or diluted in the Sample Diluent. Food samples should be stored frozen if they are not to be analyzed immediately.

All measured concentrations will be multiplied by 1.05 for the addition of the concentrated hydrochloric acid, followed by further multiplication for any subsequent dilution with Sample Diluent.

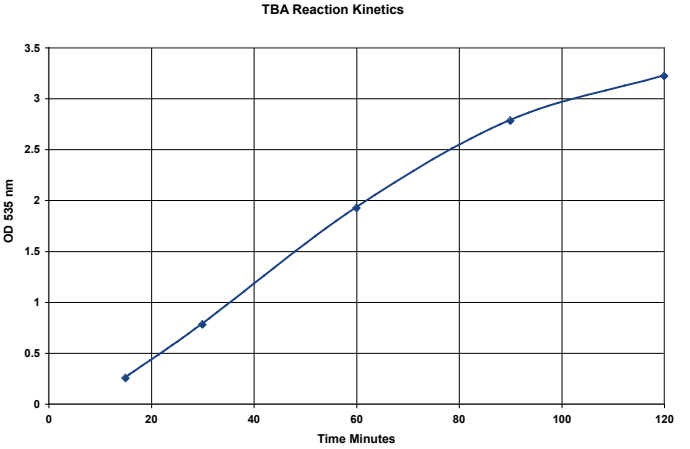
ASSAY CHARACTERISTICS

The Sample Diluent is an acidic solution of hydrochloric acid. The TBA reaction requires all samples to be acidified in our Sample Diluent for the characteristic pink color to be formed in response to MDA and other reactive aldehydes.

The spectra of the pink-colored product is shown to the right. We recommend reading the plate at 535 nm but suitable results can be obtained by reading from 530 to 545 nm.



Kinetically, the reaction is linear from 15 minutes to over an hour. After about 90 minutes the OD still increases but not in a linear fashion. The graph to the right shows the OD generated by a 100 µM standard at 37°C with TBA.

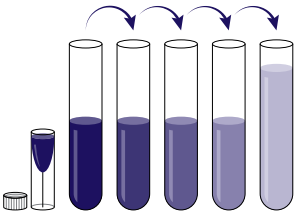


REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Standard Preparation

Malondialdehyde (MDA) Standards are prepared by labeling tubes as #1 through #7. Briefly vortex to mix the vial of MDA stock standard. Pipet 380 µL of Standard Diluent into tube #1 and 200 µL into tubes #2 to #7. Carefully add 20 µL of the Stock Standard to tube #1 and vortex completely. **The MDA stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #7. The concentration of MDA in the tubes will be 100, 50, 25, 12.5, 6.25, 3.125, and 1.563 µM.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Sample Diluent (µL)	**380**	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**20**	200	200	200	200	200	200
Final Conc (µM)	100	50	25	12.5	6.25	3.125	1.563

ASSAY PROTOCOL

We recommend all standards and samples be run in duplicate to allow the end user to accurately determine MDA concentration.

Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

1. Pipet 50 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 50 µL of Standard Diluent into duplicate wells as the Zero standard.
3. Add 50 µL of TBA Substrate to each well using a repeater pipet.
4. Cover the plate with the plate sealer and shake at 37°C for one hour. We recommend shaking at around 700–900 rpm.
   Note: if the assay is not shaken, it will result in ~25% lower signal.
5. Read the plate immediately at 535 nm (Acceptable Range 530-545 nm). The assay does not have an endpoint.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

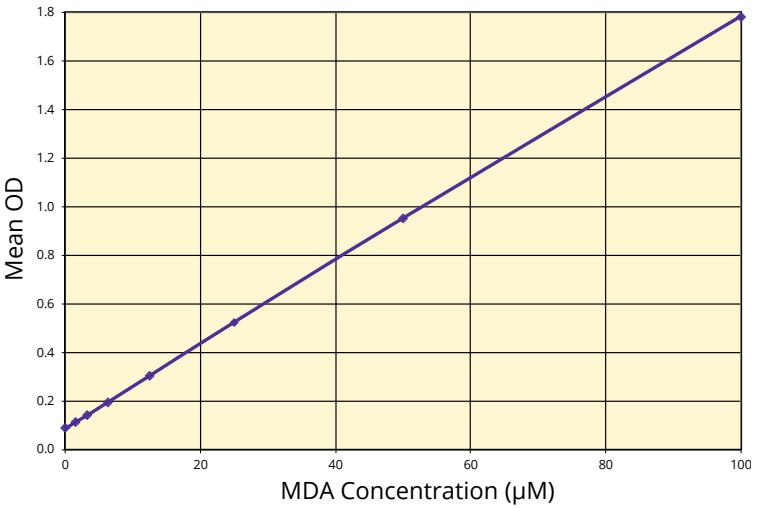
https://www.myassays.com/arbor-assays-detectx-tbars-mda-universal-colorimetric-detection-kit-k077.assay

TYPICAL DATA

Sample	Mean OD	TBARS/MDA Conc. (µM)
Zero	0.087	0
Standard 1	1.783	100
Standard 2	0.953	50
Standard 3	0.525	25
Standard 4	0.305	12.5
Standard 5	0.195	6.25
Standard 6	0.140	3.125
Standard 7	0.115	1.563
Sample 1	0.537	25.79
Sample 2	0.939	49.14

Always run your own standard curves for calculation of results. Do not use this data.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

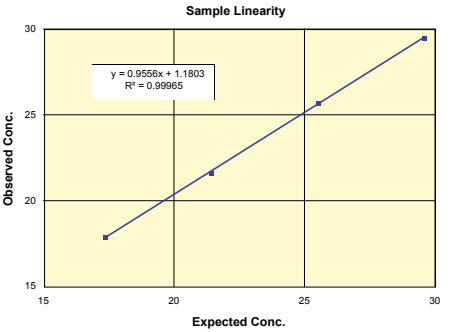
Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.36 µM.**

The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration sample. **The Limit of Detection was determined as 0.620 µM.**

Linearity

Linearity was determined by taking two rat serum samples with known MDA concentrations and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High	Low	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
80%	20%	29.62	29.46	99.5
60%	40%	25.55	25.67	100.5
40%	60%	21.47	21.56	100.4
20%	80%	17.40	17.84	102.5
			Mean Recovery	**100.7%**



Intra Assay Precision

Three samples were run in replicates of 16 in an assay. The mean and precision of the calculated concentrations were:

Sample	MDA Conc. (µM)	%CV
1	23.5	12.9
2	48.9	3.5
3	67.1	12.7

Inter Assay Precision

Three samples were run in duplicate in 20 assays run over multiple days by several operators. The mean and precision of the calculated concentrations were:

Sample	MDA Conc. (µM)	%CV
1	23.4	13.8
2	48.3	4.5
3	68.3	11.6

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

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ASSAYS™
FOR SERIOUS SCIENCE

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