

# Data sheet

## TBARS Assay kit (Colorimetric/Fluorometric)

Cat. No: CA995

### Introduction

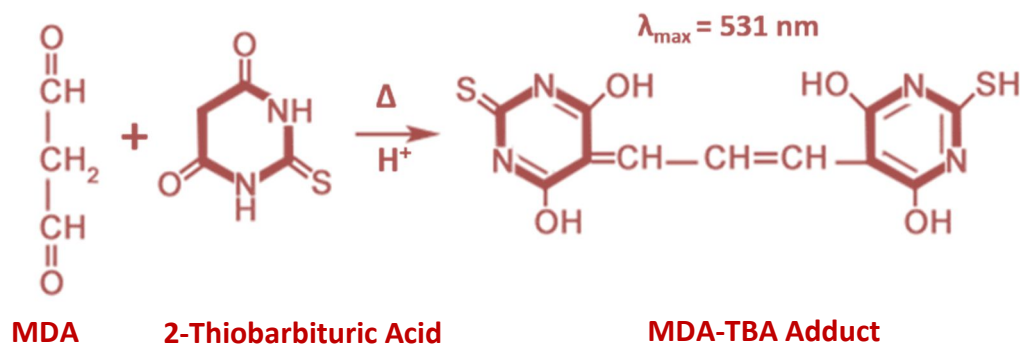
Oxidative stress in the cellular environment results in the formation of highly reactive and unstable lipid hydroperoxides. Decomposition of the unstable peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehyde (MDA), which can be quantified colorimetrically following its controlled reaction with thiobarbituric acid. Thiobarbituric Acid Reactive Substances (TBARS) assay was proposed over 40 years ago and is now the most commonly used of method to screening and monitoring lipid oxidation. TBARS method has been used to evaluate a wide range of samples that include human and animal tissues and fluids, drugs, foods and natural products. The sensitivity of measuring Thiobarbituric Acid Reactive Substances (TBARS) has made this assay the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. Even though there remains a controversy cited in literature regarding the specificity of TBARS toward compounds other than MDA, it still remains the most widely employed assay used to determine lipid peroxidation.

This assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); TBA reacts with MDA to form a pink chromogen, which can be detected spectrophotometrically at 532 nm.

### Kit Contents

	Amount
Thiobarbituric Acid (TBA)	4 x 0.53 g
Diluent 1 (contain acetic acid)	40 ml
Diluent 2 (contain NaOH)	20 ml
SDS Solution	10 ml
MDA Standard (10mM)	250 µl
H <sub>2</sub> O <sub>2</sub> solution	20 ml
96-Well Solid Black plate	1
96-Well Solid Clear plate	1
Adhesive strips	2

 Store unopened kit at 2-8 °C.



Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)

*(Continued on reverse side)*

  
the product.

## Reagent Preparation

- ✓ **Thiobarbituric Acid:** Each vial contains 530 mg of thiobarbituric acid (TBA). It is ready to use to prepare the Color Reagent.
- ✓ **Diluent 1:** The bottle contains 40 mL of concentrated acetic acid. Dilute 40 mL of **Diluent 1** with 160 mL of HPLC-grade water. This solution is used in preparing the Color Reagent and is stable for at least three months at room temperature.
- ✓ **Diluent 2:** The bottle contains a solution of sodium hydroxide (20 mL). Dilute 20 mL of **Diluent 2** with 180 mL of HPLC-grade water. This diluted NaOH solution is used in preparing the Color Reagent. The diluted NaOH solution is stable for at least three months at room temperature. Store the diluted NaOH solution in a plastic container suitable for corrosive materials.
- ✓ **Malondialdehyde Standard:** The vial contains 10 mM Malondialdehyde (MDA) in water. It is ready to use to prepare the standard curve. Store -20°C.
- ✓ **SDS Solution:** The bottle contains a solution of sodium dodecyl sulfate (SDS). The solution is ready to use as supplied.
- ✓ **H<sub>2</sub>O<sub>2</sub>:** The bottle contains Hydrogen peroxide solution 30 % (w/w) in H<sub>2</sub>O. The solution is ready to use as supplied.

### To prepare the **Color Reagent:**

The following amount of Color Reagent is sufficient to evaluate 24 samples. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 530 mg of TBA and add to ≥150 mL beaker containing 50 mL of diluted **Diluent 1**. Add 50 mL of diluted **Diluent 2** and mix until the TBA is completely dissolved. The solution is stable for 24 hours.

## Sample collection & Storage

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately.

*Typically, normal human serum has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 μM.*

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately.

*Typically, normal human plasma has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 μM.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately.

*Typically, normal human urine has a lipid peroxide level (expressed in terms of MDA) of 0.8-2 μmol/g creatinine.*

**Tissue Homogenates** - Weigh out approximately 25 mg of tissue into a 1.5 mL centrifuge tube. Add 250 μL of RIPA Buffer containing protease inhibitors. Homogenize or sonicate the tissue on ice. Centrifuge the tube at 1,600 x g for 10 minutes at 4°C. Use the supernatant for analysis. Tissue homogenates do not need to be diluted before assaying.

**Cell Lysates** - Collect 2 x 10<sup>7</sup> cells in 1 mL of cell culture medium or PBS. Homogenize or sonicate the cells on ice. Use the whole homogenate in the assay, being sure to use the culture medium as a sample blank. Cell lysates do not need to be diluted before assaying.



Samples should be tested immediately. If samples are not tested immediately aliquot and store at -70°C.

Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)



the product.

## Standard curve

### Standard preparation

Malondialdehyde is provided as a solution of the malondialdehyde tetrabutylammonium (MDA-TBA) salt. When mixed with the Color Reagent, the MDA-TBA molecule is acidified and generates MDA quantitatively.

1. 125 $\mu$ M MDA Standard Stock (**Colorimetric**): Add 125  $\mu$ L of 10 mM MDA to 9.87 mL dH<sub>2</sub>O. Prepare immediately prior to use.
2. 12.5  $\mu$ M MDA Standard Stock (**Fluorometric**): Add 12.5  $\mu$ L of 10 mM MDA to 9.98 mL dH<sub>2</sub>O. Prepare immediately prior to use.

**Table 1: Colorimetric Standard Curve Preparation**

Tube	MDA Stock( $\mu$ l)	Water( $\mu$ l)	MDA concentration( $\mu$ M)
A	0	1000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

**Table 2: Fluorometric Standard Curve Preparation**

Tube	MDA Stock ( $\mu$ l)	Water( $\mu$ l)	MDA concentration( $\mu$ M)
A	0	1000	0
B	5	995	0.0625
C	10	990	0.125
D	20	980	0.25
E	40	960	0.5
F	80	920	1
G	200	800	2.5
H	400	600	5

Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)

## Assay Procedure

- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The final volume of the assay is 150  $\mu$ L in all wells.
- The assay is performed at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples and standards be assayed at least in duplicate.
- It is recommended that the samples and standards be kept at 4°C after preparation to increase sensitivity and reproducibility.
- Monitor the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm.

1. Label vial caps with standard number or sample identification number.
2. Add 100  $\mu$ L of sample or standard to appropriately labeled 5 mL vial.
3. Add 100  $\mu$ L of **SDS solution** to vial and swirl to mix.
4. Add 4 mL of the **Color Reagent** forcefully down side of each vial.
5. Cap vials and place vials in foam or some other holder to keep the tubes upright during boiling.
6. Add vials to vigorously boiling water. Boil vials for one hour.
7. After one hour, immediately remove the vials and place in ice bath to stop reaction. Incubate on ice for 10 minutes.
8. After 10 minutes, centrifuge the vials for 10 minutes at 1,600 x g at 4°C. Vials may appear clear or cloudy. Cloudiness will clear upon warming to room temperature. Vials are stable at room temperature for 30 minutes.
9. Load 150  $\mu$ L (in duplicate) from each vial to either the clear plate (colorimetric version) or to the black plate (fluorometric version).
10. Read the **absorbance at 530-540 nm** or read **fluorescence** at an excitation wavelength of **530 nm** and an emission wavelength of **550 nm**.

## Data Analysis

### Calculations (Colorimetric/fluorometric):

1. Calculate the average absorbance (or fluorescence) of each standard and sample.
2. Subtract the absorbance value (or fluorescence value) of the standard A (0  $\mu$ M) from itself and all other values (both standards and samples). This is the corrected absorbance (or fluorescence).
3. Plot the corrected absorbance values (or fluorescence value) (from step 2 above) of each standard as a function of MDA concentration.
4. Calculate the values of MDA for each sample from the standard curve.

Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)



the product.

$$\text{MDA}(\mu\text{M}) = \left[ \frac{(\text{Corrected absorbance/fluorescence}) - (\text{y-intercept})}{\text{Slope}} \right]$$

Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)

Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)