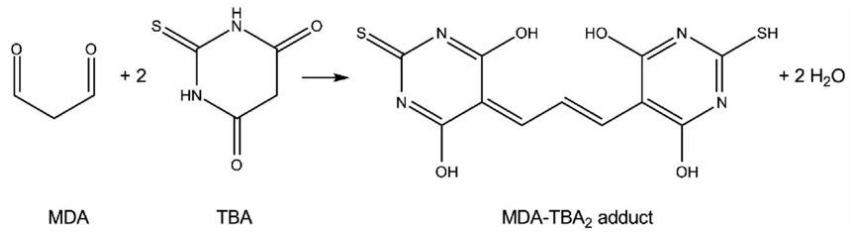
About This Kit

TPR's MDA assay Kit is a well-established tool for screening MDA as an end product of lipid peroxidation. MDA present in samples reacts with Thiobarbituric Acid (TBA) and generates a MDA-TBA adduct, which can simply quantified colorimetrically (530-540 nm) or fluorometrically (Ex/Em = 532/553 nm). This kit can be used for all biological samples e.g. **plasma, serum, urine, tissue homogenates, and cell lysates.**



Kit Component

Item Label	Item	48 Tests Kit	96 Tests Kit
Reagent 1 **(R1)**	Thiobarbituric Acid	500 mg	1000 mg
Reagent 2 (**R2**)	HOAC **(**5X**)**	10 mL	20 ml
Reagent 3 (**R3**)	Alkali **(**10X**)**	5 ml	10 ml
Reagent 4 (**R4**)	Detergent	6 ml	12 ml
Reagent 5 (**R5**)	Standard (500 µM)	0.5 ml	1 ml
Reagent 6 (**R6**)	BHT (100X)	0.5 ml	0.5 ml
-	96 Well Microplate	1 Plate	1 Plate
-	Instruction Mannual	1 Mannual	1 Mannual

Materials Required (Not Provided)

- Distilled water or HPLC-grade water.
- 1.5 ml screw-cap centrifuge tubes.
- Pipettes and tips

Required Instrumentation

- Multiwell micro-plate reader (capable of measuring absorbance between 530-540 nm or measureing fluorescence using an excitation wavelength of 530 nm and an emission wavelength of 550 nm.)
- Vortex mixer
- Heater device

Kit Performance

- **Range:** Under the standardized conditions of the assay described in this manual, the dynamic range of the kit is 0-50 µM.
- **Precision:** Human serum sample with replication No.10 showed the intra and inter assay coefficient of variation 6.7% and 7.2% respectively.

Storage and Stability

- This kit will perform as specified if **stored at 4°C**
- Use before the **expiration date**

Warning and precaution

- Thiobarbituric Acid has a strong mercaptan odor. Do not breathe dust. Avoid contact with skin and eyes.
- HOAC is corrosive and can cause burns. In case of contact with eyes, rinse immediately with copious amounts of water and seek medical advice. Wear suitable protective clothing.
- BHT is harmful if swallowed and irritating to the eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with copious amounts of water and seek medical advice. Wear protective clothing.

Sample Preparation

The multi-disciplinary interest in measuring MDA has resulted in a myriad sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail. However, general guidelines are provided below for representative sample types.

Important Note: All samples should be assayed immediately upon collection or stored at -80°C for up to 1-2 months.

PLASMA

1. Collect 5 ml of whole blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 3000 x g for 10 minutes at 4°C. Carefully pipette off the plasma layer.
3. Add 10 µl of R6 to 1 ml of plasma and store on ice.

SERUM

1. Collect 7 ml of whole blood without using an anticoagulant.
2. Allow blood to clot for 10 minutes at 25°C.
3. Centrifuge the blood at 3000 x g for 10 minutes at 4°C and pipette off the serum.
4. Add 10 µl of R6 to 1 ml of serum and store on ice.

Note: It is important to minimize hemolysis in Plasma/serum samples.

URINE

Urine does not require any special treatments. Samples must be clarified by centrifugation prior to assay.

TISSUE HOMOGENATES

1. Weigh out approximately 100 mg of wet tissue into a 2 ml centrifuge tube.
2. Add 0.9 ml of ice cooled 150 mM KC1 containing 10 µl of R6.
3. Homogenize using a glass or Teflon Potter-Elvehjem homogenizer on ice.
4. Sonicate for 15 sec. at 40 V setting.
5. Centrifuge the tube at 1600 x g for 10 minutes at 4°C. Use the supernatant for analysis.

Note: Hemoglobin interferes with the assay; therefore, if practical, blood should be removed by perfusion with an appropriate buffer.

Note: The MDA concentration shall be normalized to the protein content of the homogenate and reported as µM MDA/mg protein.

1. Collect 1 x 10^6 cells in 1 ml of cell culture medium or buffer of choice containing 10 µl of R6, such as PBS.
2. Homogenize or sonicate the cells on ice.
3. Use the whole homogenate directly in the assay, being sure to use the culture medium as a sample blank.

Reagent Preparation

1) **R2 ready reagent**: Add 40 ml distilled water to 10 ml R2 (48 Tests Kit)/Add 80 ml distilled water to 20 ml R2 (96 Tests Kit). The ready R2 reagent is stable for at least three months at room temperature.

2) **R3 ready reagent**: Add 45 ml distilled water to 5 ml R3 (48 Tests Kit)/Add 90 ml distilled water to 10 ml R3 (96 Tests Kit). The ready R3 reagent is stable for at least three months at room temperature. Store Ready R3 solution in a plastic container suitable for corrosive materials.

3) **Chromogenic reagent**: Mix 500 mg of R1 with 50 ml R2 ready reagent and 50 ml R3 ready reagent (48 Tests Kit)/ Mix 1000 mg of R1 with 100 ml R2 ready reagent and 100 ml R3 ready reagent (96 Tests Kit). Warm and swirl slowly until powder dissolves completely. This reagent is stable for 24 hours (**Do not refrigerate**). Chromogenic solution can be prepared as required with the same proportion; for example 250 mg R1 + 25 ml ready R2 and 25 ml ready R3.

4) **R6 (100X)**: Ready to Use. In lower sample volumes, R6 reagent can be used with the same proportion; for examples 5 µl of R6 with 500 µl.

5) **Standard Preparation:**

- **Colorimetric Standard Preparation**

Dilute 250 µl of Standard (**R5**) with 750 µl of water to obtain a MDA stock solution of 125 µM. Take eight clean microtubes and label them A-H. Add the amount of MDA stock solution and water to each tube as described below.

Tube	MDA stock solution (µl)	Water (µl)	MDA Concentration (µM)
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

- **Fluorometric Standard Preparation**

Dilute 25 µl of the Standard (**R5**) with 975 µl of water to obtain a MDA stock solution of 12.5 µM. Take eight clean microtubes and label them A-H. Add the amount of MDA stock solution and water to each tube as described on next page.

Tube	MDA stock solution (µl)	Water (µl)	MDA Concentration (µM)
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	1.0
G	200	800	2.5
H	400	600	5.0

Things to Note

- Allow all reagents to equilibrate to room temperature before performing the assay.
- The R4 reagent will take at least 1 hour to equilibrate to room temperature if stored at 2-8°C. If R4 is cloudy, briefly heat at 37°C to become a clear solution.
- It is recommended that all samples and standards be assayed at least in duplicate.
- Regulation of tube temperature at 4°C increases sensitivity and reproducibility.

Assay Protocol

- Shake all samples for homogenation.
- Label all test tubes.
- Add 100 µl standards/samples to related tubes.
- Add 100 µl of R4 to all tubes, swirl to mix and, Incubate for 5 minutes.
- Add 200 µl Ready Chromogenic Solution to all tubes.
- Cap tubes and place in foam or some other holder to keep the tubes upright during boiling.
- Place tubes on vigorously boiling water for 1 hour.
- After one hour, immediately place tubes on ice bath for 10 minutes to stop reactions.
- Centrifuge tubes at 10,000 x g for 10 minutes at 4°C.
- Pipette 200 µl of supernatant into the microplate.
- If supernatants are cloudy, briefly incubate at 37°C to become clear.
- Read the absorbance at 530-540 nm or read fluorescence at (Ex/Em 530/550 nm).

Colorimetric/Fluorometric Calculation

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

$$\text{MDA (µM)} = \frac{(Corrected\ absorbance) - (\text{y-intercept})}{Slope}$$