

## ab118970 Lipid Peroxidation (MDA) Assay kit (Colorimetric/Fluorometric)

For the measurement of Lipid Peroxidation in plasma, cell culture and tissue extracts.

For research use only – not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:

[www.abcam.com/ab118970](http://www.abcam.com/ab118970)

(use [abcam.cn/ab118970](http://abcam.cn/ab118970) for China, or [abcam.co.jp/ab118970](http://abcam.co.jp/ab118970) for Japan)

### Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
MDA Lysis Buffer	25 mL	-20°C	-20°C
Phosphotungstic Acid Solution	12.5 mL	-20°C	-20°C
BHT Stock/BHT (100X)	1 mL	-20°C	-20°C
Developer VII/TBA Solution	4 vials	-20°C	+4°C
MDA Standard/MDA Standard (4.17M)	100 µL	-20°C	-20°C

### Storage and Stability:

Store kit at -20°C in the dark immediately on receipt. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Reconstituted Developer VII/TBA is stable for 1 week. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

### Materials Required, Not Supplied:

- Microplate reader capable of measuring absorbance at OD532 nm or fluorescence at Ex/Em = 532/553 nm
- 96-well full clear plate (colorimetric assay) and black (fluorometric assay) flat-bottom clear plates.
- Dounce homogenizer (if using tissue)
- Glacial Acetic Acid
- 42 mM H<sub>2</sub>SO<sub>4</sub> – for plasma and serum sample preparation
- Sonicator (Optional) – to help solubilize Developer VII/TBA solution
- n-Butanol (Optional) – to increase assay sensitivity
- 5M NaCl (Optional) – to increase assay sensitivity
- 2N perchloric acid (Optional) – for alternative cell/tissue preparation protocol

### Reagent Preparation:

Briefly centrifuge small vials at low speed prior to opening.

**1. MDA Lysis Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Aliquot buffer so that you have enough to perform the desired number of assays.

**2. Phosphotungstic Acid Solution:** Ready to use as supplied. Equilibrate to room temperature before use.

**3. BHT Stock/BHT (100X):** Ready to use as supplied. Equilibrate to room temperature before use. Aliquot solution so that you have enough to perform the desired number of assays.

**4. Developer VII/TBA Solution:** Reconstitute one vial of Developer VII/TBA (250 mg) in 7.5 mL Glacial Acetic Acid (use only Glacial acetic acid, as regular acetic acid affects Developer VII/TBA stability due to its high-water content). Transfer slurry to another tube and then adjust the final volume to 25 mL with ddH<sub>2</sub>O. Mix well to dissolve. Sonicate in a RT water bath if required. Reconstituted solution is stable for 1 week at +4°C or -20°C. We do not recommend aliquoting as it may result in inconsistencies.

**Δ Note:** In case of precipitate formation, sonicate in a water bath (RT). Alternatively, Developer VII/TBA can also be dissolved in 1.5 mL of 50% glacial acetic acid in water, then making up volume to 25 mL with ddH<sub>2</sub>O (use molecular grade deionized distilled water). Mix well. If there are still precipitates, use 600 µL as directed (Generation of MDA-Developer VII/TBA adduct, step 1.1), any particle in suspension will dissolve at 95°C when incubated for 60 minutes.

### 5. MDA Standard/MDA Standard (4.17M):

Ready to use as supplied. Aliquot standard so that you have enough to perform the desired number of assays.

### Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

### For colorimetric assay:

1. Prepare a 0.1 M MDA standard by diluting 10 µL 4.17 M MDA Standard in 407 µL of ddH<sub>2</sub>O.
2. Prepare a 2 mM MDA standard by diluting 10 µL 0.1 M MDA Standard in 490 µL of ddH<sub>2</sub>O.
3. Using 2 mM MDA standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of MDA Standard (µL)	Volume ddH <sub>2</sub> O (µL)	Final volume standard in well (µL)	Amount of MDA standard in well (nmol/well)
1	0	600	200	0
2	6	594	200	4 (20 µM)
3	12	588	200	8 (40 µM)
4	18	582	200	12 (60 µM)
5	24	576	200	16 (80 µM)
6	30	570	200	20 (100 µM)

Each dilution has enough standard to set up duplicate readings (2 x 200 µL).

### For fluorometric assay:

1. Prepare a 0.1 M MDA standard by diluting 10 µL 4.17M MDA Standard in 407 µL of ddH<sub>2</sub>O.
2. Prepare a 2 mM MDA standard by diluting 10 µL 0.1 M MDA Standard in 490 µL of ddH<sub>2</sub>O.
3. Prepare a 0.2 mM standard by diluting 10 µL 2 mM Standard in 90 µL of ddH<sub>2</sub>O.
4. Using 0.2 mM MDA Standard, prepare standard curve dilutions as described in the table in a microplate or microcentrifuge tubes.

Standard #	Volume of	Volume	Final volume	Amount of MDA
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	MDA Standard (μL)	ddH <sub>2</sub> O (μL)	standard in well (μL)	standard in well (nmol/well)
1	0	600	200	0
2	6	594	200	0.4 (2 μM)
3	12	588	200	0.8 (4 μM)
4	18	582	200	1.2 (6 μM)
5	24	576	200	1.6 (8 μM)
6	30	570	200	2.0 (10 μM)

Each dilution has enough standard to set up duplicate readings (2 x 200 μL).

### Sample Preparation:

#### **Cell (adherent or suspension) samples:**

1. Harvest the number of cells needed for each assay (initial recommendation = 2x10<sup>6</sup> cells).
2. Wash cells with cold PBS.
3. Prepare Lysis Solution: Mix 300 μL of MDA Lysis Buffer with 3 μL BHT Stock/BHT (100X). BHT stops further sample peroxidation while processing.
4. Homogenize cells in 303 μL Lysis Solution (Buffer + BHT) using a Dounce homogenizer (10-50 passes) on ice. The DNA released from the cells could make the lysate viscous. Sonicate the lysate before centrifugation to shear the DNA and reduce the viscosity of the solution. The cells can be observed under microscope for efficient lysis, a shiny ring around nuclei indicate cells are still intact (perform 30-50 additional passes). If 70 – 80% of the nuclei do not have the shiny ring, proceed to the next step.
5. Centrifuge at 13,000 x g for 10 minutes to remove insoluble material). **Collect supernatant.**

#### **Tissue samples:**

1. Harvest the amount of tissue needed for each assay (initial recommendation = 1-10 mg).
2. Wash tissue in cold PBS.
3. Prepare Lysis Solution as above
4. Homogenize tissue in 303 μL Lysis Solution (Buffer + BHT) with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
5. Centrifuge at 13,000 x g for 10 minutes to remove insoluble material. **Collect supernatant.**

**Δ Note:** For samples containing high amount of proteins, we recommend this protocol:

1. Precipitate protein by homogenizing 1-10 mg tissue samples in 150 μL ddH<sub>2</sub>O + 3 μL BHT. 3μL BHT is normally miscible with water however in case of precipitate formation tissue homogenization helps in dissolution.
2. Add 1 volume of 2N perchloric acid, vortex and then centrifuge to remove precipitated protein.
3. Take 200 μL of the supernatant from each sample and add into a microcentrifuge tube.

#### **Plasma or serum samples:**

1. Gently mix 20 μL plasma or serum with 500 μL of 42 mM H<sub>2</sub>SO<sub>4</sub> in a microcentrifuge tube. Plasma can be collected using any anticoagulant.
2. Add 125 μL of Phosphotungstic Acid Solution and mix by vortexing. The acid is used to precipitates lipids specifically, the pellet should have low contamination with proteins.
3. Incubate at room temperature for 5 minutes.
4. Centrifuge at 13,000 x g for 3 minutes.

5. **Collect the pellet** and resuspend on ice with 100 μL ddH<sub>2</sub>O (with 2 μL BHT Stock/BHT (100X)). Use 200 μL ddH<sub>2</sub>O, if the pellet doesn't dissolve, sonicate in mild water bath. If lipids form a suspension as these are not soluble in water, just proceed with the suspension. The Developer VII/TBA will react with the MDA in these lipid particles. Sonicate if necessary.
6. Adjust the final volume to 200 μL with ddH<sub>2</sub>O.

**Δ Note:** Mild plasma hemolysis shouldn't affect the assay. If hemolysis is observed we recommend fluorometric method.

**Δ Note:** Urine samples can also be used directly with this kit. The samples should be assayed immediately after collection for best results, however samples stored at -70°C can be used.

### Assay Procedure:

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.
- Follow procedure for enhanced sensitivity when working with plasma and other samples where low MDA is expected.

#### **1. Generation of MDA-TBA adduct:**

- 1.1 Add 600 μL of Developer VII/TBA reagent into each vial or well containing 200 μL standard and 200 μL sample.
- 1.2 Incubate at 95°C for 60 minutes. Cool to room temperature in an ice bath for 10 minutes.

**Δ Note:** Occasionally samples will exhibit a turbidity which can be eliminated by filtering them through a 0.2 μm filter. Developer VII/TBA can also react with other compounds in samples giving other colored compounds, these however should not interfere with quantitation of the Developer VII/TBA-MDA adduct.

- 1.3 Take 200 μL of the reaction mix (containing MDA-TBA adduct) and add into a 96-well microplate for analysis (the end range for the standard curve will be:
  - Colorimetric: 0-1-2-3-4-5 nmol MDA/well
  - Fluorometric: 0-0.1-0.2-0.3-0.4-0.5 nmol MDA/well
- 1.4 Proceed to step 3 (Measurement).

#### **2. Optional step for enhanced sensitivity:**

**Δ Note:** Use this step for plasma and other samples where MDA-TBA adduct concentration is low. After performing n-butanol precipitation step, transfer sample to a black clear bottom plate and perform fluorometric measurement.

- 2.1 Add 300 μL n-butanol to the 800 μL extract from step 1.2 to precipitate MDA-TBA adduct. If there is no separation, add 100 μL of 5 M NaCl to the mixture and vortex vigorously.
- 2.2. Centrifuge for 3 minutes at 16,000 x g at RT. Transfer top layer (containing n-butanol) to a new tube.
- 2.3. Evaporate n-butanol by freeze-dry or by incubating sample overnight in a dry-oven at 55°C. Use of a vacuum dry oven would provide a faster result.
- 2.4. Dissolve the MDA-TBA adduct in 200 μL ddH<sub>2</sub>O and then place into the 96-well plate microplate for analysis.

### 3. Measurement:

Measure absorbance immediately on a microplate reader at OD 532 nm for colorimetric assay and RFU at Ex/Em = 532/553 nm for fluorometric assay.

**Δ Note:** For fluorometric assay, we recommend setting the instrument sensitivity to high with a slit width of 5 nm.

### Data Analysis:

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards and sample readings. This is the corrected absorbance.
3. Plot the corrected values for each standard as a function of the final concentration of MDA.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Apply the corrected sample OD reading to the standard curve to get MDA amount ( $y = mx + b$ ) in the sample wells.
6. Concentration of MDA in the test samples is calculated as:

$$MDA\ Concentration = \left( \frac{A}{[mg\ or\ mL]} \right) \times 4 \times D = \frac{nmol}{mL} \text{ or } \frac{nmol}{mg}$$

Where:

A = Amount of MDA in sample calculated from the standard curve (nmol).

mg = Original tissue amount used (e.g. 10 mg).

mL = Original plasma volume used (0.020 mL).

4\* = Correction for using 200  $\mu$ L of the 800  $\mu$ L Reaction Mix.

D = Sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

\*This correction factor may need to be adjusted depending on the amount of supernatant obtained after homogenization and centrifugation. The total amount of supernatant that can be collected depends on the cell debris, insoluble material and tissue type.

For example, when using tissue extracts, if 300  $\mu$ L of supernatant is recovered after homogenization and centrifugation and 200  $\mu$ L is carried forward for downstream analysis, a correction factor of 6 is required:

$$MDA\ Concentration = \left( \frac{A}{[mg\ or\ mL]} \right) \times 6 \times D = \frac{nmol}{mL} \text{ or } \frac{nmol}{mg}$$

**Δ Note:** For tissue samples, concentration can also be expressed in nmol/mg or nmol/ $\mu$ g of protein if a protein quantification has been performed.

### Technical Support

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