Accelerating Scientific Discovery

Elite™ Intracellular Colorimetric Lipid Peroxidation Assay Kit

Catalog Number: **CA-LP200** | Size: **200 assays**

1. Description

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids and other membrane lipids. It can disrupt membrane integrity, alter cell signaling, and generate reactive aldehydes that modify proteins and nucleic acids. Malondialdehyde (MDA), one of the major end products of lipid peroxidation, is widely used as a quantitative biomarker of oxidative stress in biological samples.

The Elite™ Intracellular Colorimetric Lipid Peroxidation (MDA) Assay Kit provides a rapid and convenient method for quantifying MDA without the prolonged heating and strongly acidic conditions required by conventional thiobarbituric acid reactive substances (TBARS) assays. The kit uses a selective chromogenic reagent that reacts with MDA to generate a blue product. The color intensity is proportional to the MDA concentration and is measured at 695–700 nm using an absorbance microplate reader.

2. Key Advantages

- Rapid, heating-free protocol with results in approximately 30 minutes
- Colorimetric detection using a standard absorbance microplate reader
- Improved selectivity for MDA with minimal interference from many other aldehydes
- Compatible with 96-well and 384-well microplate formats
- Broad MDA standard range: 50–1000 µM

3. Kit Components

Component	Quantity / Format
Component A: Elite™ MDA Chromogenic Reagent	1 vial
Component B: Dilution Buffer	1 bottle (10 mL)
Component C: MDA Standard	1 vial (lyophilized)
Component D: Reaction Solution	1 bottle (10 mL)

4. Storage

Store all kit components frozen at −20 °C and protect from light. After preparation, divide stock solutions into single-use aliquots when practical. Avoid repeated freeze-thaw cycles. Allow all reagents to thaw at room temperature before use.

5. Materials Required but Not Supplied

- Clear, flat-bottom 96-well microplate (or clear 384-well microplate)
- Absorbance microplate reader capable of measuring at 695–700 nm, preferably with path-length correction

6. Assay Protocol (for One 96-Well Plate)

Add 50 µL standards or samples ▶ Add 10 µL Elite™ MDA Chromogenic Reagent ▶ Incubate at RT for 10-30 minutes ▶ Add 40 µL Reaction Solution ▶ Read absorbance at 695-700 nm

Unless otherwise noted, prepare all solutions using high-purity water. Protect the chromogenic reagent from light. Store unused prepared stock solutions at −20 °C in single-use aliquots and avoid repeated freeze-thaw cycles.

Step 1. MDA Standard Stock Solution (100 mM)

Add 100 µL of high-purity water to the MDA Standard (Component C). Mix thoroughly until fully dissolved. Store unused stock solution at −20 °C in single-use aliquots.

Step 2. Preparation of MDA Standards

Add 10 µL of the 100 mM MDA stock solution to 990 µL of Dilution Buffer (Component B) to prepare a 1000 µM MDA working standard. Prepare standards at 1000, 800, 600, 400, 200, 100, 50, and 0 µM using Dilution Buffer.

Step 3. Sample Preparation

Prepare cell lysates or other biological samples using a procedure compatible with MDA recovery. Clarify samples by centrifugation when necessary. Dilute samples with Dilution Buffer so that measured values fall within the MDA standard range. Include an appropriate sample blank when sample color or turbidity may contribute to absorbance.

Step 4. Preparation of HRP Working Solution (1x)

Add 2.5 µL of 2000X HRP stock solution into 5 mL of Assay Buffer (Component B) to prepare 1X HRP working solution.

Step 4. Sample Experimental Assay

Arrange MDA standards, blank controls, and test samples in duplicate or triplicate according to the suggested plate layout below.

Table 1. Suggested Plate Layout

BL	BL	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD1	NS1	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD2	NS2	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD3	NS3	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD4	NS4	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD5	NS5	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD6	NS6	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD7	NS7	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS

BL = Blank Control; SD = MDA Standard (SD1-SD7); TS = Test Sample

Table 2. Reagent Addition Guide (each Well)

Well Type	Reagent	Volume
SD1–SD7	MDA serial dilution (1000 to 50 µM)	50 µL
BL	Dilution Buffer (Component B)	50 µL
TS	Test Sample	50 µL

Step 5. Run Assay

5.1. Add 50 µL of each MDA standard, blank control, or test sample to the designated wells of a clear-bottom 96-well microplate.

5.2. Add 10 µL of Elite™ MDA Chromogenic Reagent (Component A) to each well. Mix gently without introducing bubbles.

5.3. Incubate the plate for 10–30 minutes at room temperature, protected from light.

Note: Use the same incubation time for all standards and test samples.

5.4. Add 40 µL of Reaction Solution (Component D) to each well, bringing the final assay volume to 100 µL/well.

For a 384-well plate, use 25 µL of standard or sample, 5 µL of Component A, and 20 µL of Component D for a final volume of 50 µL/well.

5.5. Measure absorbance at 695–700 nm using an absorbance microplate reader. Path-length correction may improve consistency when supported by the instrument.

7. Data Analysis

Subtract the absorbance of the blank control from each MDA standard and test sample. Plot the blank-corrected absorbance values against the corresponding MDA concentrations to generate a standard curve. Determine the MDA concentration of each test sample from the fitted standard-curve equation and multiply by the applicable sample dilution factor. Report results as µM MDA or normalize to sample volume, total protein, cell number, or tissue mass, as appropriate for the experiment.

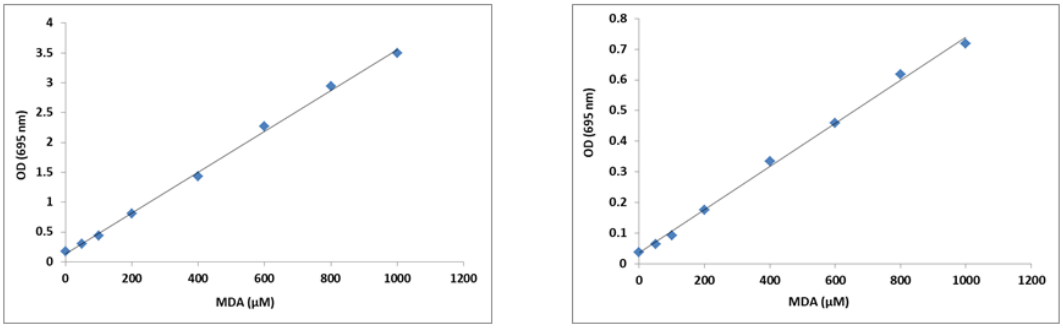

Figure 1. Representative MDA standard curves measured in a clear-bottom 96-well microplate at approximately 695–700 nm. Instrument path-length correction can influence the apparent absorbance scale but should preserve the concentration-dependent response.