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Lipid Peroxidation (MDA) Assay Kit

Cat #: orb219867 (manual)

Detection and Quantification of Malondialdehyde (MDA) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

INTRODUCTION

Quantification of lipid peroxidation is essential for assessing oxidative stress in various pathophysiological processes. Lipid peroxidation generates malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) as natural byproducts. Measurement of these end products is one of the most widely accepted methods for evaluating oxidative damage.

The Lipid Peroxidation (MDA) Assay Kit provides a convenient and sensitive method for detecting MDA in a variety of sample types. In this assay, MDA reacts with thiobarbituric acid (TBA) to form an MDA–TBA adduct. This adduct can be readily quantified using colorimetric detection at 532 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer I	10 ml x 1	4 °C
Reaction Buffer II	1 ml x 1	4 °C, keep in dark
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	5 ml x 1	4 °C
Standard (1 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 532 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Hot air circulation oven

REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.
To prepare the standard curve for the orb219867 Lipid Peroxidation (MDA) Assay Kit, perform a two-fold serial dilution starting from the 1 mmol/L standard provided with the kit.

As indicated in the technical manual, ethanol should be used as the diluent for these steps. To ensure accuracy and maintain the stability of the MDA standard, use absolute **(100%) ethanol** for the dilution process.

2-Fold Serial Dilution Guide

You can follow this table to prepare the seven required concentrations. Each step involves mixing a specific volume of the previous concentration with an equal volume of ethanol.

Standard Concentration	Volume of Standard to Add	Volume of Ethanol to Add	Total Volume
1 mmol/L (Stock)	500 ul (from vial)	0 ul	500 ul
0.5 mmol/L	250 ul of 1mmol/L	250 ul	500 ul
0.25 mmol/L	250 ul of 0.5 mmol/L	250 ul	500 ul
0.125 mmol/L	250 ul of 0.25 mmol/L	250 ul	500 ul
0.062 mmol/L	250 ul of 0.125 mmol/L	250 ul	500 ul

Note: This table is for reference only. Please prepare an appropriate amount of the standard according to the actual experiment.

Dye Reagent A: Add 5 ml distilled water and put it in water bath of 70 °C, shake it occasionally to dissolve before use. Store at 4 °C for 1 month.

Dye Reagent Working Solution: When Dye Reagent A is cold, add 5 ml Dye Reagent B, mix. Store at 4 °C for 1-2 weeks or -20 °C for 1-2 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

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SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1g tissue, homogenize with 1ml Assay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly or dilute with Assay Buffer.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Standard	Sample**	Blank
Reaction Buffer I	100 µl	100 µl	100 µl
Standard	10 µl	--	--
Sample	--	10 µl	--
Assay Buffer	--	--	10 µl
Mix.			
Reaction Buffer II	10 µl	10 µl	10 µl
Dye Reagent Working Solution	100 µl	100 µl	100 µl
Mix, put the plate into the hot air circulation oven, 90 °C for 30 minutes, when cold, record absorbance measured at 532nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \text{ (mmol/L)}$$

Calculate the initial concentration according to sample preparation procedure.

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2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample}} \quad (\mu mol/mg)$$

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \quad (\mu mol/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \quad (\mu mol/mg)$$

2.4 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \quad (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = µmol/ml

$V_{Standard}$: the volume of standard in assay procedure, µl

V_{Sample}: the volume of sample in assay procedure, µl

V_{Assay}: the volume of Assay Buffer, µl

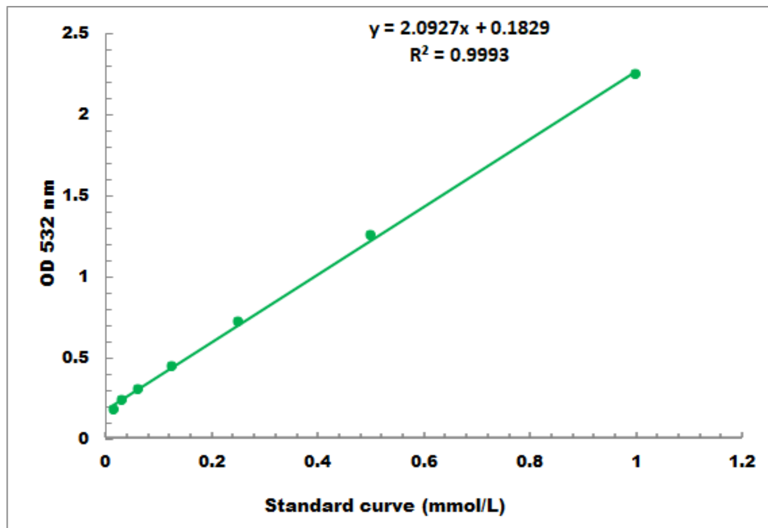
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L

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