Amplite™ Fluorimetric Monoamine Oxidase Assay Kit *Red Fluorescence*

Catalog number: 11303
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: Horseradish Peroxidase (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: MAO Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component E: Plasma Amine Oxidase Standard (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial (2.5 Units)
Component F: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Monoamine oxidases (MAO) are a family of flavin-containing amine oxidoreductases that catalyze the oxidation of monoamines. They are found bound to the outer membrane of mitochondria in numerous tissues including liver, intestinal mucosa, and nerves. In humans there are two types of MAO: MAO-A and MAO-B. MAO-A is particularly important in the metabolism of monoamines ingested in food. MAOs play a major role in the inactivation of neurotransmitters. MAO dysfunction has been associated with depression, schizophrenia, substance abuse, attention deficit disorder, migraines, and irregular sexual maturation. The Amplite™ Monoamine Oxidase Assay Kit provides a quick and sensitive method for the measurement of monoamine oxidase and semicarbazide-sensitive amine oxidase (SSAO) activities in blood samples and other biological samples. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The signal can be easily read by either a fluorescence microplate reader or an absorbance microplate reader. With the Amplite™ Monoamine Oxidase Assay Kit, we have detected as little as 10 ?U/mL SSAO in a 100 µL reaction volume. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

AT A GLANCE

Protocol summary

1. MAO standards or test samples (50 µL)
2. Add MAO working solution (50 µL)
3. Incubate at room temperature for 30-60 min
4. Read fluorescence intensity at Ex/Em = 540/590 nm(cut off 570 nm)

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540nm
Emission:	590nm
Cutoff:	570nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red stock solution (250X):
Add 40 µL of DMSO (Component F) into the vial of Amplite™ Red substrate (Component A). The stock solution should be used promptly.

Note The Amplite™ Red substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite™ Red substrate is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH 7 – 8. The provided assay buffer, pH 7.4, is recommended.

2. HRP stock solution (200X):
Add 100 µL of Assay Buffer (Component B) into the vial of horseradish peroxidase (Component C).

3. Plasma Amine Oxidase (PAO) standard solution (20 U/mL):
Add 125 µL of Assay Buffer (Component B) into the vial of Plasma Amine Oxidase Standard (Component E).

PREPARATION OF STANDARD SOLUTION

PAO standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11303

Add 50 µL of 20 U/mL PAO standard solution into 950 µL of Assay Buffer (Component B) to get 1000 mU/mL PAO standard solution (PAO7). Take 1000 mU/mL PAO standard solution and perform 1:3 serial dilutions to get remaining serially diluted PAO standards (PAO6 - PAO1). Note: Higher concentrations of PAO may cause reduced fluorescence signal due to the over oxidation of Amplite™ red substrate (to a non-fluorescent product).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Amplite™ Red stock solution (250X), 25 µL of HRP stock solution (200X) and 25 µL of MAO Substrate (Component D) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.07 mL Monoamine Oxidase (MAO) working solution. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of PAO standards and test samples in a solid black 96-well microplate. PAO = plasma amine oxidase standard (PAO1 - PAO7, 1 to 1000 mU/mL); BL = blank control; TS = test sample.

BL	BL	TS	TS
PAO1	PAO1	...	...
PAO2	PAO2	...	...
PAO3	PAO3		
PAO4	PAO4		
PAO5	PAO5		
PAO6	PAO6		
PAO7	PAO7		

Table 2. Reagent composition for each well

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Well	Volume	Reagent
PAO1 - PAO7	50 µL	serial dilution (1 to 1000 mU/mL)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	sample

1. Prepare plasma amine oxidase standards (PAO), blank controls (BL), and test samples (TS) into a 96-well solid black microplate according the the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of MAO working solution into each well of the PAO standard, blank control, and test samples to make the total PAO assay volume of 100 µL/well. For a 384-well plate, add 25 µL of MAO working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Excitation = 530 - 570, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, cutoff = 570 nm).

Note The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. However, the absorption detection will have a lower sensitivity compared to that of the fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate PAO samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

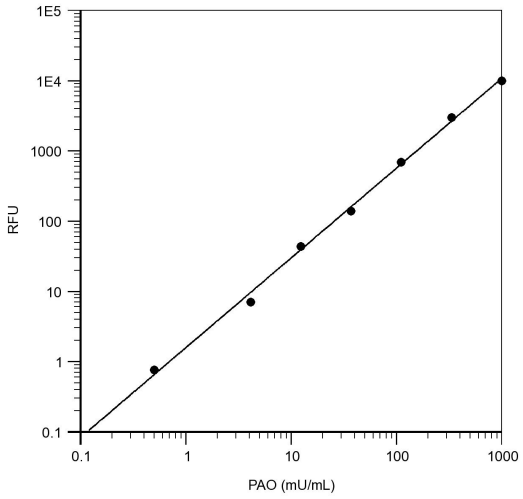

Figure 1. PAO dose response was obtained with Amplite™ Fluorimetric Monoamine Oxidase Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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