Amplite™ Fluorimetric Monoamine Oxidase Assay Kit
Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11303 (200 assays)	Keep at -20 °C and protect from light	Fluorescence microplate readers

Introduction

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 the 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. MAOs dysfunction have been associated with depression, schizophrenia, substance abuse, attention deficit disorder, migraines, and irregular sexual maturation. Monoamine oxidase inhibitors are one of the major classes of drug prescribed for the treatment of depression.

The Amplite™ Fluorimetric Monoamine Oxidase Assay Kit provides a quick and sensitive method for the measurement of monoamine oxidase and semicarbazide-sensitive amine oxidase (SSAO or plasma amine oxidase PAO) activity in blood samples and other biological samples. The kit uses our Amplite™ Red substrate which makes it recordable in a dual mode, the signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530-570 nm/590 -600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576±5 nm. With the Amplite™ Fluorimetric Monoamine Oxidase Assay kit, as little as 10 mU/mL PAO might be detected 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 without a separation step.

Kit Components

Components	Amount
Component A: Amplite™ Red (light sensitive)	1 vial
Component B: Assay Buffer	20 mL
Component C: Horseradish Peroxidase (lyophilized)	1 vial
Component D: MAO Substrate	1vial (50 µL)
Component E: Plasma Amine Oxidase Standard (lyophilized)	1 vial (2.5 Units)
Component F: DMSO	1vial (200 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

MAO standards or test samples (50 µL) → Add MAO assay mixture (50 µL) → Incubate at room temperature for 30-60 min → Read fluorescence intensity at Ex/Em = 540/590 nm

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

1. Prepare stock solutions:

1.1 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. Any remaining solution should be aliquoted and frozen at -20 °C.
Note 1: Avoid repeated freeze-thaw cycles.
Note 2: 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

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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.

1.2 HRP stock solution (200X): Add 100 µL of Assay Buffer (Component B) into the vial of horseradish peroxidase (Component C).
Note: The unused HRP solution should be divided into single use aliquots and stored at -20 ^oC.

1.3 20 U/mL Plasma Amine Oxidase (PAO) stock solution: Add 125 µL of Assay Buffer (Component B) into the vial of Plasma Amine Oxidase Standard (Component E).
Note: The unused PAO stock solution should be divided into single use aliquots and stored at -20 ^oC.

2. Prepare assay mixture:

Prepare assay mixture according to the following tables and protect from light.

Table 1. Assay mixture for one 96-well solid black plate (2X)

Components	**Volume**
Amplite™ Red Stock Solution (250X, from Step 1.1)	20 µL
HRP Stock Solution (200X, from Step 1.2)	25 µL
MAO Substrate (Component D)	25 µL
Assay Buffer (Component B)	5 mL
Total volume	5.07 mL

3. Prepare serially diluted PAO standards (0 to 1000 mU/mL):

3.1 Add 50 µL of 20 U/mL PAO stock solution (from Step 1.3) into 950 µL of Assay Buffer (Component B) to get 1000 mU/mL PAO standard solution.

3.2 Take 300 µL of 1000 mU/mL PAO standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 mU/mL serially diluted PAO standards.

3.3 Add PAO standards and/or PAO-containing test samples into a 96-well solid black microplate as described in Tables 2 and 3

Table 2. Layout of PAO standards and test samples in a solid black 96-well microplate

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

Note: PAO = Plasma Amine Oxidase standards, BL=Blank control, TS=test samples.

Table 3. Reagent composition for each well

PAO Standard	**Blank Control**	**Test Sample**
Serial Dilutions* (50 µL)	Assay Buffer (Component B): 50 µL	50 µL

**Note 1: Add the serially diluted Plasma Amine Oxidase standards from 1 mU/mL to 1000 mU/mL into each well from PAO1 to PAO7 in duplicate.*
Note 2: High concentration of PAO may cause reduced fluorescence signal due to the over oxidation of Amplite™ red substrate (to a non-fluorescent product).

4. Run PAO assay:

4.1 Add 50 µL of assay mixture (from Step 2) into each well of the PAO standard, blank control, and test samples (see Step 3, Table 1 and 2) to make the total PAO assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay mixture into each well.

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4.2 Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

4.3 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570/590-600 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. The absorption detection has lower sensitivity compared to fluorescence reading.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with monoamine oxidase reactions. The typical data are shown in Figure 1 (PAO standard curve).
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

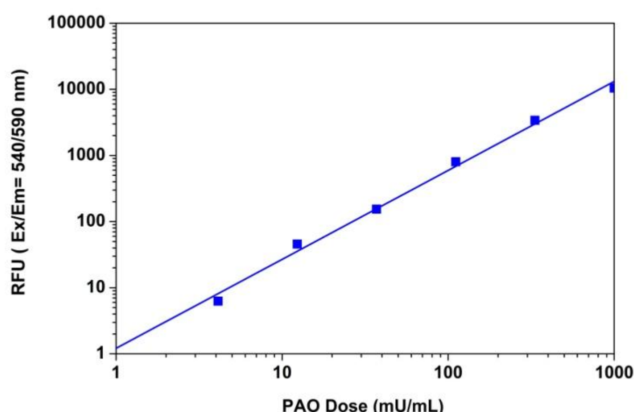

Figure 1. PAO dose response was obtained with Amplite™ Fluorimetric Monoamine Oxidase Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 mU/mL PAO was detected with 45 minutes incubation time (n=3).

References

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