Amplite® Fluorimetric Aldehyde Quantitation Kit

Catalog number: 10052
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 10052)
Component A: AldeLight™ Blue	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (30 mL)
Component C: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (6 mL)
Component D: Aldehyde Standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Component E: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

The formation, reactivity and toxicity of aldehydes originating from the peroxidation of lipids of cellular membranes have received great attention in recent years. Rapid and accurate measurement of aldehydes is an important task for biological research, chemical research, food industry and environmental pollution surveillance. There are a few reagents or assay kits available for quantifying the number of aldehydes. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS. Both our Amplite® Colorimetric Aldehyde Quantitation Kit (10051) and Amplite® Fluorimetric Aldehyde Quantitation kit (10052) are used for quantifying aldehydes at higher pH. Kit 10052 uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. Kit 10052 is much more sensitive than Kit 10051. This fluorimetric kit provides a sensitive mix-and-read method to detect as little as 0.1 nanomole of aldehyde in a 100 µL assay volume (1 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare Aldehyde Standards and/or test samples (50 µL)
2. Add AldeLight™ Blue working solution (50 µL)
3. Incubate at RT for at least 30 minutes
4. Add 25 µL of Reaction Buffer
5. Monitor fluorescence increase at Ex/Em = 365/435 nm

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

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	420 nm
Emission	435 nm
Excitation	365 nm
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

AldeLight™ Blue stock solution (250X)

Add 40 µL of DMSO (Component E) into the vial of AldeLight™ Blue (Component A) to make 250X AldeLight™ Blue stock solution.

Aldehyde standard solution (10 mM)

Add 1 mL of ddH_2O into the vial of Aldehyde Standard (Component D) to make a 10 mM aldehyde standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10052

Aldehyde standard
Take 10 mM aldehyde standard solution and perform 1:10 dilutions in ddH2O to get 1000 uM Aldehyde standard solution (AS7). Then perform 1:3 serial dilutions to get remaining aldehyde standard (AS6 - AS1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of 250X AldeLight™ Blue stock solution into 5 mL of Assay Buffer (Component B), and mix well to make AldeLight™ Blue working solution.

Note 5 mL of AldeLight™ Blue working solution is enough for one plate. The reaction mixture is not stable, and best used within 2 hours.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Aldehyde Standards and test samples in a solid black 96-well microplate. AS= Aldehyde Standards (AS1 - AS7, 1 to 1000 µM); BL= Blank Control; TS= Test Samples.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilution (1 to 1000 µM)
BL	50 µL	ddH_2O

TS	50 µL	test sample

Protocol

1. Prepare Aldehyde standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Optional:The sensor will react with the thiol, so treat samples (including the control) with 2-5 mM H2O2 for 30 min if the sample contains thiol prior to addition of AldeLight™ Blue.
3. Add 50 µL of AldeLight™ Blue working solution to each well of aldehyde standard, blank control, and test samples to make the total aldehyde assay volume of 100 µL/well. For a 384-well plate, add 25 µL of AldeLight™ Blue working solution into each well instead, for a total volume of 50 µL/well.
4. Incubate the reaction mixture at room temperature for 30 minutes or more, protected from light.
5. Add 25 µL of Reaction Buffer (Component C) into each well.
6. Monitor the fluorescence increase at Ex/Em = 365/435 nm using a fluorescence plate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

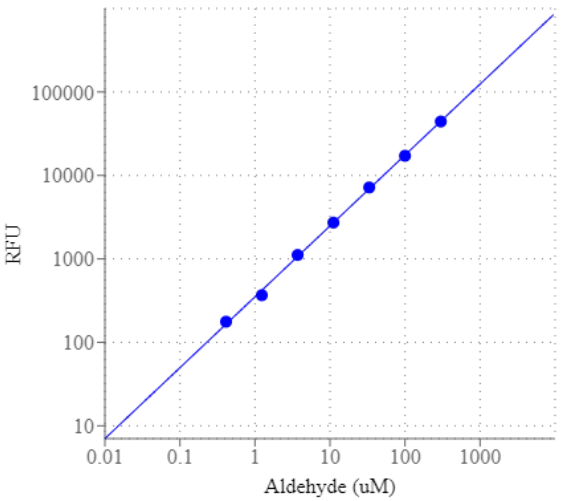

Figure 1. Aldehyde dose response was measured in a solid black 96-well plate with Amplite® Fluorimetric Aldehyde Quantitation Kit using a Gemini fluorescence microplate reader (Molecular Devices).

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