Amplite™ Fluorimetric Aldehyde Quantitation Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 10052 (200 assays)	Keep at -20 °C Avoid exposure to light	Fluorescence microplate readers

Introduction

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 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 0.3 nanomole of aldehyde in a 100 µL assay volume (3 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 read by a fluorescence microplate reader at Ex/Em = 365/435 nm.

Kit Key Features

Broad Application:	Used for quantifying aldehydes in a variety of applications, such as enzyme reactions.
Sensitive:	Detect as little as 0.3 nanomole of aldehyde in a 100 µL assay volume.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: AldeLight™ Blue	1 vial
Component B: Assay Buffer	1 bottle (30 mL)
Component C: Reaction Buffer	1 vial (6 mL)
Component D: Aldehyde Standard	1 vial
Component E: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare enzyme reaction solution (50 µL) → Add AldeLight™ Blue reaction mixture (50 µL)
→ Incubate at RT for 15 to 30 minutes → Add 25 µL of Reaction Buffer
→ Monitor fluorescence increase at Ex/Em = 365/435 nm

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

Ordering: sales@aatbio.com; 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; 408-733-1055

1. Prepare 250X AldeLight™ Blue stock solution:

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

Note: The unused AldeLight™ Blue stock solution should be divided into single use aliquots, and stored at -20°C.

2. Prepare AldeLight™ Blue reaction mixture:

Add 20 µL of 250X AldeLight™ Blue stock solution (from Step 1) into 5 mL of Assay Buffer (Component B), and mix them well.

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

3. Prepare serial dilutions of aldehyde standard (0 to 1 mM):

3.1 Add 1 mL of Assay Buffer (Component B) into the vial of Aldehyde Standard (Component D) to make a 10 mM aldehyde standard stock solution.

Note: The unused 10 mM Aldehyde standard stock solution should be divided into single use aliquots and stored at -20°C.

3.2 Take 100 µL of 10 mM aldehyde standard stock solution (from Step 3.1) to perform 1:10, and 1:3 serial dilutions to get 1000, 300, 100, 30, 10, 3, 1 and 0 µM serial dilutions of aldehyde standard.

3.3 Add serially diluted aldehyde standards and aldehyde-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1. Layout of Aldehyde Standards and test samples in a solid black 96-well microplate

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

Note: AS= Aldehyde Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

Aldehyde Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serially diluted Aldehyde standards from 1 µM to 1000 µM into wells from AS1 to AS7 in duplicate.*

4. Run aldehyde assay:

4.1 Add 50 µL of AldeLight™ Blue reaction mixture (from Step 2) into each well of aldehyde standard, blank control, and test samples (see Step 3.3) to make the total aldehyde assay volume of 100 µL/well.

Note: For a 384-well plate, add 25 µL of test sample and 25 µL of AldeLight™ Blue reaction mixture into each well.

4.2 Incubate the reaction mixture at room temperature for 15 to 30 minutes, protected from light.

4.3 Add 25 µL of Reaction Buffer (Component C) into each well (from Step 4.2).

4.4 Monitor the fluorescence increase at Ex/Em = 365/435 nm using a fluorescence plate reader.

Data Analysis

The fluorescence in blank wells (0 µM Aldehyde Standard and AldeLight™ Blue reaction mixture only) is used as a control, and subtracted from the values of those wells with the aldehyde reactions. An aldehyde standard curve is shown in Figure 1.

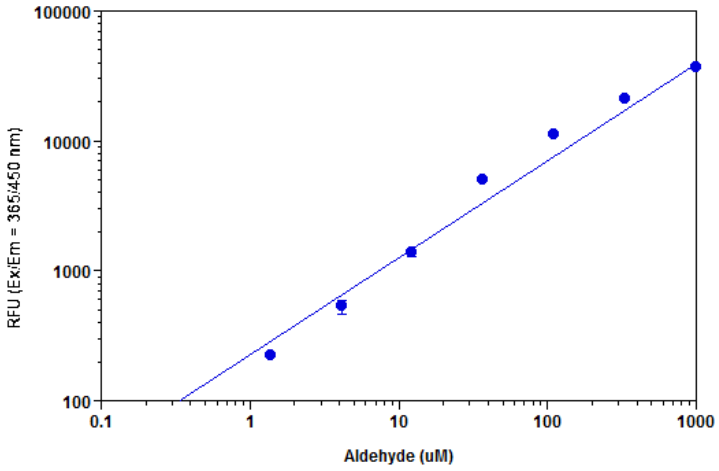

Figure 1. Aldehyde dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Aldehyde Quantitation Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 3 µM of aldehyde can be detected with 15 minutes incubation (n=3). *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

References

1. Trevor M. Kitson. (1985) High concentrations of aldehydes slow the reaction of cytoplasmic aldehyde dehydrogenase with thiol-group modifiers Biochem. J. 228, 765.
2. Crabb DW, Matsumoto M, Chang D, You M (2004). Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. The Proceedings of the Nutrition Society 63 (1): 49.
3. Steinmetz CG, Xie P, Weiner H, Hurley TD (1997). Structure of mitochondrial aldehyde dehydrogenase: the genetic component of ethanol aversion. Structure 5 (5): 701.
4. O'Donnell JM, Kudej RK, LaNoue KF, Vatner SF, Lewandowski ED. (2004) Limited transfer of cytosolic NADH into mitochondria at high cardiac workload. Am J Physiol Heart Circ Physiol, 286, H2237.
5. Zurek G, and karst U (2000). 2,4-Dinitro-3,5,6-trideuterophenylhydrazones for the quantitation of aldehydes and ketones in air samples by liquid chromatography-mass spectrometry. J of chromatography A, 869, 251.
6. Ou Z, Ogamo A, Guo L, Konda Y, Harigaya Y, and Nakagawa Y. (1995). Identification and quantitation of choline glycerophospholipids that contain aldehyde residues by fluometric high-performance liquid chromatography. Analytical biochemistry 227, 289.