Elite™ Aldehyde Detection Kit (Blue Fluorescence)

CATALOG NUMBER: CA-A052, 200 assays

Description

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.

The Elite™ Aldehyde Detection kit is used for quantifying aldehydes at higher pH. This kit uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. 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 Components

- **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)

Storage

Keep **Component A** at -20 °C and avoid exposure to light; **Component D** at -20 °C; **Component B, C, E** at 4 °C. All components are stable for 6 months after receipt if stored properly.

Materials Required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

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Assay Protocol

1. **Preparation of 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. **Preparation of 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 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. **Preparation of 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								
FS1	FS1										
FS2	FS2										
FS3	FS3										
FS4	FS4										
FS5	FS5										
FS6	FS6										
FS7	FS7										

Note: FS= Aldehyde Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

Aldehyde Standards	**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 FS1 to FS7 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.

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Data Analysis

The fluorescence in blank wells (with 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.

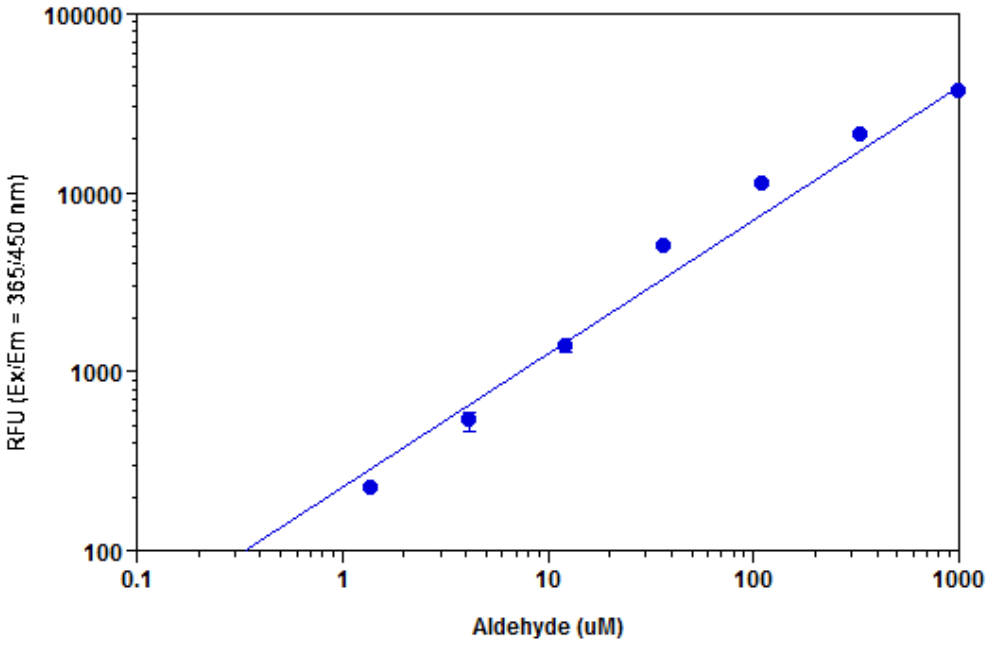

Figure 1. Aldehyde dose response was measured in a solid black 96-well plate with Elite™ Fluorimetric Aldehyde Quantitation Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 3 µM of aldehyde can be detected with 15 minutes incubation (n=3). Note: The fluorescence background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

References

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