Elite™ Formaldehyde Detection Kit (Green Fluorescence)

CATALOG NUMBER: CA-F057, 200 assays

Description

Formaldehyde is an organic compound with the formula CH2O. It is one of the well-identified volatile chemical contaminants responsible for indoor pollution and "building sick" syndrome disease and was recently classified as carcinogenic. The main sources for the CH2O pollution in air include painting, coating material and cigarette smoking. Rapid and accurate measurement of formaldehyde is an important task for biological research, food industry, chemical research and environmental pollution surveillance. There are a few reagents or assay kits available for quantifying formaldehyde. Most of the existing formaldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS.

The Elite™ Fluorimetric Formaldehyde Detection kit uses a proprietary fluorogenic dye that generates a green fluorescent product upon reacting with formaldehyde. The kit provides a sensitive, one-step fluorometric method to detect as little as 1 uM of formaldehyde in a 100 µL assay volume (Figure 1). 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 Ex/Em = 410/525 nm.

Kit Components

- **Component A:** AldeLight™ Green — 1 vial
- **Component B:** Assay Buffer — 1 bottle (30 ml)
- **Component C:** 37.2% Formaldehyde Standard (12.3M) — 1 vial (100 µl)
- **Component D:** DMSO — 1 vial (100 µl)

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Shelf Life

All reagents are stable for at least 6 months after receipt when stored properly at the recommended conditions.

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 for One 96-Well Plate

1. **Preparation of 500x AldeLight™ Green stock solution:**

   Add 20 µl of DMSO (**Component D**) into the vial of AldeLight™ Green (**Component A**) to make 500x AldeLight™ Green stock solution.

   Note: The unused AldeLight™ Green stock solution should be divided into single use aliquots and stored at -20 °C (avoid light).

2. **Preparation of AldeLight™ Green reaction mixture:**
   Add 10 µl of 500x AldeLight™ Green stock solution (from step 1) into 5 ml of Assay Buffer (**Component B**), and mix well.

   Note: 5 ml of AldeLight™ Green 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 formaldehyde standard (0 to 300 µM):**

   3.1. Add 5 µL of 37.2% Formaldehyde Standard (**Component D**) into 0.5 mL of Assay Buffer (from **Component B**) to make 123 mM stock solution.

   3.2. Add 12.2 µL of 123 mM Formaldehyde Standard Solution (from Step 3.1) into 0.5 mL of Assay Buffer (from **Component B**) to make 3 mM stock solution.

   3.3. Take 3 mM Formaldehyde Standard Solution (from Step 3.2) to perform 1:10, and 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3, 0.1, and 0 µM standard formaldehyde solutions.

   3.4. Add formaldehyde standards and formaldehyde-containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

Table 1. Layout of fomaldehyde 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= Formaldehyde Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

Formaldehyde Standard	**Blank Control**	**Test Sample**
Serial Dilutions: 50 µl	Assay Buffer: 50 µl	50 µl

Note: Add the serially diluted formaldehyde standards from 0.1 µM to 100 µM into wells from FS1 to FS7 in duplicate.

4. **Run formaldehyde assay:**

   4.1. Add 50 µl of AldeLight™ Green reaction mixture (from Step 2) into each well of formaldehyde standard, blank control, and test samples (see Step 3.4) to make the total formaldehyde assay volume of 100 µl/well.

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

   4.2. Incubate the reaction mixture at room temperature for 20 to 60 minutes (protected from light).

   4.3. Monitor the fluorescence increase at Ex/Em = 410/525 nm using a fluorescence plate reader..

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Data Analysis

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

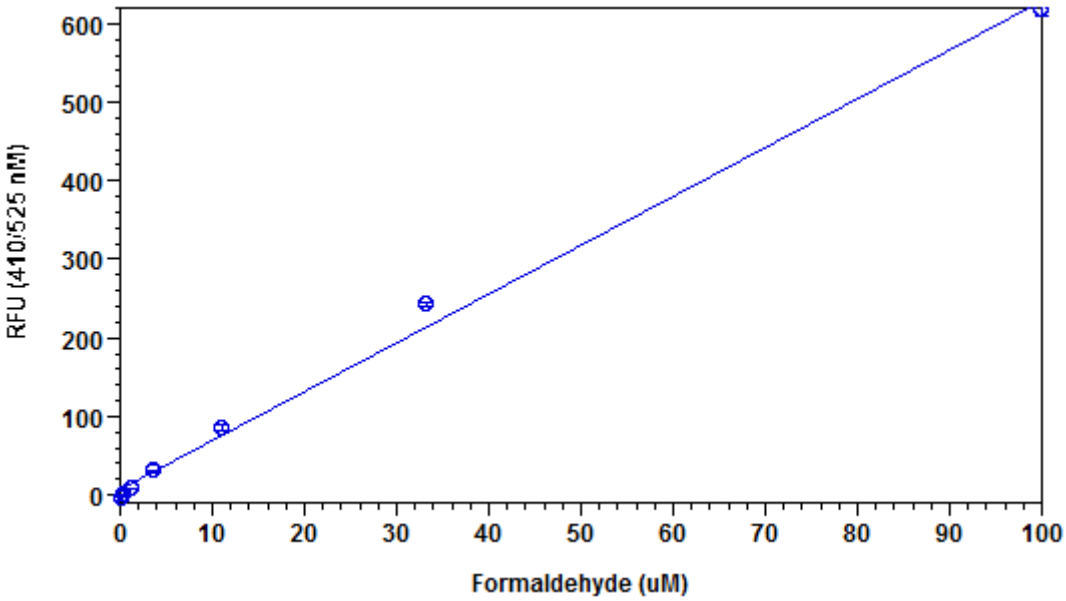

Figure 1. Formaldehyde dose response was measured in a solid black 96-well plate with Elite™ Formaldehyde Detection Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 µM of formaldehyde can be detected with 30-minute incubation (n=3). Note: The fluorescence background 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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