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Product Information

Fluorometric Aldehyde Assay Kit

Catalog Number **MAK141**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Aldehydes are highly reactive molecules, which play a role in many physiological, pathological and commercial processes. They have been implicated as environmental pollutants, and in neurodegeneration and DNA damage. A method for rapid and accurate measurement of aldehydes is an important tool for biological and chemical research, the food industry, and environmental pollution surveillance.

The Fluorometric Aldehyde Assay Kit provides a simple and direct procedure for measuring aldehydes in samples in which the pH is physiological and higher. Aldehyde reacts with a fluorogenic dye, resulting in a fluorometric (λ_{ex} = 365/λ_{em} = 435 nm) product proportional to the amount of aldehydes present. The Fluorometric Aldehyde Assay Kit has a lower limit of detection of 0.3 nanomole of aldehyde in a 100 µL assay volume (3 µM).

Components

The kit is sufficient for 200 assays in 96 well plates.

Aldehyde Detection Reagent Catalog Number MAK141A	1 vl
Assay Buffer Catalog Number MAK141B	30 mL
Reaction Buffer Catalog Number MAK141C	6 mL
Aldehyde Standard Catalog Number MAK141D	1 vl
DMSO Catalog Number MAK141E	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Allow reagents to come to room temperature before use. Briefly centrifuge vials before opening.

Aldehyde Detection Reagent Stock Solution – Reconstitute with 40 µL of DMSO to generate the 250× Detection Reagent Stock Solution. Mix well by pipetting, divide into single-use aliquots, and store at –20 °C.

Aldehyde Standard – Reconstitute with 1 mL of Assay Buffer to generate a 10 mM Aldehyde Standard stock solution. Mix well by pipetting, divide into single-use aliquots, and store at –20 °C.

Storage/Stability

The kit is shipped under ambient conditions, storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Aldehyde Standards for Fluorometric Detection
Dilute 10 µL of the 10 mM Standard with 90 µL of Assay Buffer to prepare a 1,000 µM standard solution. Further dilute the 1,000 µM standard solution by 3-fold serial dilutions with Assay Buffer. Add 50 µL of the diluted standard solutions into a 96 well plate, generating 0 (blank), 1.4, 4.1, 12.3, 37, 111, 333, and 1,000 µM standards.

Sample Preparation
Add up to 50 µl of sample to wells. Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction for One 96-well Plate
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
250× Detection Reagent Stock	20 µL
Assay Buffer	5 mL

Note: The Master Reaction Mix is enough for one plate. The Master Reaction Mix is not stable and best used within 2 hours.

2. Add 50 µL of the Master Reaction Mix to each of the standard, blank control, and sample wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 15–30 minutes at room temperature. Protect the plate from light during the incubation.

3. Add 25 µL of Reaction Buffer into each well.

4. Measure the fluorescence intensity (λ_{ex} = 365/ λ_{em} = 435 nm).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) Aldehyde standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Aldehyde standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Using the corrected measurement, the concentration of aldehyde present in the samples may be determined from the standard curve.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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