

## Product Information

### Colorimetric Aldehyde Assay Kit

Catalog Number **MAK139**

Storage Temperature  $-20^{\circ}\text{C}$

## 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 Colorimetric Aldehyde Assay Kit provides a simple and direct procedure for measuring aldehydes in a variety of samples. Aldehydes reacts with a dye, resulting in a colorimetric (405 or 550 nm) product proportional to the amount of aldehyde present. The Colorimetric Aldehyde Assay Kit has a lower limit of detection of 1 nanomole of aldehyde in a 100  $\mu\text{L}$  assay volume. This kit has been used for monitoring activities of oxidases that convert an amino group to an aldehyde group.

### Components

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

Aldehyde Detection Reagent Catalog Number MAK139A	2 ea
Assay Solution Catalog Number MAK139B	10 mL
Aldehyde Standard Catalog Number MAK139C	1 vL
Dilution Buffer Catalog Number MAK139D	20 mL

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates or white plates with clear bottoms for colorimetric assays.
- Spectrophotometric multiwell plate reader

### Precautions and Disclaimer

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

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Aldehyde Detection Reagent, Assay Solution, Dilution Buffer – Allow them to come to room temperature before use.

Aldehyde Standard – Reconstitute with 1 mL of Dilution Buffer to generate a 10 mM Aldehyde Standard stock solution. Mix well by pipetting, then aliquot and store at  $-20^{\circ}\text{C}$ .

### Storage/Stability

The kit is shipped under ambient conditions and storage at  $-20^{\circ}\text{C}$ , protected from light, is recommended.

### Procedure

#### Aldehyde Standards for Colorimetric Detection

Dilute 100  $\mu\text{L}$  of the 10 mM Standard with 900  $\mu\text{L}$  of Assay Buffer to prepare a 1,000  $\mu\text{M}$  standard solution. Further dilute the 1,000  $\mu\text{M}$  standard solution by 10-fold and 3-fold serial dilutions with Dilution Buffer. Add 50  $\mu\text{L}$  of the diluted standard solutions into a 96 well plate, generating 0 (blank), 1, 3.3, 10, 33, 100, 333, and 1,000  $\mu\text{M}$  standards.

### Sample Preparation

Add 0–50  $\mu\text{L}$  of samples into wells. For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. If analyzing enzyme reactions, for example, fructose-1,6-biphosphate aldolase and fructose-1,6-biphosphate, prepare a 50  $\mu\text{L}$  of enzyme reaction and incubate the enzyme reaction at 37 °C for at least one hour.

Note: Samples from enzyme reactions may need to be optimized in regards to the components of the reaction (for example, buffer solution). Both BSA (>0.001%) and TWEEN® 20 (>0.01%) will interfere with the assay and should be avoided during sample preparation. For most reactions, the Dilution Buffer can also be used for running enzyme reactions.

### Assay Reaction for One 96 well Plate

1. Set up the Master Reaction Mix by adding 5 mL of Assay Solution to 1 bottle of Aldehyde Detection Reagent. 50  $\mu\text{L}$  of the Master Reaction Mix is required for each reaction (well).

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  $\mu\text{L}$  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 30–60 minutes at room temperature. Protect the plate from light during the incubation.
3. Measure the absorbance at either 405 or 550 nm.  
Note: Samples with lower aldehyde concentrations should be read at 405 nm while samples with higher concentrations should be read at 550 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**

<b>Problem</b>	<b>Possible Cause</b>	<b>Suggested Solution</b>
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 colorimetric assays, use clear plates or white plates with clear bottoms
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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