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Product Information

Aconitase Activity Assay Kit

Catalog Number **MAK051**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The aconitases are a family of iron-sulfur containing enzymes that catalyze the isomerization of citrate to isocitrate. Two aconitases, encoded by different genes, have been identified in eukaryotes. Aconitase 1 (ACO1, c-aconitase) is a cytosolic enzyme, while Aconitase 2 (ACO2, m-aconitase) is a mitochondrial enzyme that functions in the tricarboxylic acid cycle. Aconitases are reversibly inactivated by oxidative stress, and aconitase activity in cells and tissues has been used as a biomarker for oxidative damage.

The Aconitase Activity Assay kit provides a simple and direct procedure for measuring Aconitase activity in a variety of samples. Aconitase activity is determined in a coupled enzyme reaction in which citrate is converted to isocitrate by aconitase. This results in a colorimetric (450 nm) product proportional to the enzymatic activity present. One unit of aconitase is the amount of enzyme that will isomerize 1.0 μmole of citrate to isocitrate per minute at pH 7.4 at 25 °C.

Components
The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer 25 mL
Catalog Number MAK051A

Substrate 1 vl
Catalog Number MAK051B

Developer 1 vl
Catalog Number MAK051C

Enzyme Mix 0.6 mL
Catalog Number MAK051D

Cysteine 1 vl
Catalog Number MAK051E

$(NH_4)Fe(SO_4)_2$ 1 vl
Catalog Number MAK051F

Isocitrate Standard, 100 mM 100 μL
Catalog Number MAK051G

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

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

Assay Buffer – Allow buffer to come to room temperature before use.

Substrate – Reconstitute with 220 μL of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution.

Developer – Reconstitute with 1.1 mL of Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution.

Aconitase Activation Solution – Reconstitute cysteine and $(NH_4)Fe(SO_4)_2$ each with 0.5 mL of Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at –20 °C.

To prepare Aconitase Activation Solution, mix 0.1 mL each of the reconstituted cysteine and $(NH_4)Fe(SO_4)_2$ solutions, and mix well by pipetting. Activation solution should be **prepared fresh**. Keep on ice while in use.

Storage/Stability
The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Isocitrate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Isocitrate Standard solution with 490 µL of the Assay Buffer to prepare a 2 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 2 mM standard solution into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 nmole/well standards. Add Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (20–40 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold Assay Buffer. Centrifuge the samples at 800 × *g* for 10 minutes at 4 °C to remove insoluble material. The supernatant can be used for the c-aconitase assay. For the m-aconitase assay, centrifuge the supernatant at 20,000× *g* for 15 minutes at 4 °C, collect the pellet, and dissolve in 0.1 mL cold Assay Buffer. Sonicate pellet for 20 seconds. Store samples at –80 °C and keep on ice while in use.

To activate samples, add 10 µL of Aconitase Activation Solution to 100 µL of sample and incubate on ice for 1 hour.

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.

Add 2–50 µL of activated samples to wells. Bring samples to a final volume of 50 µL with Assay Buffer. To control for background activity, a sample blank may be set up for each sample by omitting the Substrate (Table 1). The blank readings can be subtracted from the sample readings.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well). The enzyme mix should be kept on ice while in use.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Assay Buffer	46 µL	48 µL
Enzyme Mix	2 µL	2 µL
Substrate	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation

3. Incubate the plate at 25 °C for 30–60 minutes.

4. Add 10 µL of the Developer to each well, mix, and incubate at 25 °C for 10 minutes. Measure the absorbance at 450 nm.

Results

Calculations

Correct for the background by subtracting the value obtained for the 0 (blank) Isocitrate standard from all sample readings. Background values can be significant and must be subtracted from all readings. Plot the Isocitrate standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample readings to obtain the corrected measurement. Using the corrected measurement, the amount of isocitrate generated by the aconitase in the sample may be determined from the standard curve.

The aconitase activity of a sample may be determined by the following equation:

$$\text{Aconitase Activity (milliunit/mL)} = \frac{B \times \text{Sample Dilution Factor}}{T \times V}$$

B = Amount (nmole) of isocitrate generated
T = time reaction incubated in minutes
V = pretreated sample volume (mL) added to well

Aconitase activity is reported as nmole/min/mL = milliunit/mL. One unit of aconitase is the amount of enzyme that will isomerize 1.0 μmole of citrate to isocitrate per minute at pH 7.4 at 25 °C.

Example:
Isocitrate (B) = 5.84 nmole
T = 45 minutes
Sample volume (V) = 0.05 mL
Sample dilution is 1

Aconitase activity is:

$$\frac{5.84 \times 1}{45 \times 0.05} = 2.59 \text{ milliunits/mL}$$

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 colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided
	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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