Product Information

Aconitase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2226-100 100 assays	Cap Color	Part Number
Assay Buffer	30 ml	WM	K2226-C-1
Substrate (lyophilized)	1 vial	Blue	K2226-C-2
Developer (lyophilized)	1 vial	Purple	K2226-C-3
Enzyme Mix	200 µl	Green	K2226-C-4
Cysteine-HCl (lyophilized)	1 vial	Red	K2226-C-5
$(NH_4)Fe(SO_4)_2$ (lyophilized)	1 vial	Brown	K2226-C-6
Isocitrate Standard (100 mM)	100 µl	Yellow	K2226-C-7

II. Introduction:

Aconitase (aconitate hydratase) is an iron-sulfur protein that catalyzes the stereo-specific isomerization of citrate to isocitrate via cis-aconitate in the TCA cycle. The active aconitase has an $[Fe_4S_4]^{2+}$ cluster. There is two aconitases: a cytosolic (c-) aconitase and a mitochondrial (m-), which are related but distinctly different enzymes and are coded on different chromosomes. Cells or other biological samples treated with pro-oxidants can cause loss of aconitase activity, which can be used to measure oxidative damage.

The Aconitase Activity Colorimetric Assay Kit provides a highly sensitive, simple, fast and convenient way for detection of aconitase activity in various samples based on colorimetric method. In the assay, aconitase converts citrate into isocitrate, which is further processed generating a product that converts a nearly colorless probe into an intensely colored product (λmax = 450nm). The kit is suitable for high throughput screening.

III. Storage and Handling:

Store the kit at 4ÁC, protected from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Substrate: Dissolve with 220 µl ddH_2O; sufficient for 100 assays. Store at 4ÁC.

Developer: Dissolve with 1.1 ml Assay Buffer before use; sufficient for 100 assays. Store at 4ÁC.

Aconitase Activation Solution: Dissolve cysteine-HCl and $(NH_4)_2Fe(SO_4)_2$ with 0.5 ml Assay Buffer separately, and store at -20ÁC.Use within one month. Take out 0.1 ml cysteine-HCl and $(NH4)_2Fe(SO_4)_2$ solutions and mix together to prepare fresh activation solution.

Ensure that the Assay Buffer is at room temperature before use. Keep samples, Enzyme Mix and Aconitase solution on ice during the assay.

V. Aconitase Activity Assay:

1. Sample Preparations:

Homogenize 20 - 40 mg tissue or 10^6 Cells on ice in 0.1 ml cold Assay Buffer; Centrifuge at 800 xg for 10 min at 4°C; Collect the supernatant for caconitase assay. For m-aconitase assay, centrifuge the supernatant at 20,000 x g for 15 min at 4ÁC and collect the pellet, dissolve into 0.1 ml cold Assay Buffer, sonicate for 20 sec. Keep samples at -80ÁC for storage.

Add 10 µl activation solutions to 100 µl sample; incubate on ice for 1 hr to activate aconitase in the sample.

Add 2 - 50 µl activated samples into each well, and adjust volume to 50 µl. We suggest using a background control group as well as several doses of your sample to ensure the readings are within the linear range.

2. Isocitrate Standard Curve:

Dilute 10 µl with 490 µl assay buffer to prepare 2 mM isocitrate standard solution. Add 0, 2, 4, 6, 8, 10µl 2 mM Isocitrate Standard solution into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well Isocitrate standard. Bring the final volume to 50 µl with Assay Buffer

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

	Sample Reaction Mix	Background Mix
Assay Buffer	46 µl	48 µl
Enzyme Mix	2 µl	2 µl
Substrate	2 µl	---

Add 50 µl of the Sample Reaction Mix to each test samples, background control and Isocitrate standards. Mix well and incubate at 25°C for 30-60 min. Add 10µl Developer to each well, mix and incubate at 25°C for 10 min. Measure OD 450nm.

4. Calculation: Plot the Isocitrate standard curve. $\Delta OD = OD_{sample} - OD_{background}$, apply the ΔOD to the Isocitrate standard curve to get B nmol of isocitrate generated by aconitase in 30 - 60 min.

Aconitase Activity = B/ (T X V) x Sample Dilution Factor = nmol/min/ml = mU/ml

Where: B is the isocitrate amount from Standard Curve (in nmol).

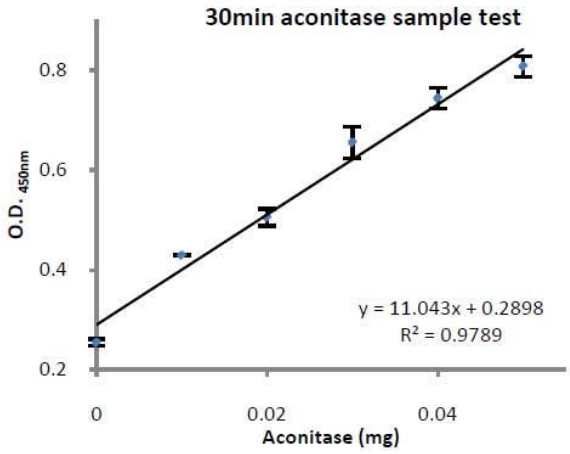
T is the time incubated (in min).

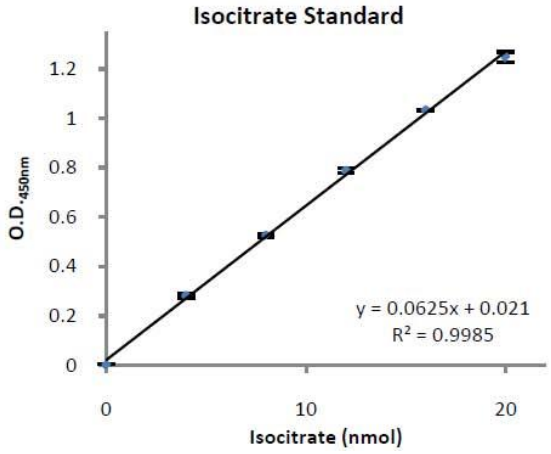
V is the pretreated sample volume added into the reaction well (in ml).

Unit definition: One unit of Aconitase is the amount of enzyme that will isomerize 1.0 µmol of Citrate to Isocitrate per min at pH 7.4 at 25°C.

III. Reagent Reconstitution and General Consideration:

Store kit at -20°C Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. Keep samples and amylase positive control on ice during the assay. Amylase Positive Control: Dissolve into 50 µl Assay Buffer, and store at -20°C.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

	• Use of a different 96-well plate	of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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