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Product datasheet

Aconitase Activity Assay Kit ab109712

18 References 3 Images

Overview

Product name	Aconitase Activity Assay Kit
Detection method	Colorimetric
Sample type	Cell culture extracts, Tissue
Assay type	Enzyme activity
Assay time	2h 00m
Product overview	Aconitase Activity Assay Kit ab109712 is a simple, reproducible, and sensitive tool for assaying aconitase from tissue homogenates or cell lysates. Unlike other aconitase assays this is not a coupled reaction and therefore only aconitase activity is required and measured. In the aconitase activity assay protocol, aconitase catalyzes an equilibrium between citrate, cis-aconitate and iso-citrate. These reactions are monitored by measuring the increase in absorbance at 240 nm associated with the formation of the cis-aconitate which has an extinction coefficient of 2.2 OD/mM per well. Therefore the rate of cis-aconitate production is proportional to aconitase activity. Aconitase preservation solution, assay buffer, reagents and an essential UV microplate are provided for this measurement. The entire assay can be completed within 2 hours. Note – mitochondrial and cytoplasmic aconitase activities are indistinguishable. Therefore, to measure the mitochondrial activity only, first isolate mitochondria, or for both activities fractionate the cells into cytoplasmic and mitochondrial.
Notes	Previously called Aconitase Enzyme Activity Microplate Assay Kit. Aconitase (aconitate hydratase; EC 4.2.1.3) is an iron-sulfur protein that catalyzes the reversible inter-conversion of citrate and isocitrate, via a cis-aconitate intermediate, in both the TCA and glyoxylate cycles. The enzyme contains a [4Fe-4S] cluster which interacts directly with the substrates. In eukaryotes there are both mitochondrial and cytosolic forms of the enzyme. The mitochondrial form functions not only in the TCA cycle, but also to stabilize mtDNA thereby influencing mitochondrial gene expression. The cytosolic form can function as an aconitase as well as an iron regulatory protein. The active form of the enzyme is inhibited by citrate analogs, and fluoracetate. Other inhibitors

include oxidative stress agents such as peroxynitrite, hydrogen peroxide and superoxide, which inactivate the enzyme by changing the [4Fe-4S] to a [3Fe-4S] cluster. Aconitase is considered a good marker of mitochondrial and cellular oxidative stress. This change in mitochondrial aconitase can lead to a decreased energy production, whereas in cytosolic aconitase it triggers binding of the enzyme to mRNA iron response elements resulting in increased expression of iron uptake proteins and decreased transcription of iron sequestering protein.

A hydroxyl scavenging solution (Aconitase preservation solution) is supplied with this aconitase assay kit to maintain aconitase activity during sample preparation. An inactivated [3Fe-4FS] aconitase may be activated in vitro by the addition of iron and cysteine.

Platform Microplate reader

Properties

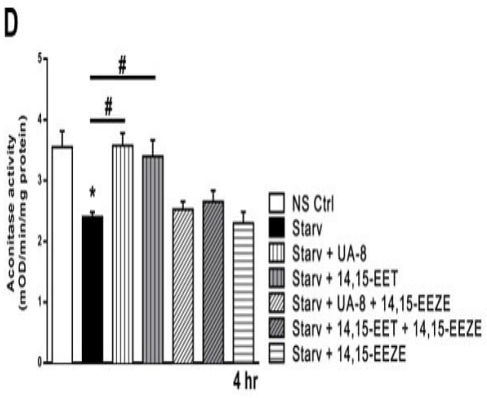
Storage instructions Store at +4°C. Please refer to protocols.

Components	96 tests
Detergent	1 x 1ml
96-well UV microplate	1 unit
Aconitase Preservation Solution	1 x 20ml
Buffer	1 x 50ml
Isocitrate (25X)	1 x 800µl
Manganese (100X)	1 x 200µl

Relevance Aconitase (aconitate hydratase; EC 4.2.1.3) is an iron-sulfur protein containing an $[Fe_4S_4]^{2+}$ cluster that catalyzes the stereospecific isomerization of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle, a non-redox-active process. Tissue contains two aconitases, a mitochondrial (m-) and a cytosolic (c-) aconitase. They are related, but distinctly different enzymes and are coded for on different chromosomes. Loss of aconitase activity in cells or other biological samples treated with prooxidants has been interpreted as a measure of oxidative damage.

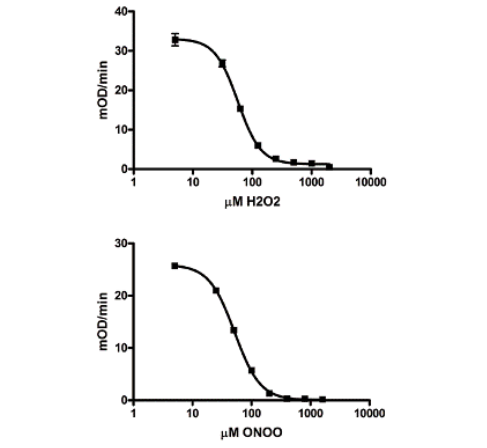
Cellular localization ACO1: Cytoplasmic ACO2: Mitochondrial

Images



Mitochondrial aconitase enzymatic activities were measured using ab109712

Mitochondria were isolated from HL-1 cells following starvation protocol and approximately 50µg of mitochondrial preparation were placed in each microplate well. Equal amounts of the substrate isocitrate were added to all wells and the absorbance at 240nm was recorded for 30 minutes. The catalytic activity was measured by the rate of formation of cis-aconitate as detected by the increase in absorbance.



ELISA - Aconitase Enzyme Activity Microplate Assay Kit (ab109712)

Figure 1. Bovine heart mitochondria were treated with increasing concentrations of hydrogen peroxide and peroxynitrite to generate aconitase activity IC50's for these oxidative stress agents.

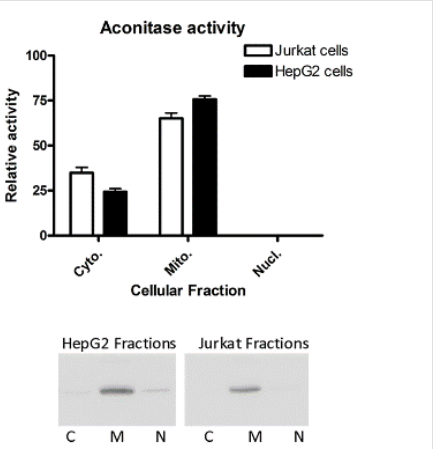


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Figure 2. Using cellular fractionation kit **ab109719**, whole cells were separated into cytoplasmic, mitochondrial and nuclear fractions.

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