CREATIVE BIOMART®
Assay Kit

Aconitase Enzyme Activity Microplate Assay Kit

Product Information

Cat.No. Kit-0044

Product Overview

Aconitase Enzyme Activity Microplate Assay Kit is a simple, reproducible, and sensitive tool for assaying aconitase from tissue homogenates or cell lysates. Unlike other assays this is not a coupled reaction and therefore only aconitase activity is required and measured. Aconitase catalyzes an equilibrium between aconitate, 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.

Description

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 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.

Applications

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Functional Studies

Storage

Store UV microplate at room temperature.Store all other components store at 4°C.

Kit Components

Components 96 tests10X Detergent 1 x 1ml96-well UV microplate 1 unitAconitase preservation solution 1 x 20mlBuffer 1 x 50mlIsocitrate (25X) 1 x 800µlManganese (100X) 1 x 200µl

Compatible Sample Types

Cell culture extracts, Tissue

Assay time

2h 00m

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