

Aconitase Activity Assay Kit

Product Information

Cat.No.

Kit-0042

Size

96 rxn

Description

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. Therefore, aconitase is considered a good marker of mitochondrial and cellular oxidative stress. This inhibition of 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-4S] aconitase may be activated in vitro by the addition of iron and cysteine. The Aconitase Activity Assay Kit measures the activity of aconitase in a sample by following the conversion of isocitrate to cis-aconitate as indicated by an increase in absorbance at UV 240 nm with an extinction coefficient of 2.2 OD/mM per well. Note: Mitochondrial and cytoplasmic aconitase activities are indistinguishable. Therefore, to measure the mitochondrial activity only, first isolate mitochondria, but to measure for both activities, separate the cells into cytoplasmic and mitochondrial fractions. The entire assay can be completed within 2 hours. The intra-assay variation and inter-assay variation with this assay are each less than 10% and 15% respectively.

Storage

Store all components at 2 to 8°C. Do not freeze.

Kit Components

50 ml, Buffer (Tube 1), 20 ml, Aconitase preservation solution, 800 µl, 25X Isocitrate, 200 µl, 100X

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Manganese, 1 ea, UV Microplate, 1 ml, Detergent, This kit contains sufficient materials for 96 measurements (one 96-well microplate).

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