

## Product Data Sheet

# K2226 Aconitase Activity Colorimetric Assay Kit

## Kit Contents

Components	K2226-100 100 assays	Part Number
Aconitase Preservation Solution	1 x 20 mL	K2226-C-1
Isocitrate (25X)	1 x 800 $\mu$ L	K2226-C-2
Detergent	1 x 1 mL	K2226-C-3
Buffer	1 x 50 mL	K2226-C-4
96-well UV microplate	1 x 1 Unit	K2226-C-5
Manganese (100X)	1 x 200 $\mu$ L	K2226-C-6

## Introduction

Aconitase Activity Assay Kit is a simple, reproducible, and sensitive tool for assaying aconitase from tissue homogenates or cell lysates.

## Storage

Shipped at conditions	Blue Ice
Appropriate long-term storage conditions	Multi
Storage information	Please refer to protocols

## Notes

Aconitase Activity Assay Kit 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 isocitrate. 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.

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.

## Caution

**FOR RESEARCH PURPOSES ONLY.**

**NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

*Specific storage and handling information for each product is indicated on the product datasheet. Most APEx BIO products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Short term storage of many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality of the reagents. Upon receipt of the product, follow the storage recommendations on the product data sheet.*

**APEx BIO Technology**

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