

TECHNICAL DATA SHEET**Cat # MS745****Aconitase Enzyme Activity Microplate Assay Kit**

Rev.0

LOT #:

APPLICATIONS: Aconitase activity

SPECIES CROSS-
REACTIVITY: All

KIT COMPONENTS:

Item	MS745
Aconitase preservation solution	20 mL
Detergent (for cultured cell preparation only)	1 mL
Buffer	50 mL
Isocitrate (25X)	800 µL
Manganese (100X)	200 µL
96-well UV microplate	1

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

COUNTRY OF ORIGIN: USA

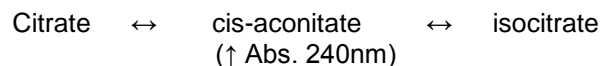
BACKGROUND:

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.

Inhibition of aconitase activity

The active form of the enzyme is inhibited by citrate analogs, and fluoracetate. Other inhibitors include oxidative stress agents such as peroxyxynitrite, 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 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. In Friedreich's ataxia decreased functional frataxin leads to increased cellular free iron, oxidative stress and decreased aconitase activity.

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.



Note: This product is for research purposes only. It is not to be used in humans or for diagnostic purposes.

WARRANTY – MitoSciences Inc warranties that products will perform as indicated in the published Technical Data Sheet for 1 month from date of purchase when stored according to specifications and when used consistent with recommended protocols. If you experience results which materially differ from those described, send evidence of the non-performing product for replacement of the original product purchased or a credit toward any other of the company's products or services.

The MitoSciences assay MS745 measures the activity of aconitase in a sample by following the conversion of isocitrate to cis-aconitate as an increase in absorbance at UV 240 nm with an extinction coefficient of 2.2 OD/mM per well. Aconitase preservation solution, assay buffer, reagents and a special UV microplate are provided for this measurement.

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.

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.

Note: This product is for research purposes only. It is not to be used in humans or for diagnostic purposes.

WARRANTY – MitoSciences Inc warrants that products will perform as indicated in the published Technical Data Sheet for 1 month from date of purchase when stored according to specifications and when used consistent with recommended protocols. If you experience results which materially differ from those described, send evidence of the non-performing product for replacement of the original product purchased or a credit toward any other of the company's products or services.

MitoSciences Inc., 1850 Millrace Drive, Suite 3A, Eugene, OR 97403, 541-284-1800