

Mit Complex II/III Activity Assay Kit

Product Information

Cat.No.

Kit-0247

Product Overview

Complex II/III Activity Assay measures the reduction of excess cytochrome c (550 nm absorbance) as catalyzed by complex III. This assay is coupled to succinate co-enzyme Q oxidoreductase (complex II) for the generation of QH₂. Due to the dependence on complex II activity, a counterscreen for complex II activity should be performed in order to truly measure complex III activity. For the use of this kit with other types of tissue mitochondria, please see Mitochondrial (Tissue) Isolation Kit.

Description

Complex III (CoQ cytochrome c oxidoreductase) is an essential protein for mitochondrial oxidative phosphorylation. Complex III functions as both a gatekeeper for mitochondrial respiration, and as a major source of reactive oxygen species III. Complex III accepts electrons from complexes I and II in the form of QH₂, the reduced form of the electron carrier ubiquinone. Once bound to complex III, QH₂ undergoes a series of redox reactions, known as the Q-cycle. During the Q-cycle, electrons are passed from QH₂ onto cytochrome c via the Rieske iron-sulfur protein and cytochrome c1 resulting in the translocation of 4H⁺ and the generation of O₂•⁻. This assay is designed to measure the complex III-dependent reduction of cytochrome c. Because the mechanism is coupled to complex II, it is also sensitive to complex II inhibitors. It is therefore recommended that all potential complex III inhibitors be pre-screened against complex II.

Usage

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use. NOTE: It is recommended that gloves be worn at all time when working with isolated mitochondria and mitochondrial inhibitors.

Storage

Stability: 1 year; Storage: -80°C; This kit will perform as specified if stored as directed in the Materials

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Supplied section and used before the expiration date indicated on the outside of the box.

Kit Components

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below. Mitochondrial Complex III Activity Assay Buffer: 2 vials/10 ml, -20°C; Cytochrome c Assay Reagent: 1 vial/3 mg, -80°C; Bovine Heart Mitochondria Assay Reagent: 1 vial/100 µl, -80°C; Succinate Assay Reagent: 1 vial/100 µl, -20°C; Half Volume 96-Well Clear Plate 1 plate Room temperature