abcam

Product datasheet

MitoTox™ Complex II + III OXPHOS Activity Assay Kit ab109905

32 References 2 图像

概述

产品名称	MitoTox™ Complex II + III OXPHOS Activity Assay试剂盒
检测方法	Colorimetric
检测类型	Direct
检测时间	0h 30m
种属反应性	**与反应:** Cow, Mammals
产品概述	MitoTox™ Complex II+ III OXPHOS Activity Assay Kit (ab109905) is designed for testing the direct inhibitory effect of compounds on Complex III activity in only 30 minutes. The assay is performed using whole bovine heart mitochondria, a rich source of OXPHOS complex. Succinate (electron donor of Complex II) and oxidized cytochrome c (electron acceptor of Complex III) are added to the mitochondria to start the electron transfer pathway that takes place during oxidative phosphorylation. The rate of couple Complex II + III reaction is measured by monitoring the conversion of oxidized cytochrome c into reduced form, which can be observed as increase in absorbance at OD 550 nm. The assay requires rotenone and KCN (not supplied): rotenone (Complex I inhibitor) blocks electron transfer to ubiquinone, ensuring that all reduction of cytochrome c happens via Complex II. The addition of KCN (Complex IV inhibitor) ensures that there is no re-oxidation of cytochrome c. The intra-assay and inter-assay variation of this assay are both < 10%. Inhibitory effects of compounds on Complex III activity can be tested in two different ways: 1. Screening format, where up to 23 compounds can be tested at a single concentration in triplicate; 2. Dose response (IC_{50}) format, where two compounds known to affect Complex III activity can be tested at 11 different data points in triplicate. Testing for mitochondrial function has become a key aspect of drug discovery. Mitochondria can be affected by drug treatment, resulting into cardio- and hepatotoxic side effects that can lead to drug withdrawal from the market. Therefore, there is increasing emphasis on testing the impact on mitochondria early on in the drug development process to reduce failure rates during preclinical and clinical phases.
说明	Store Bovine Heart Mitochondria and Cytochrome c (oxidized) at -80°C. Store all other components at 4°C. **Related products**

Review the **mitochondrial assay guide**, or the full **metabolism assay guide** to learn about more assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also how to assay metabolic function in live cells using your plate reader.

平台 Microplate reader

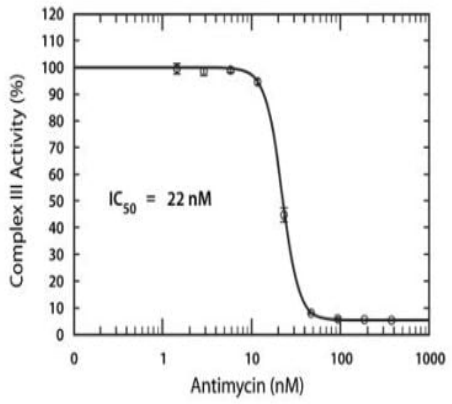
性能

存放说明 Please refer to protocols.

组件	96 tests
12-channel reagent reservoirs	2 units
1X Complex III Mito Buffer	1 x 1ml
1X Succinate Solution	1 x 12ml
96-well microplate	1 unit
Bovine heart mitochondria (5 mg/mL)	1 x 300µl
Cytochrome c (III)	1 x 550µl
Single-channel reagent reservoirs	2 units

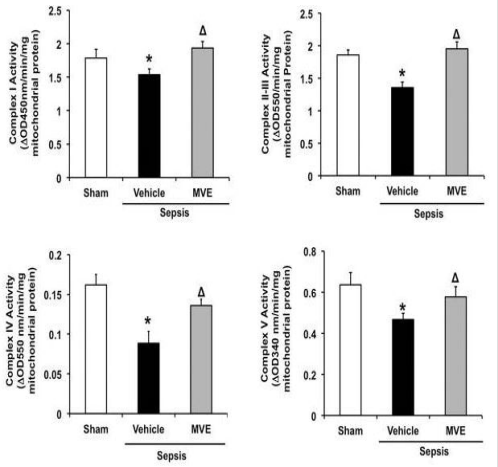
相关性 Complex II (succinate-ubiquinone oxidoreductase), one of the mitochondrial respiratory chain complexes, transfers electrons from succinate generated during the citric acid cycle to Complex III (ubiquinolcytochrome c oxidoreductase), via a mobile electron shuttle, ubiquinone. Complex III transfers electrons to Complex IV (cytochrome c oxidase) via another mobile electron shuttle, cytochrome c.

图片



MitoTox™ Complex III OXPHOS Activity Assay (ab109905)

Typical dose response curve for antimycin A. Assay was performed following the Dose Response Assay Procedure using antimycin A, a well known Complex III inhibitor. Antimycin A was prepared in DMSO to generate a 10 mM stock. Starting with a 150 µM final concentration in well, 1:4 serial dilutions of antimycin A were generated.



Mitochondrial ROS dependent functional deficiency and structural impairment in cardiac mitochondria after sepsis.

Image courtesy of Yao X et al.PLoS One. 2015; 10(10): e0139416. doi: 10.1371/journal.pone.0139416.

Mitchondiral fractions from the heart tissue of Rats infected by S. pneumoniae, or given PBS sham control, were subjected to measurements of complex I-V activities. Complex I was measured with **ab109721** (top left), Complex II + III were measured using ab109905 (top right), Complex IV was measured using **ab109911** (bottom left) and Complex V was measured using **ab109714** (bottom right).

Freshly isolated mitochondrial pellets were resuspened in PBS supplemented with 10% detergent provided in the kits. Protein concentrations of these mitochondrial lysates were estimated and 25 µg (for complex I, IV and V) or 100 µg (for complex II+III) mitochondrial protein was used per reaction. Enzyme activities were measured spectrophotometricly in triplicate and expressed as changes of absorbance per minute per mg protein

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