abcam

Product datasheet

MitoBiogenesis™ In-Cell ELISA Kit (Fluorescent) ab140359

2 References 4 Images

Overview

Product name MitoBiogenesis™ In-Cell ELISA Kit (Fluorescent)

Detection method Fluorescent

Precision

Intra-assay

Sample	n	Mean	SD	CV%
COX-1 (HRP)				4.62%
SDH-A (AP)				6.02%

Sample type Cell culture extracts

Assay type Cell-based (quantitative)

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Mouse, Rat, Cow, Human

Product overview ab140359 is an In-Cell ELISA (ICE) assay kit that uses quantitative immunocytochemistry to measure levels of COX1 and SDHA levels in cultured cells. Cells are fixed in a microplate and targets of interest are detected with highly specific, well-characterized antibodies. Relative target levels are quantified using secondary antibodies conjugated to either horseradish peroxidase (HRP) or alkaline phosphatase (AP) which generate signal through the use of two spectrally distinct fluorogenic substrates. Fluorescence is measured using a standard fluorescent spectrophotometer and relative levels of target proteins are quantified. Optionally, antibody signal intensity can be normalized to the total cell amount using Janus Green stain. In-Cell ELISA (ICE) technique generates quantitative data with specificity similar to Western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of fluorescence detection and the ability to run up to 96 samples in parallel. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their post-translational modifications, and thus essentially eliminates changes during sample handling, such as preparation of protein extracts.

Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought

separately.

Notes

"Cytochrome c oxidase is the component of the respiratory chain that catalyzes the reduction of oxygen to water. Subunits 1-3 form the functional core of the enzyme complex. CO I is the catalytic subunit of the enzyme. Electrons originating in cytochrome c are transferred via the copper A center of subunit 2 and heme A of subunit 1 to the bimetallic center formed by heme A3 and copper B. Catalytic activity carries out the following reaction;

4 ferrocytochrome c + O_2 + 4 H^+ = 4 ferricytochrome c + 2 H_2O.

Flavoprotein (FP) subunit of succinate dehydrogenase (SDH) that is involved in complex II of the mitochondrial electron transport chain and is responsible for transferring electrons from succinate to ubiquinone (coenzyme Q). Can act as a tumor suppressor. Catalytic activity carries out following reaction;

Succinate + ubiquinone = fumarate + ubiquinol.

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.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate

Properties

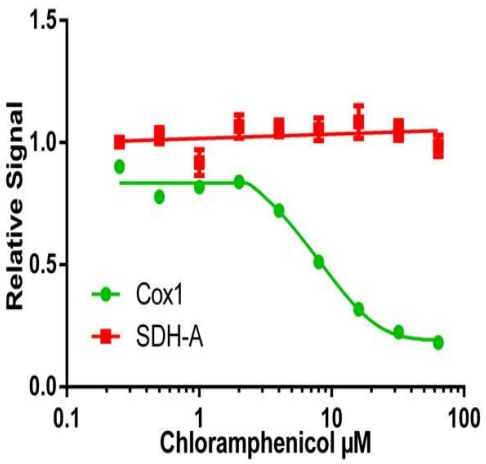
Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
1000X Anti-Mouse IgG/AP-Labeled Secondary Antibody	1 x 20µl
1000X HRP Labeled Secondary Antibody (Anti-Mouse IgG2a)	1 x 20µl
100X Primary Antibody Cocktail	1 x 120µl
100X Triton X-100	1 x 1.5ml
10X Blocking Buffer	1 x 15ml
10x Phosphate buffered Saline (PBS)	1 x 100ml
10X Quenching Solution	1 x 1.5ml
400X Fluorescent Substrate Cocktail	1 x 50µl
400X Tween-20	1 x 2ml
8000X Hydrogen Peroxide	1 x 50µl

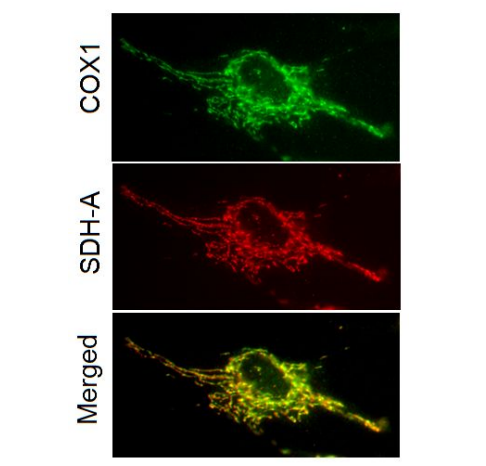
Components	1 x 96 tests
Fluorescent Substrate Buffer	1 x 12ml
1X Janus Green Stain	1 x 17ml

Images



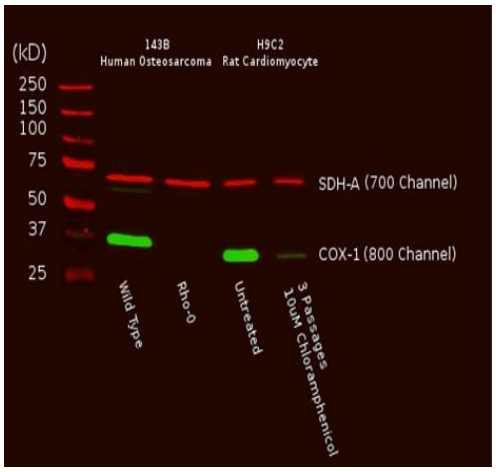
Inhibition of mitochondrial biogenesis by chloramphenicol

The IC50 of a drug's effect on mitochondrial protein translation can be determined quickly using the MitoBiogenesis™ ICE Kit. In this example, cells were seeded at 6,000 cells/well, allowed to grow for 6 days in a drug dilution series and then relative amounts of COX-I (HRP signal), and SDH-A (AP-signal) were measured in each well. Chloramphenicol inhibits mtDNA-encoded (HRP signal) COX-I protein synthesis relative to nuclear DNA-encoded (AP signal) SDH-A protein synthesis by 50% at 8.1 µM, %CV = 4.33% for HRP (COX-1) signal and 3.13% for AP (SDH-A) signal.



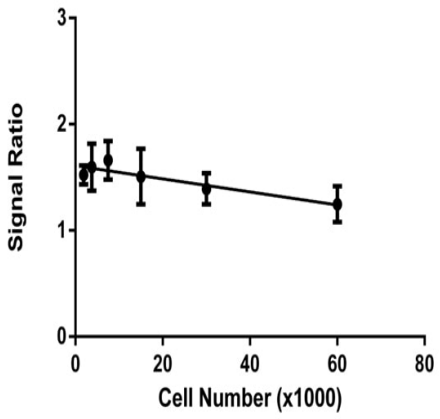
Antibody specificity demonstrated by immunocytochemistry

Two-color immunocytochemical labeling of cultured cells with the two primary monoclonal antibodies specific for COX-I and SDH-A. The two antibodies exhibit striking and specific co-localization in the mitochondria, consistent with the known mitochondrial expression of both proteins.



Antibody specificity demonstrated by Western blot

A Western blot of total cell protein (10 µg) from Human or rat cultured cells was probed with the primary and secondary antibodies and scanned with a LI-COR® Odyssey® imager. The two mitochondrial proteins targeted by the two primary mAbs were labeled and visualized specifically despite the presence of thousands of other proteins. Furthermore, reduction of mtDNA levels in human Rho0 (mtDNA-depleted) cells, or inhibition of mitochondrial protein translation by chloramphenicol in rat cells result in specific reduction of COX-I protein while nuclear DNA-encoded SDH-A is unaffected.



Quantitative measurement of the COX-I/SDH-A protein expression ratio

At all cell concentrations, a consistent ratio of mtDNA-encoded protein expression COX-I (HRP signal) to nuclear DNA-encoded mitochondrial protein expression SDH-A (AP signal) is observed in untreated cells. Therefore, normalizing COX-I levels to SDH-A levels simplifies data analysis and eliminates the need to perform all tests at the same cell concentration.

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