

MitoBiogenesis™ In-Cell ELISA Kit (IR) ab110216

★★★★★ [1 Abreviews](#) [5 References](#) [画像数 7](#)

製品の概要

製品名 MitoBiogenesis™ In-Cell ELISA Kit (IR)

検出方法 IR

サンプルの種類 Adherent cells

アッセイタイプ Cell-based (quantitative)

ステップ Multiple steps standard assay

種交差性 **交差種:** Mouse, Rat, Cow, Human

製品の概要 For identifying inhibitors and activators of mitochondrial biogenesis in adherent cultured cells. Each kit contains sufficient reagents to analyze two 96-well plates of fixed human, rat, mouse, or bovine cells. This kit utilizes IRDyes® for detection, and so requires a LI-COR® Odyssey® or Aeries® imaging system. An alternate colorimetric version of this kit is available for use with standard plate readers - MitoBiogenesis™ In-Cell ELISA Kit (Colorimetric) ([ab110217/MS643](#)).

In-Cell ELISA Kits use quantitative immunocytochemistry to measure protein levels or post-translational modifications in cultured cells. Cells are fixed in a 96-well plate and targets of interest are detected with highly-specific, well-characterized monoclonal antibodies, and levels are quantified with IRDye®-labeled Secondary Antibodies. IR imaging and quantitation is performed using a LI-COR® Odyssey® or Aeries® system.

Cells (human, rat or mouse) are seeded in 96- or 384-well microplates, and after exposure to experimental compounds for several cell doublings, the levels of two mitochondrial proteins are measured simultaneously in each well. The two proteins are each subunits of a different oxidative phosphorylation enzyme complex, one protein being subunit I of Complex IV (COX-I), which is mtDNA-encoded, and the other being the 70 kDa subunit of Complex II (SDH-A), which is nDNA-encoded. Complex IV includes several proteins which are encoded in the mitochondrion, while the proteins of Complex II are entirely encoded in the nucleus. Optionally, total protein levels can also be measured.

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Plates are available in our ICE (In-Cell ELISA) Support Pack ([ab111542](#)) which can be bought separately

特記事項

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.

試験プラットフォーム

Microplate

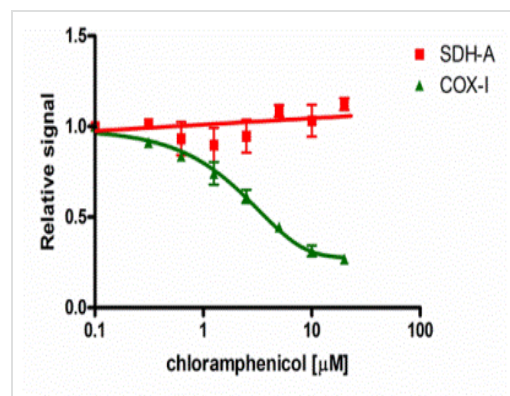
製品の特性

保存方法

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

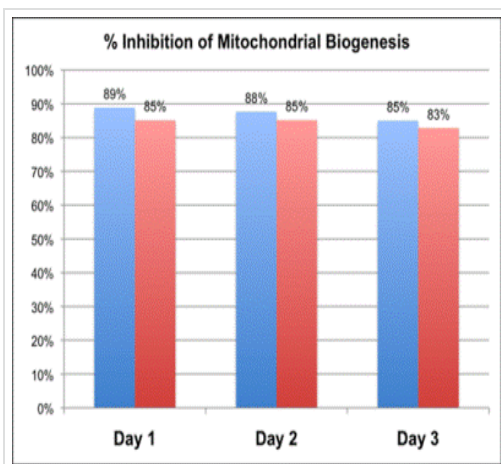
| 内容 | 2 x 96 tests |
|--|--------------|
| 1000X IRDye-labeled Secondary Antibodies | 1 x 24µl |
| 100X Triton X-100 | 1 x 0.5ml |
| 10X Blocking Buffer | 1 x 15ml |
| 10X Phosphate Buffered Saline | 1 x 100ml |
| 200X Primary Antibodies | 1 x 0.1ml |
| 400X Tween-20 | 1 x 2ml |
| Janus Green Stain | 1 x 11ml |
| Plate Seals | 2 units |

画像



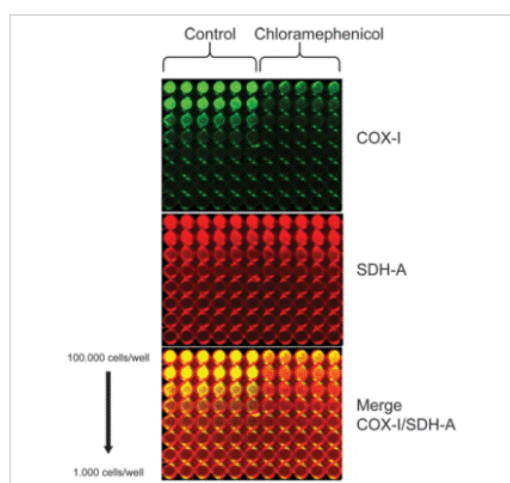
In-Cell ELISA - MitoBiogenesis™ In-Cell ELISA Kit
(IR) (ab110216)

Inhibition of mitochondrial biogenesis by chloramphenicol The IC₅₀ of a drug's effect on mitochondrial protein translation can be determined quickly using the MitoBiogenesis™ In-Cell ELISA Kit (IR). In this example, cells were seeded at 3000 cells/well, allowed to grow for 3 cell doublings in a drug dilution series and then the relative amounts of COX-I, and SDH-A were measured in each well. Chloramphenicol inhibits mtDNA-encoded COX-I protein synthesis relative to nuclear DNA-encoded SDH-A protein synthesis by 50% at 3.5 µM.



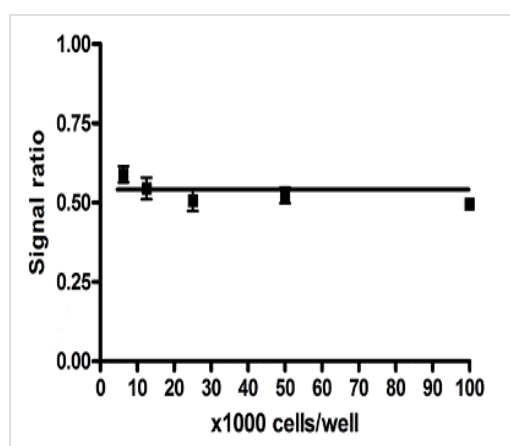
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Assay reproducibility demonstrated by experiments conducted on three separate days. HepG2 cells were grown for 7 days in the presence of 10 μ M chloramphenicol and then measured in duplicate on 3 different days using the MitoBiogenesis™ In-Cell ELISA Kit. The assay was able to record inhibition of mitochondrial biogenesis with an average intra-day CV of 2.3% and an average day-to-day CV of 1.9%.



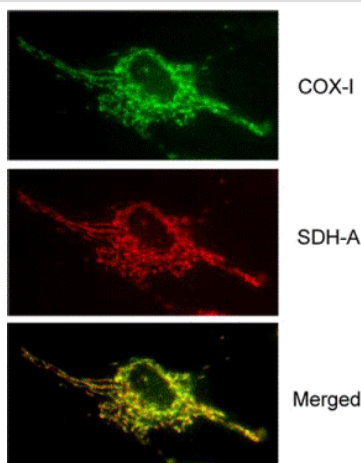
In-Cell ELISA - MitoBiogenesis™ In-Cell ELISA Kit
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Visual representation of inhibition of mtDNA-encoded protein synthesis. Visually-imaged levels of mtDNA-encoded COX-I are shown in the green 800 channel while levels of nuclear DNA-encoded SDH-A are shown in the red 700 channel. The merged image shows the relative ratios of COX-I/SDH-A protein expression in each microwell. Wells with normal levels of both proteins exhibit a yellow merged color due to approximately equal red and green signals. In contrast, wells with low levels of COX-I and normal levels of SDH-A exhibit an orange color due to the relative lack of green (COX-I) fluorescence. The specific inhibition of mtDNA-encoded protein synthesis by chloramphenicol is thus easily observed.



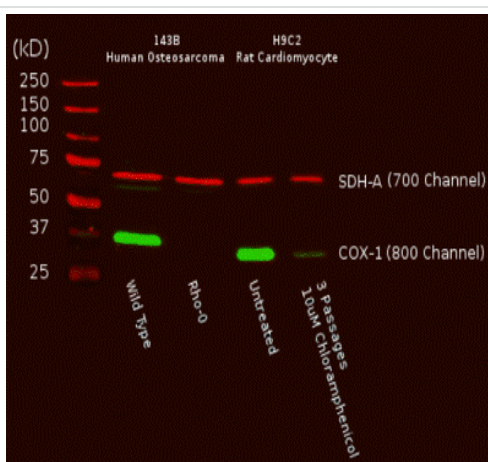
In-Cell ELISA - MitoBiogenesis™ In-Cell ELISA Kit
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Quantitative measurement of the COX-I/SDH-A protein expression ratio. At all cell concentrations, a constant ratio of mtDNA-encoded protein expression (COX-I) to nuclear DNA-encoded mitochondrial protein expression (SDH-A) 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.



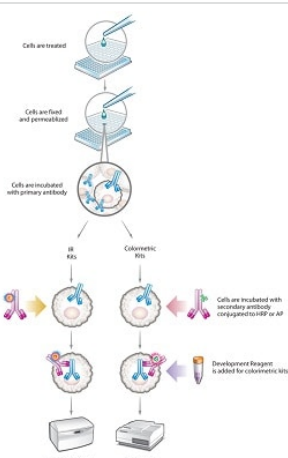
Immunocytochemistry - MitoBiogenesis™ In-Cell ELISA Kit (IR) (ab110216)

Antibody specificity demonstrated by immunocytochemistry. Two-color immunocytochemical labeling of cultured cells with the two MS642 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.



Western blot - MitoBiogenesis™ In-Cell ELISA Kit (IR) (ab110216)

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 both result in specific reduction of COX-I protein while nuclear DNA-encoded SDH-A is unaffected.



In-Cell ELISA - MitoBiogenesis™ In-Cell ELISA Kit (IR) (ab110216)

Cells are grown to ~80% confluency in a 96- or 384-well plate, a drug/other treatment is applied to stimulate a cellular response. The cells are then fixed and permeabilized, effectively "freezing" them. Primary antibodies are then added which bind to their intended targets within the mitochondria or other subcellular compartment. After incubation, the unbound primary antibodies are washed away and secondary antibodies are added. These secondaries are conjugated to either IRDyes® or to an enzyme label (HRP or AP) for the colorimetric versions of the assays. Unbound secondaries are washed away, reaction buffer is added for the colorimetric assays, and the signal is read on a suitable instrument for the kit type.

» [In-cell ELISA diagram](#) in PDF format

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