ab170194 – MitoBiogenesis™ Immunocytochemistry Kit

Instructions for Use

For the measurement of MTCO1 and SDHA Mouse, Rat, Cow, Human and Caenorhabditis elegans samples only.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's MitoBiogenesis™ Immmunocytochemistry kit is designed for to evaluate drug-induced effects on mitochondrial biogenesis early in the safety screening process. The assay allows for imaging and qualitative analysis between mtDNA encoded protein MTCO1 and nDNA encoded SDHA proteins in cells.

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.

The MitoBiogenesis™ Immunocytochemistry kit enables researchers to evaluate in-vivo levels of target proteins detected by highly specific, well-characterized monoclonal antibodies that are labeled with Alexa® fluorophores. Thus, minimizing potential changes during sample preparation and handling, such as preparation of protein extracts.

2. ASSAY SUMMARY

Culture cells on coverslips

Fix cells with 4% paraformaldehyde

Perform antigen retrieval and wash coverslips with 1X PBS

Permeabilize cells with PBS + 0.1% TritonX-100. Rinse coverslip with 1X PBS

Block cells in 10X Blocking Solution for 1 hour at room temperature

Incubate coverslips with 1X Antibody Solution overnight at 4ºC, protected from light. Rinse the coverslip with PBS + 0.1% Triton® X-100

(optional) Stain nucleus by adding DAPI in final wash and incubate 10 minutes at room temperature, protected from light.

Mount coverslips with antifade mounting medium onto slides; view slides with fluorescence microscope when mounting medium is sufficiently dried.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X PBS	100 mL	4ºC
100X Triton® X-100	500 µL	4ºC
100X Blocking Solution	10 mL	4ºC
Antigen Retrieval Buffer	25 mL	4ºC
100X Antibody Cocktail (Mouse anti-MTCO1 Alexa®488 and Mouse anti-SDHA Alexa®594 Antibody)	250 µL	4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cells of interest and media.
- Sterile 12-well tissue culture plate.
- Sterile glass coverslips.
- Broad tipped forceps.
- Coverglass staining jar.
- Microscope slides.
- Paraformaldehyde solution.
- DAPI (optional).
- Nanopure water or equivalent.
- Fluorescence Antifade Mounting Media.
- Water bath (capable of reaching 95^0C).
- Microscope with appropriate light source and filters to view Alexa® 488 and Alexa® 594 signals:

	Absorption Max (nm)	Emission Max (nm)	Emission Color
MTCO1 Alexa® 488	346	442	Green
SDHA Alexa® 594	590	617	Red

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Correct preparation of Antigen Retrieval is critical for antibody performance.
- Prepare 10X Blocking Solution fresh every time.
- Use of an adherent cell lines at subconfluent density is recommended to allow for cell spreading and clear visualization of mitochondria.

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1. 1X PBS

Prepare 1X PBS by adding 50 mL of 10X PBS into 450 mL of molecular biology grade water.

9.2. 1X Triton X-100

Prepare 1X Triton® X-100 by adding 500 µL 100X Triton® X 100 into 49.5 mL 1X PBS.

9.3. 10X Blocking Solution

Prepare 10X Blocking Solution by adding 8 mL 100X Blocking Solution into 72 mL 1X PBS.

9.4. 1X Antibody Solution

Prepare 1X Antibody Solution by adding 250 µL 100X Primary antibody cocktail to 24.75 mL 10X Blocking Solution.

10. SAMPLE PREPARATION

In a sterile 12-well tissue culture plate place an appropriately sized sterile glass coverslip at the bottom of each well needed for experiment.

10.1. Add 500 µL – 1.0 mL of cell suspension over each coverslip.

10.2. Grow cells at 37^0C in humidified 5% CO2 incubator until desired cell density is reached.

10.3. Perform desired treatment if desired.

10.4. Aspirate culture media.

10.5. Gently wash the cells twice with warm 1X PBS, do not let cells dry out.

10.6. **Fixation of Cells**

10.6.1. Aspriate final PBS wash and add 500 µL – 1.0 mL 4% paraformaldehyde in PBS to each well.

10.6.2. Incubate at room temperature for 20 minutes.

10.6.3. Wash coverslips three times with 1X PBS.

10.6.4. Coverslips can be stored in 1X PBS + 0.02% sodium azide at 4^0C for several days. Seal plate with parafilm to ensure coverslips do not dry out.

10.7. Proceed with assay procedure when desired.

11. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

  Note: Enough reagents are provided for 50 tests in a 500 µL volume in a standard 12-well tissue culture plate for each step.

11.1. Perform Antigen Retrieval

11.1.1. Preheat Antigen Retrieval Buffer to 95^{0}C. Typically done by heating buffer in a coverglass staining jar placed in a 95^{0}C waterbath.

11.1.2. Using broad tipped forceps, transfer coverslips to the coverglass staining jar containing heated antigen retrieval buffer.

11.1.3. Incubate coverslips for 10 minutes at 95^{0}C.

11.1.4. Transfer coverslips back to 12-well tissue culture plate. Be sure to place coverslip so cells are facing up.

11.1.5. Wash coverslips three times with 1X PBS.

11.2. Permeabilize Cells

11.2.1. Incubate cells in 1X Triton X-100 for 15 minutes at room temperature.

11.2.2. Wash coverslips three times with 1X PBS.

11.3. Blocking Cells

11.3.1. Incubate coverslips in 500 µL 10X Blocking Solution for 1 hour at room temperature.

11.4. Staining coverslips with antibodies

11.4.1. Remove 10X Blocking Solution.

11.4.2. Add 500 µL 1X Antibody Solution to each coverslip.

11.4.3. Incubate coverslips with Antibody Solution overnight at 4^0C or at room temperature for 2 hours, protected from light. Seal plate to prevent evaporation if incubating overnight.

11.4.4. Wash coverslips with 1X Triton X-100 for 5 minutes for a total of three times.

11.4.5. (Optional) After final wash dilute DAPI to a final concentration of 300 ng/mL in 10X Blocking Solution and incubate coverslips 10 minutes protected from light.

11.5. Mounting coverslips to microscope slide

11.5.1. Add drop of mounting media to slide.

11.5.2. Transfer coverslip and transfer to slide so that cells are facing down in mounting media.

11.5.3. Store slides in dark, until mounting media is dried

11.5.4. Visualize the cells using fluorescence capable microscope.

12. TYPICAL DATA

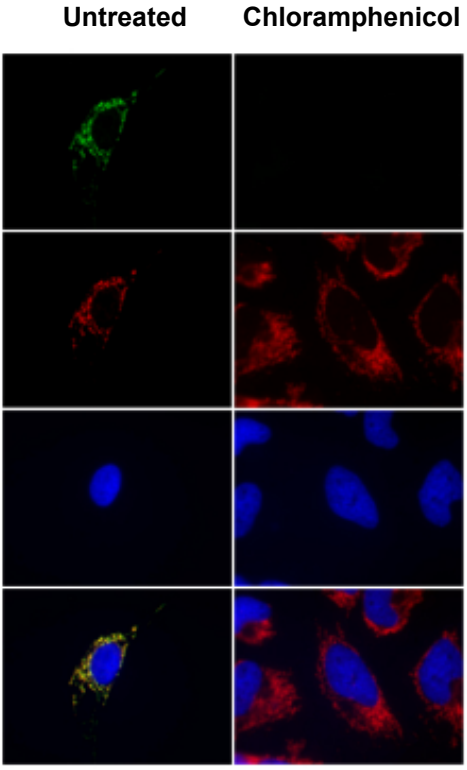

Figure 1. HeLa cell ICC using MitoBiogenesis™ Immunocytochemistry kit antibodies. Cells were treated with 10 µM chloramphenicol for a period of 6 days. Results of immunostaining of HeLa cells showing MTCO1 Alexa® 488 antibody (ab154477, grn) on untreated (A) and chloramphenicol treated (B). SDHA Alexa® 594 antibody (red) on untreated (C) and chloramphenicol treated (D). DAPI staining of nucleus (blue) on untreated (E) and chloramphenicol treated (F). Merge of color channels for untreated (G) and chloramphenicol treated (H) cells.

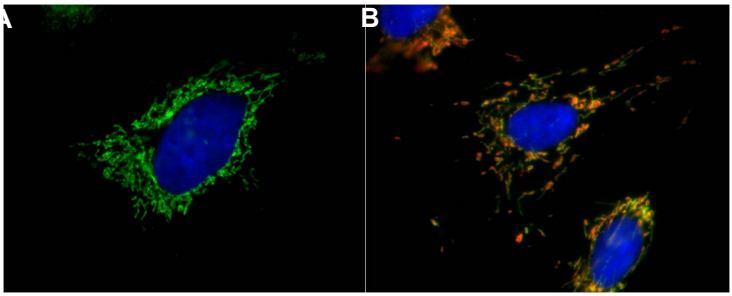

Figure 2. Immunocytochemistry localization of MTCO1 protein. A) Staining of HeL cells with Immunocytochemistry with HeLa cells (100X) were stained with anti-MTCO1 Alexa® 488 antibody (1.0 µg/mL, ab154477) in green and DAPI in blue, as a nuclear stain. B) Immunocytochemistry with HDFn (100X) cells were stained with Anti-MTOC1 Alexa® 488 antibody (1.0 µg/mL, ab154477) in green, Anti-HSP60 (1/1000, ab46798) as red, and DAPI in blue, as a nuclear stain. Secondary antibody used was goat anti-rabbit Dylight-594 (1/1000, ab96897).

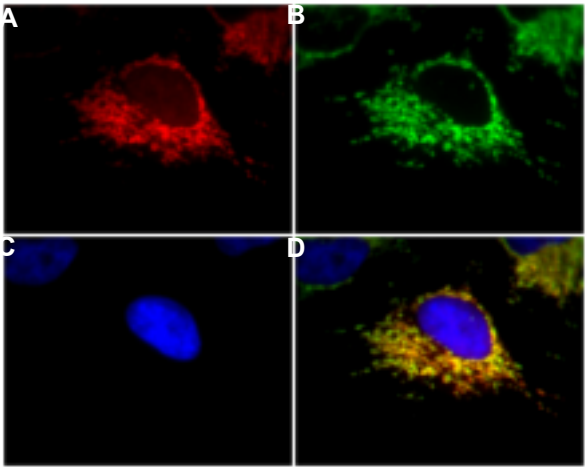

Figure 3. Immunocytochemistry localizaiton of SDHA protein. Immunocytochemistry with HeLa cells (100X). A) HeLa stained with anti-SDHA Alexa® 594 antibody (1.0 µg/mL). B) HeLa stained with Anti-HSP60 (1/1000, ab46798), Secondary antibody used was goat anti-rabbit Alexa® 488 (1/1000, ab150077). C) DAPI as nuclear stain (1/10000). D) Merge of color channels to show specificity of signal to mitochondria.

13. SPECIES REACTIVITY

The combined kit has so far been demonstrated with Human samples only. However, the mouse monoclonal antibodies provided in this kit have been shown to detect the expression of MTCO1 and SDHA proteins in Human, Mouse, Rat, Cow, and Caenorhabditis elegans samples.

14. ASSAY SPECIFICITY

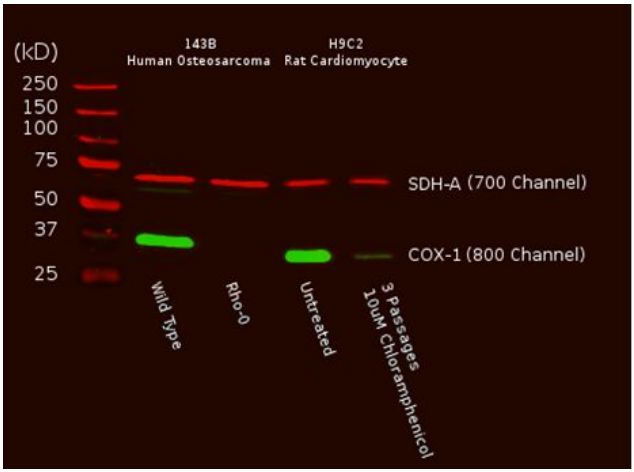

Figure 4. A Western blot of total cell protein. A total of (10 µg) from Human or rat cultured cells was probed with the primary and secondary antibodies and scanned with a LI-COR® Odyssey® imager. The two mitochondrial proteins targeted by the two primary mAbs were labeled and visualized specifically 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.

15. TROUBLESHOOTING

Problem	Solution
High Background	Incubate samples with Antibody Cocktail at room temperature for 2 hours protected from light on rocker plate 300-400 rpm, rather than the recommened 4^{0}C overnight.
	Increase the volume of 0.1% Triton® X-100 in PBS used in wash steps to maximum of sample well (about 2 mL for standard 12-well plate). Also increase the number of washes from to 5, place plate on rocker plate 300-400 rpm, and allow plate to incubate for 10 minutes between washes. After addition of Antibody Cocktail remember to protect samples from light.
Low Fluorescent Signal (unrelated to treatment)	Increase the concentration of Antibody Cocktail used up to a 5X solution, instead of the recommended 1X solution. This will alter the total number of samples that can be tested in the kit.
High Fluorescent Signal (unrelated to treatment)	Decrease the concentration of Antibody Cocktail used down to a 0.2X solution, instead of the recommended 1X solution. This will alter the total number of samples that can be tested in the kit.
	Incubate samples with Antibody Cocktail at room temperature for 2 hours protected from light on rocker plate 300-400 rpm, rather than the recommened 4^{0}C overnight.

16. **NOTES**

The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to use products containing Alexa Fluor® dyes for purposes other than research, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@lifetech.com.

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