**Overview**

**Product name**
Anti-MTCO1 antibody [1D6E1A8]

**Description**
Mouse monoclonal [1D6E1A8] to MTCO1

**Host species**
Mouse

**Specificity**
In mouse liver lysate a specific band below 37 kDa was detected.

**Tested applications**
Suitable for: ICC/IF, IHC-FoFr, IHC-P, WB, ICC, Flow Cyt, IHC-Fr

**Species reactivity**
Reacts with: Mouse, Rat, Goat, Cow, Human, Pig, Caenorhabditis elegans, Zebrafish, Quail, Rhesus monkey, Chinese hamster

**Immunogen**
Other Immunogen Type corresponding to Human MTCO1. Biochemically Purified Human Complex IV

**Positive control**
ICC/IF: Pig retinal pigment epithelial cells; Rat cerebellum primary cells. IHC-P: Human skeletal muscle and colon tissue; Rat pancreas tissue; Pig smooth muscle tissue. WB: Mouse, human, bovine and rat heart mitochondria lysate. Flow Cyt: HEK-293 cells.

**General notes**

**Western blot protocol advice:**
For best results with this antibody in Western blot, do not boil samples before loading onto the gel. Boiling of the sample will cause a loss of signal.

Hydrophobic intrinsic membrane proteins such as the core mtDNA-encoded proteins of the mitochondrial OXPHOS complexes tend to run faster in SDS-PAGE than predicted by their amino acid composition. This is likely due to incomplete unfolding of the protein and a more negative charge:mass ratio.

This antibody clone [1D6E1A8] is manufactured by Abcam. If you require a different buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com.

**Properties**

**Form**
Liquid

**Storage instructions**
Shipped at 4°C. Store at +4°C.

**Storage buffer**
Preservative: 0.02% Sodium azide
Constituent: HEPES buffered saline

**Purity**
Ammonium Sulphate Precipitation

**Purification notes**
Near homogeneity as judged by SDS-PAGE. The antibody was produced in vitro using hybridomas grown in serum-free medium, and then purified by biochemical fractionation.
Clonality: Monoclonal
Clone number: 1D6E1A8
Isotype: IgG2a
Light chain type: kappa

Applications

Our Abpromise guarantee covers the use of ab14705 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>✭✭✭✭✭</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 23840470</td>
</tr>
<tr>
<td>IHC-P</td>
<td>✭✭✭✭✭</td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
<tr>
<td>WB</td>
<td>✭✭✭✭✭</td>
<td>Use a concentration of 0.5 µg/ml. Detects a band of approximately 40 kDa (predicted molecular weight: 57 kDa).</td>
</tr>
<tr>
<td>ICC</td>
<td>✭✭✭✭✭</td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>✭✭✭✭✭</td>
<td>Use 1µg for 10^6 cells. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>✭✭✭✭✭</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Target

Function
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.

Pathway
Energy metabolism; oxidative phosphorylation.

Involvement in disease
Defects in MT-CO1 are a cause of Leber hereditary optic neuropathy (LHON) [MIM:535000]. LHON is a maternally inherited disease resulting in acute or subacute loss of central vision, due to optic nerve dysfunction. Cardiac conduction defects and neurological defects have also been described in some patients. LHON results from primary mitochondrial DNA mutations affecting the respiratory chain complexes.
Defects in MT-CO1 are a cause of anemia sideroblastic acquired idiopathic (AISA) [MIM:516030]; a disease characterized by inadequate formation of heme and excessive accumulation of iron in mitochondria.
Defects in MT-CO1 are a cause of mitochondrial complex IV deficiency (MT-C4D) [MIM:220110]; also known as cytochrome c oxidase deficiency. A disorder of the mitochondrial respiratory chain with heterogeneous clinical manifestations, ranging from isolated myopathy to severe multisystem disease affecting several tissues and organs. Features include hypertrophic cardiomyopathy,
hepatomegaly and liver dysfunction, hypotonia, muscle weakness, exercise intolerance, developmental delay, delayed motor development and mental retardation. A subset of patients manifest Leigh syndrome.

Defects in MT-CO1 are associated with recurrent myoglobinuria mitochondrial (RM-MT) [MIM:550500]. Recurrent myoglobinuria is characterized by recurrent attacks of rhabdomyolysis (necrosis or disintegration of skeletal muscle) associated with muscle pain and weakness, and followed by excretion of myoglobin in the urine.

Defects in MT-CO1 are a cause of deafness sensorineural mitochondrial (DFNM) [MIM:500008]. DFNM is a form of non-syndromic deafness with maternal inheritance. Affected individuals manifest progressive, postlingual, sensorineural hearing loss involving high frequencies.

Defects in MT-CO1 are a cause of colorectal cancer (CRC) [MIM:114500].

**Sequence similarities**

Belongs to the heme-copper respiratory oxidase family.

**Cellular localization**

Mitochondrion inner membrane.

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**Images**

![Western blot - Anti-MTCO1 antibody [1D6E1A8] (ab14705)](image)

- **All lanes**: Anti-MTCO1 antibody [1D6E1A8] (ab14705)
- **Lane 1**: Isolated mitochondria from Human heart at 5 µg
- **Lane 2**: Isolated mitochondria from Bovine heart at 1 µg
- **Lane 3**: Isolated mitochondria from Rat heart at 10 µg
- **Lane 4**: Isolated mitochondria from Mouse heart at 5 µg

**Secondary**

- All lanes: Goat Anti-Mouse IgG

**Predicted band size**: 57 kDa

**Observed band size**: 40 kDa

*why is the actual band size different from the predicted?*

**Additional bands at**: 70 kDa. We are unsure as to the identity of these extra bands.

Extra bands in the mouse sample (lane 4) are due to the reaction of the IgG-specific goat anti-mouse secondary antibody with residual mouse blood in the heart tissue, as it is very difficult to entirely remove the blood from these small organs.
Immunocytochemistry/ Immunofluorescence - Anti-MTCO1 antibody [1D6E1A8] (ab14705)
This image is courtesy of an Abreview submitted by Dr Vladimir Milenkovic

ab14705 staining MTCO1 in pig retinal pigment epithelial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% TX-100 and blocked with 5% serum for 20 minutes at 25°C. Samples were incubated with primary antibody (1/2000 in 1% goat serum, 0.1% TX100; PBS) for 16 hours at 4°C. An Alexa Fluor®546-conjugated Goat polyclonal to mouse IgG, dilution 1/500, was used as secondary antibody.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MTCO1 antibody [1D6E1A8] (ab14705)

IHC image of MTCO1 staining in human normal colon formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab14705, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
Overlay histogram showing HEK293 cells stained with ab14705 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab14705, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

ab14705 staining MTCO1 in rat cerebellum primary cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% TX-100 and blocked with 5% serum for 20 minutes at 25°C. Samples were incubated with primary antibody (1/2000 in 1% goat serum, 0.1% TX100; PBS) for 16 hours at 4°C. An Alexa Fluor®546-conjugated Goat polyclonal to mouse IgG, dilution 1/5000, was used as secondary antibody.

ab14705 staining rat pancreas sections by IHC-P. The tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed with citric acid pH 6. Blocking of the sample was done with 1% BSA for 10 minutes at 21°C, followed by staining with ab14705 at 1/1000 in TBS/BSA/azide for 2h at 21°C. A biotinylated goat anti-mouse polyclonal antibody at 1/200 was used as the secondary antibody. Positivity in exocrine glands appears to be intense at the cytoplasm of adjacent cells. The cells of the Islet of Langerhan to the right have a diffuse, punctate positivity.
ab14705 staining MTCO1 in skeletal muscle tissue by Immunohistochemistry (Frozen sections). Tissue sections were from a patient with a single large deletion of the mtDNA and show a mosaic of complex IV positive and complex IV negative fibers.

ab14705 staining MTCO1 in Human colon tissue by Immunohistochemistry (Frozen sections). Tissue sections from a normal ageing patient show complex IV negative crypts due to clonal expansion of colonic stem cells bearing mutations in the mtDNA-encoded gene for complex IV.

ab14705 staining MTCO1 in pig smooth muscle tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in citric acid. Samples were incubated with primary antibody (1/250 in TBS/BSA/azide) for 2 hours at 21°C. A Biotin-conjugated goat anti-mouse IgG polyclonal (1/200) was used as the secondary antibody.

The image shows the two smooth muscle layers (inner circular is above the outer longitudinal) of the small intestine. Between the two layers one can see Ganglion cells of Aurbach's plexus. Both muscle and ganglion cells are enriched with mitochondria, as expected.
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