# abcam

## Product datasheet

## Anti-ETFA antibody [2B11AE8] ab110316

## KO VALIDATED

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#### Overview

Product name Anti-ETFA antibody [2B11AE8]

**Description** Mouse monoclonal [2B11AE8] to ETFA

Host species Mouse

Tested applications

Suitable for: WB, ICC/IF, Flow Cyt, IHC-P

Species reactivity

Reacts with: Mouse, Rat, Cow, Human

**Immunogen** Recombinant full length protein. This information is considered to be commercially sensitive.

Positive control ICC: HeLa cells. Flow Cyt: HL-60 cells. IHC-P: Human heart tissue. WB: HepG2 and HEK-293T

whole cell lysates; Human liver mitochondria; Bovine heart mitochondria; Rat liver mitochondria;

Mouse liver mitochondria.

General notes

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact orders@abcam.com.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Product was previously marketed under the MitoSciences sub-brand.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.5

Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline

**Purity** Proprietary Purification

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Purification notes The antibody was produced in vitro using hybridomas grown in serum-free medium, and then

purified by biochemical fractionation. Purity >95% by SDS-PAGE.

ClonalityMonoclonalClone number2B11AE8

**Light chain type** lgG2b kappa

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab110316 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 1 µg/ml. Predicted molecular weight: 35 kDa.
ICC/IF		Use a concentration of 4 µg/ml.
Flow Cyt		Use a concentration of 1 $\mu$ g/ml. <u>ab170192</u> - Mouse monoclonal lgG2b, is suitable for use as an isotype control with this antibody.
IHC-P		Use a concentration of 5 μg/ml.

## **Target**

**Function** The electron transfer flavoprotein serves as a specific electron acceptor for several

dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-

ubiquinone oxidoreductase (ETF dehydrogenase).

Involvement in disease Glutaric aciduria 2A

**Sequence similarities**Belongs to the ETF alpha-subunit/FixB family.

**Domain** Domain I shares an identical polypeptide fold with the beta subunit ETFB though there is no

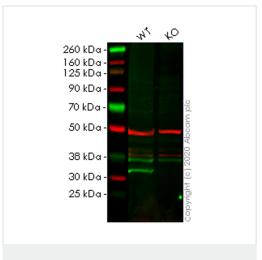
sequence similarity.

Post-translational modifications

The N-terminus is blocked.

Cellular localization Mitochondrion matrix.

## Images



Western blot - Anti-ETFA antibody [2B11AE8] (ab110316)

**All lanes :** Anti-ETFA antibody [2B11AE8] (ab110316) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2 : ETFA knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lysates/proteins at 40 µg per lane.

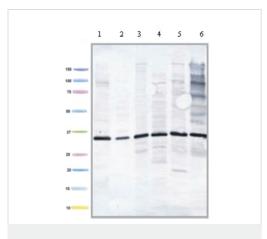
#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Predicted band size: 35 kDa
Observed band size: 35 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab110316 observed at 35 kDa. Red - loading control <u>ab52901</u> observed at kDa.

ab110316 Anti-ETFA antibody [2B11AE8] was shown to specifically react with ETFA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266513">ab266513</a> (knockout cell lysate <a href="mailto:ab257943">ab257943</a>) was used. Wild-type and ETFA knockout samples were subjected to SDS-PAGE. ab110316 and Anti-beta Tubulin [EP1331Y] - Microtubule Marker (<a href="mailto:ab52901">ab52901</a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216777">ab216777</a>) and Goat anti-Mouse lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216772">ab216777</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ETFA antibody [2B11AE8] (ab110316)

All lanes : Anti-ETFA antibody [2B11AE8] (ab110316) at 1  $\mu$ g/ml

Lane 1 : Human heart tissue

Lane 2: HepG2 whole cells

Lane 3: Human liver mitochondria

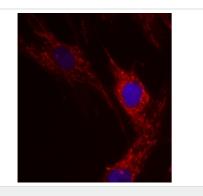
Lane 4: Bovine heart mitochondria

Lane 5: Rat liver mitochondria

Lane 6: Mouse liver mitochondria

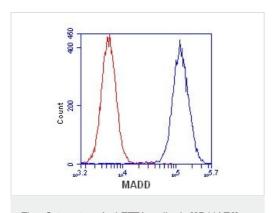
Lysates/proteins at 10 µg per lane.

Predicted band size: 35 kDa



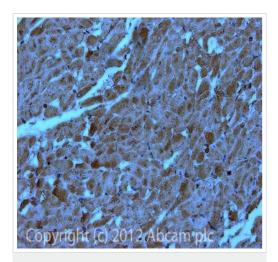
Immunocytochemistry/ Immunofluorescence - Anti-ETFA antibody [2B11AE8] (ab110316)

Immunocytochemistry image of stained HeLa cells. The cells were paraformaldehyde fixed (4%, 20 minutes) and Triton X-100 permeabilized (0.1%, 15 minutes). The cells were incubated with the antibody (ab110316, 4  $\mu$ g/mL) for 2 hours at room temperature or over night at 4°C. The secondary antibody was (red) Alexa Fluor® 594 goat anti-mouse lgG (H+L) at a 1/1000 dilution for 1 hour. 10% Goat serum was used as the blocking agent for all blocking steps. The target protein locates to the mitochondrial matrix



Flow Cytometry - Anti-ETFA antibody [2B11AE8] (ab110316)

HL-60 cells were stained with 1  $\mu$ g/mL ab110316 (blue) or an equal amount of an isotype control antibody (red) and analyzed by flow cytometry.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ETFA antibody
[2B11AE8] (ab110316)

IHC image of ETFA staining in Human heart 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 ab110316, 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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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