

## Product datasheet

# Anti-AIF antibody [E20] - Mitochondrial Marker ab32516

KO VALIDATED Recombinant RabMAb

★★★★☆ 5 Abreviews 64 References 7 Images

### Overview

Product name	Anti-AIF antibody [E20] - Mitochondrial Marker
Description	Rabbit monoclonal [E20] to AIF - Mitochondrial Marker
Host species	Rabbit
Tested applications	<b>Suitable for:</b> Flow Cyt (Intra), ICC/IF, IHC-Fr, WB, IHC-P, IP
Species reactivity	<b>Reacts with:</b> Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T and K562 cell lysate. IHC-P: Human cervical carcinoma tissue.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA</p>
Clonality	Monoclonal
Clone number	E20
Isotype	IgG

## Applications

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32516 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
Flow Cyt (Intra)		1/50. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
IHC-Fr	★★★★★ (1)	Use at an assay dependent concentration. PubMed: 23118224
WB	★★★★★ (3)	1/1000. Detects a band of approximately 67 kDa (predicted molecular weight: 67 kDa).
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

## Target

### Function

Probable oxidoreductase that has a dual role in controlling cellular life and death; during apoptosis, it is translocated from the mitochondria to the nucleus to function as a proapoptotic factor in a caspase-independent pathway, while in normal mitochondria, it functions as an antiapoptotic factor via its oxidoreductase activity. The soluble form (AIFsol) found in the nucleus induces 'parthanatos' i.e., caspase-independent fragmentation of chromosomal DNA. Interacts with EIF3G, and thereby inhibits the EIF3 machinery and protein synthesis, and activates caspase-7 to amplify apoptosis. Plays a critical role in caspase-independent, pyknotic cell death in hydrogen peroxide-exposed cells. Binds to DNA in a sequence-independent manner.

### Involvement in disease

Defects in AIFM1 are the cause of combined oxidative phosphorylation deficiency type 6 (COXPD6) [MIM:300816]. It is a mitochondrial disease resulting in a neurodegenerative disorder characterized by psychomotor delay, hypotonia, areflexia, muscle weakness and wasting.

### Sequence similarities

Belongs to the FAD-dependent oxidoreductase family.

### Post-translational modifications

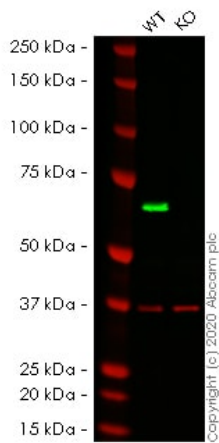
Under normal conditions, a 54-residue N-terminal segment is first proteolytically removed during or just after translocation into the mitochondrial intermembrane space (IMS) by the mitochondrial processing peptidase (MPP) to form the inner-membrane-anchored mature form (AIFmit). During apoptosis, it is further proteolytically processed at amino-acid position 101 leading to the generation of the mature form, which is confined to the mitochondrial IMS in a soluble form (AIFsol). AIFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis in a caspase-independent manner.

### Cellular localization

Mitochondrion intermembrane space. Mitochondrion inner membrane. Cytoplasm. Nucleus. Cytoplasm > perinuclear region. Proteolytic cleavage during or just after translocation into the mitochondrial intermembrane space (IMS) results in the formation of an inner-membrane-anchored mature form (AIFmit). During apoptosis, further proteolytic processing leads to a mature

form, which is confined to the mitochondrial IMS in a soluble form (AIFsol). AIFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis. Colocalizes with EIF3G in the nucleus and perinuclear region.

## Images



Western blot - Anti-AIF antibody [E20] - Mitochondrial Marker (ab32516)

**All lanes :** Anti-AIF antibody [E20] - Mitochondrial Marker (ab32516) at 1/1000 dilution

**Lane 1 :** Wild-type HEK-293T cell lysate

**Lane 2 :** AIFM1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

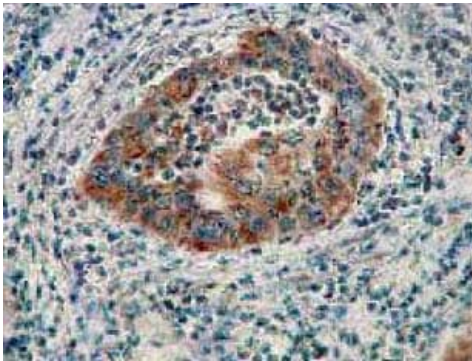
Performed under reducing conditions.

**Predicted band size:** 67 kDa

**Observed band size:** 67 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab32516 observed at 67 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

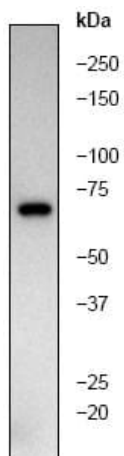
ab32516 was shown to react with AIF in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line [ab266347](#) (knockout cell lysate [ab256834](#)) was used. Wild-type HEK-293T and AIFM1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32516 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AIF antibody [E20] - Mitochondrial Marker (ab32516)

Ab32516, at a 1/500 dilution, staining AIF in paraffin embedded human cervical carcinoma tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

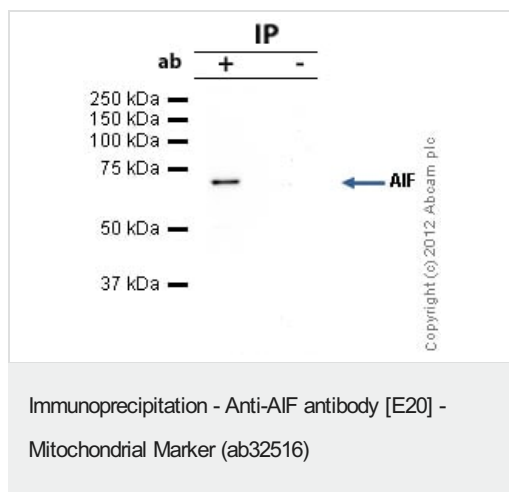


Western blot - Anti-AIF antibody [E20] - Mitochondrial Marker (ab32516)

Anti-AIF antibody [E20] - Mitochondrial Marker (ab32516) at 1/1000 dilution + K562 cell lysate

**Predicted band size:** 67 kDa

**Observed band size:** 67 kDa



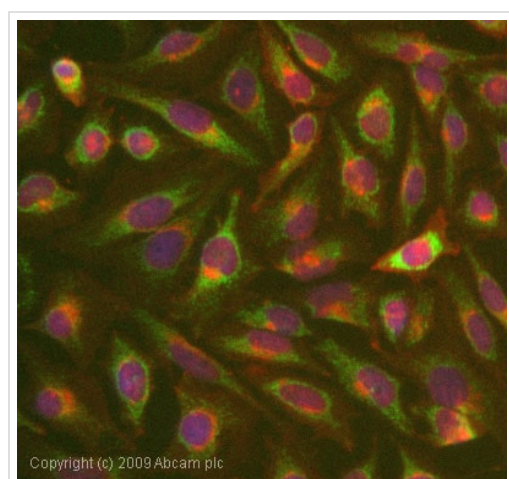
AIF was immunoprecipitated using 0.5mg K562 whole cell extract, 5µg of Rabbit monoclonal to AIF and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, K562 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

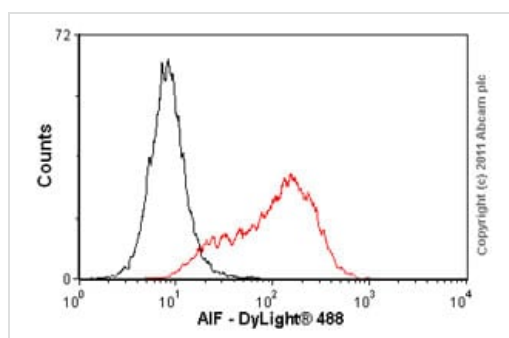
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab32516.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) ([ab99697](#)).

Band: 67kDa: AIF



ICC/IF image of ab32516 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32516, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Overlay histogram showing K562 cells stained with ab32516 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab32516, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) ([ab96899](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in K562 cells fixed with methanol (5 min)/permeabilized with 0.1% PBS-Tween used under the same conditions.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-AIF antibody [E20] - Mitochondrial Marker  
(ab32516)

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

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- Extensive multi-media technical resources to help you
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