abcam

Product datasheet

Anti-AIF antibody [E20] - BSA and Azide free ab220215

KO 評価済 リコンビナント RabMAb

画像数 6

製品の概要

製品名	Anti-AIF antibody [E20] - BSA and Azide free
製品の詳細	Rabbit monoclonal [E20] to AIF - BSA and Azide free
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P, IHC-Fr
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HEK-293T and K562 cell lysate. IHC-P: Human cervical carcinoma tissue.
特記事項	ab220215 is the carrier-free version of **ab32516**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.20 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	E20
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab220215の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

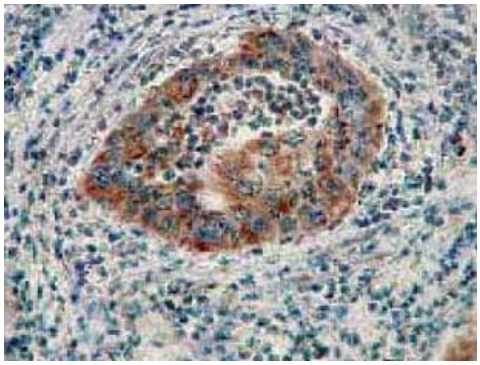
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 67 kDa (predicted molecular weight: 67 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IHC-Fr		Use at an assay dependent concentration.

ターゲット情報

機能	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 casapse-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.
関連疾患	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.

配列類似性	Belongs to the FAD-dependent oxidoreductase family.
翻訳後修飾	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.
細胞内局在	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.

画像

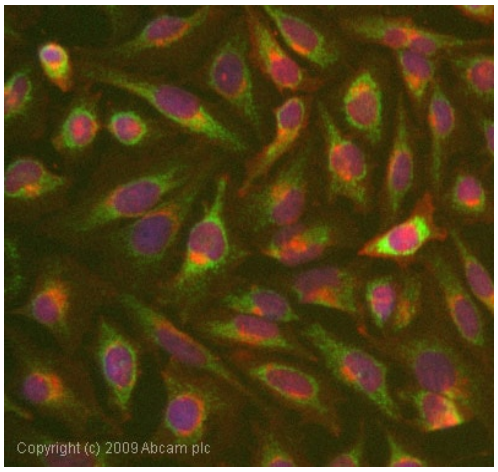


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AIF antibody [E20] - BSA and Azide free (ab220215)

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.

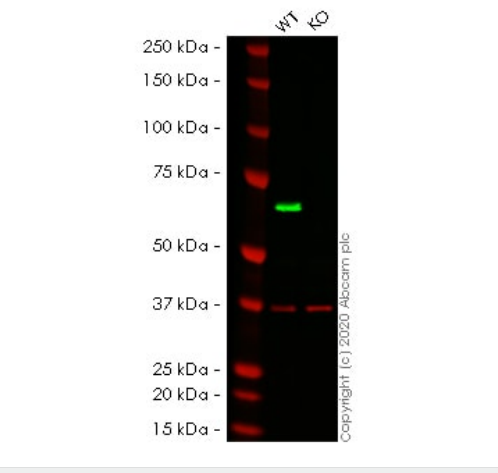
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32516**).



Immunocytochemistry/ Immunofluorescence - Anti-AIF antibody [E20] - BSA and Azide free (ab220215)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32516**).



Western blot - Anti-AIF antibody [E20] - BSA and Azide free (ab220215)

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.

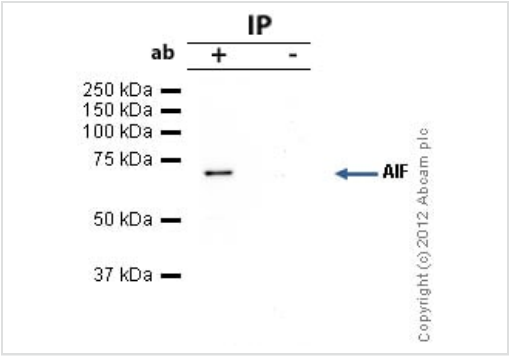
Predicted band size: 67 kDa
Observed band size: 67 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab32516**).

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.

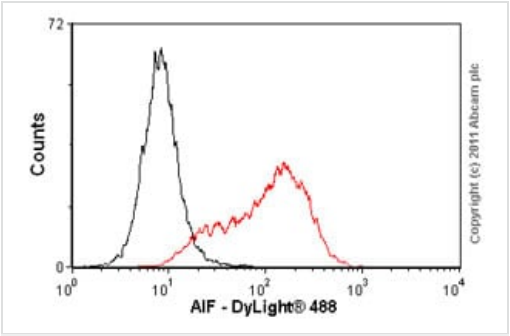
ab32516 was shown to react with AIF in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266347** (knockout cell lysate **ab256834**) was used. Wild-type HEK-293T and AIFM1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked 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.



Immunoprecipitation - Anti-AIF antibody [E20] - BSA and Azide free (ab220215)

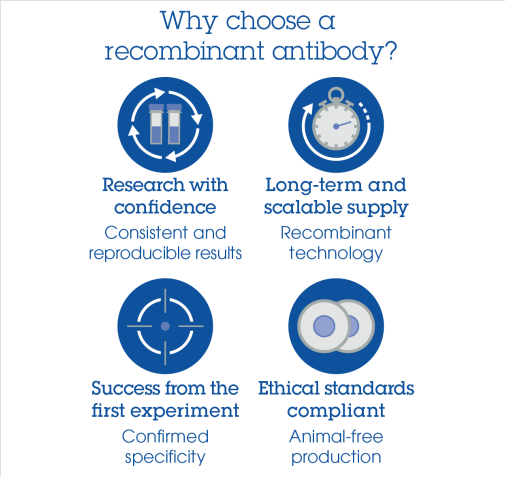
AIF was immunoprecipitated using 0.5mg K562 whole cell extract, 5µg of Rabbit monoclonal to AIF and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, K562 whole cell extract lysate diluted in RIPA 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
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32516**).



Flow Cytometry (Intracellular) - Anti-AIF antibody [E20] - BSA and Azide free (ab220215)

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/1x10^6 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32516**).



Anti-AIF antibody [E20] - BSA and Azide free

(ab220215)

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