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Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5)

Catalog # ABO14853

Specification

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	O95831
Host	**Mouse**
Isotype	**Mouse IgG1**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Additional Information

Gene ID 9131

Other Names
Apoptosis-inducing factor 1, mitochondrial, 1.6.99.-, Programmed cell death protein 8, AIFM1 (HGNC:8768), AIF, PDCD8

Calculated MW
70 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml
 Immunofluorescence, 2 µg/ml
 Immunocytochemistry/Immunofluorescence, 5 µg/ml
 Flow Cytometry, 1-3 µg/1x10^6 cells

Subcellular Localization
Mitochondrion intermembrane space. Mitochondrion inner membrane. Nucleus. Cytoplasm. Perinuclear region.

Tissue Specificity
Detected in muscle and skin fibroblasts (at protein level). Isoform 5 is frequently down-regulated in human cancers.

Protein Name
Apoptosis-inducing factor 1, mitochondrial

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.

Immunogen
A synthetic peptide corresponding to a sequence at the C-terminus of human AIF, identical to the related mouse and rat sequences.

Cross Reactivity
No cross-reactivity with other proteins.

Storage	**Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.**

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Protein Information

Name AIFM1 (HGNC:8768)

Synonyms AIF, PDCD8

Function
Functions both as NADH oxidoreductase and as regulator of apoptosis (PubMed:17094969, PubMed:20362274, PubMed:23217327, PubMed:33168626). In response to apoptotic stimuli, it is released from the mitochondrion intermembrane space into the cytosol and to the nucleus, where it functions as a proapoptotic factor in a caspase- independent pathway (PubMed:20362274). Release into the cytoplasm is mediated upon binding to poly-ADP-ribose chains (By similarity). The soluble form (AIFsol) found in the nucleus induces 'parthanatos' i.e. caspase-independent fragmentation of chromosomal DNA (PubMed:20362274). Binds to DNA in a sequence-independent manner (PubMed:27178839). Interacts with EIF3G, and thereby inhibits the EIF3 machinery and protein synthesis, and activates caspase-7 to amplify apoptosis (PubMed:17094969). Plays a critical role in caspase-independent, pyknotic cell death in hydrogen peroxide-exposed cells (PubMed:19418225). In contrast, participates in normal mitochondrial metabolism. Plays an important role in the regulation of respiratory chain biogenesis by interacting with CHCHD4 and controlling CHCHD4 mitochondrial import (PubMed:26004228).

Cellular Location
Mitochondrion intermembrane space. Mitochondrion inner membrane. Cytoplasm. Nucleus. Cytoplasm, perinuclear region. Note=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 (PubMed:15775970). Release into the cytoplasm is mediated upon binding to poly-ADP-ribose chains (By similarity) Translocation into the nucleus is promoted by interaction with (auto- poly-ADP-ribosylated) processed form of PARP1 (PubMed:33168626) Colocalizes with EIF3G in the nucleus and perinuclear region (PubMed:17094969). {ECO:0000250|UniProtKB:Q9Z0X1, ECO:0000269|PubMed:15775970, ECO:0000269|PubMed:17094969, ECO:0000269|PubMed:33168626} [Isoform 4]: Mitochondrion.

Cytoplasm, cytosol. Note=In pro-apoptotic conditions, is released from mitochondria to cytosol in a calpain/cathepsin-dependent manner.

Tissue Location
Expressed in all tested tissues (PubMed:16644725). Detected in muscle and skin fibroblasts (at protein level) (PubMed:23217327). Expressed in osteoblasts (at protein level) (PubMed:28842795). [Isoform 4]: Expressed in all tested tissues except brain.

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Images

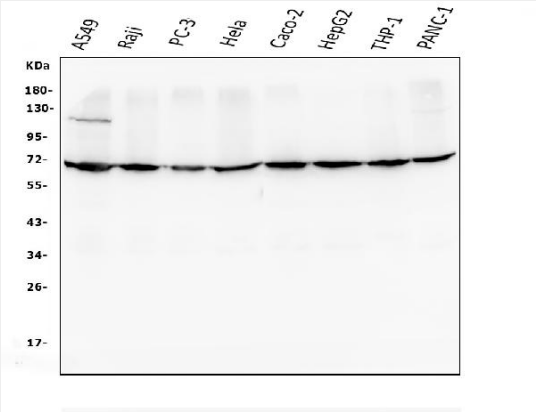

Figure 1. Western blot analysis of AIF using anti-AIF antibody (M01571-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: A549 whole cell lysates,
Lane 2: Raji whole cell lysates,
Lane 3: PC-3 whole cell lysates,
Lane 4: Hela whole cell lysates,
Lane 5: Caco-2 whole cell lysates,
Lane 6: HepG2 whole cell lysates,
Lane 7: THP-1 whole cell lysates,
Lane 8: PANC-1 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-AIF antigen affinity purified monoclonal antibody (Catalog # M01571-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5

hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for AIF at approximately 70KD. The expected band size for AIF is at 70KD.

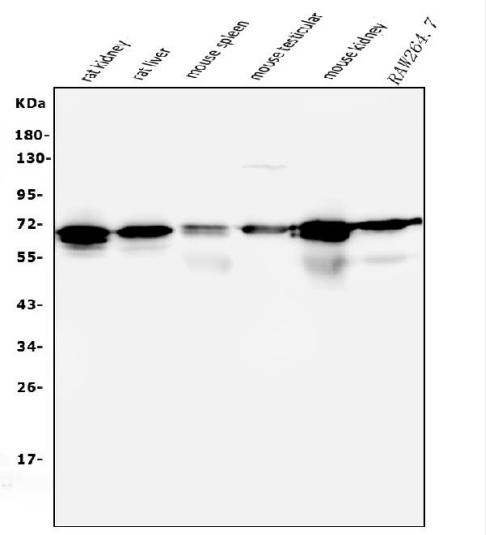

Figure 2. Western blot analysis of AIF using anti-AIF antibody (M01571-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat kidney tissue lysates,
Lane 2: rat liver tissue lysates,
Lane 3: mouse spleen lysates,
Lane 4: mouse testicular tissue lysates,
Lane 5: mouse kidney tissue lysates,
Lane 6: RAW264.7 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-AIF antigen affinity purified monoclonal antibody (Catalog # M01571-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for AIF at approximately 70KD. The expected band size for AIF is at 70KD.

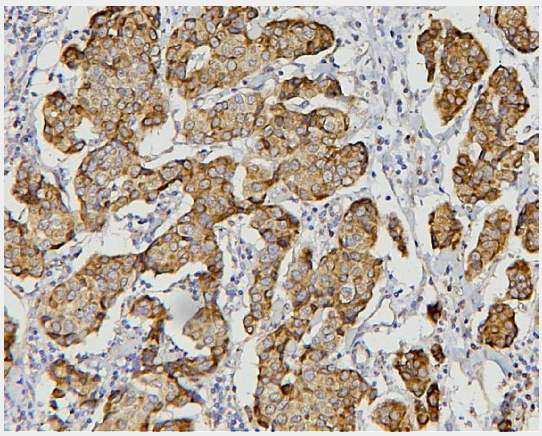

Figure 3. IHC analysis of AIF using anti-AIF antibody (M01571-1).
AIF was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated

antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-AIF Antibody (M01571-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

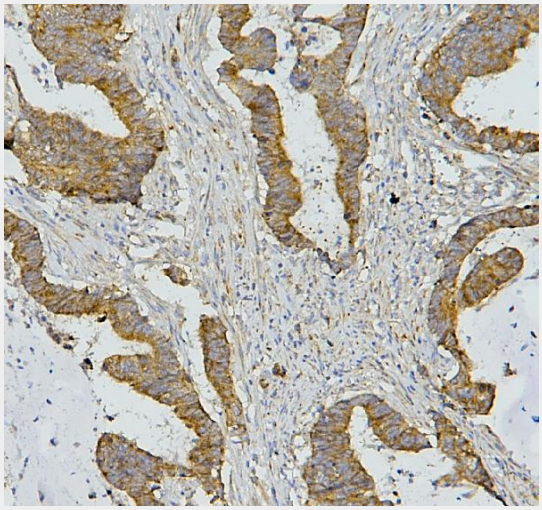

Figure 4. IHC analysis of AIF using anti-AIF antibody (M01571-1).
AIF was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-AIF Antibody (M01571-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

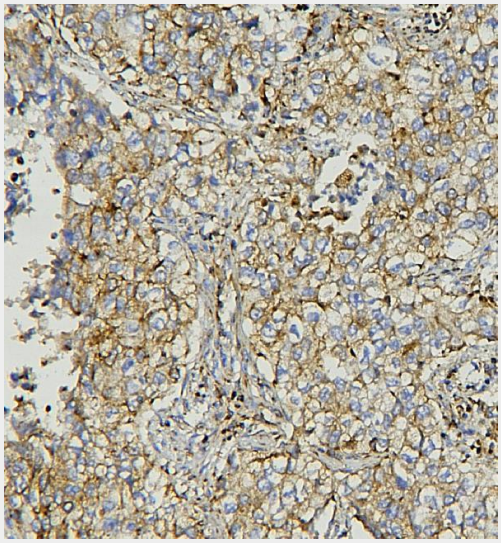

Figure 5. IHC analysis of AIF using anti-AIF antibody (M01571-1).
AIF was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-AIF Antibody (M01571-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

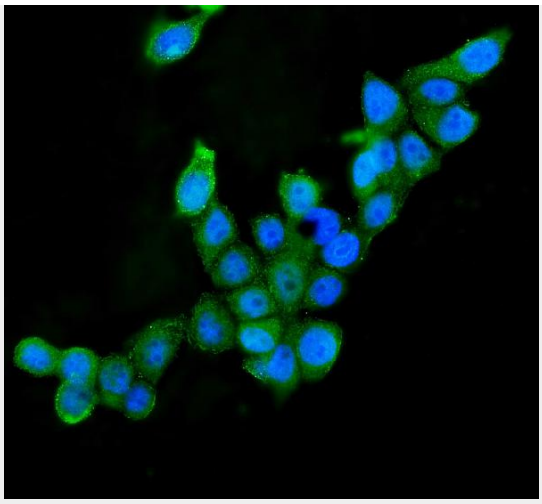

Figure 5. IF analysis of AIF using anti-AIF antibody (M01571-1).
AIF was detected in immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-AIF Antibody (M01571-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

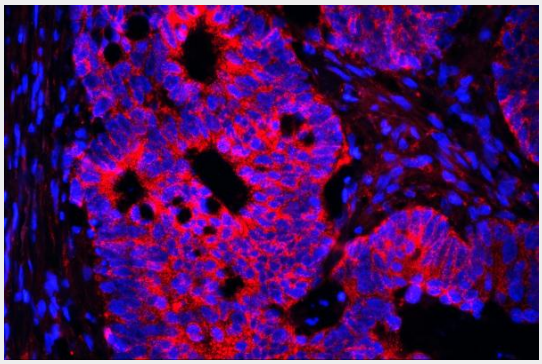

Figure 6. IF analysis of AIF using anti-AIF antibody (M01571-1).
AIF was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-AIF Antibody (M01571-1) overnight at 4°C. DyLight®550 Conjugated Goat Anti-Mouse IgG (BA1133) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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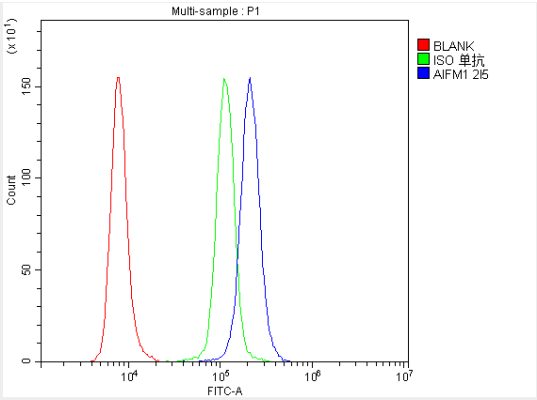

Figure 7. Flow Cytometry analysis of Raji cells using anti-AIF antibody (M01571-1).
Overlay histogram showing Raji cells stained with M01571-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-AIF Antibody (M01571-1,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-AIF/AIFM1 Antibody Picoband™ (monoclonal, 2I5) - Background

Apoptosis-inducing factor 1, mitochondrial, also known as AIF or PDCD8 is a protein that in humans is encoded by the AIFM1 gene. AIFM1 gene is mapped to Xq26.1 based on an alignment of the AIFM1 sequence with the genomic sequence. This gene encodes a flavoprotein essential for nuclear disassembly in apoptotic cells, and it is found in the mitochondrial intermembrane space in healthy cells. Induction of apoptosis results in the translocation of this protein to the nucleus where it affects chromosome condensation and fragmentation. In addition, this gene product induces mitochondria to release the apoptogenic proteins cytochrome c and caspase-9. Mutations in this gene cause combined oxidative phosphorylation deficiency 6, which results in a severe mitochondrial encephalomyopathy. A related pseudogene has been identified on chromosome 10.