abcam

Product datasheet

Anti-Fas antibody [EPR24898-74] ab271016

Recombinant RabMAb

1 References 11 Images

Overview

Product name Anti-Fas antibody [EPR24898-74]

Description Rabbit monoclonal [EPR24898-74] to Fas

Host species Rabbit

Tested applications Suitable for: WB, IHC-Fr, IP, IHC-P

Unsuitable for: Flow Cyt (Intra)

Reacts with: Mouse Species reactivity

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse spleen, skin, liver, heart, lung, thymus and stomach tissue lysates; A20 and J774A.1

whole cell lysates. IHC-P: Mouse thymus, spleen and lung cancer tissue. IHC-Fr: Mouse thymus

and liver (fresh) tissue. IP: Mouse thymus tissue lysate.

General notes 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**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR24898-74

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab271016 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		1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 37 kDa).
IHC-Fr		1/100.
IP		1/30.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

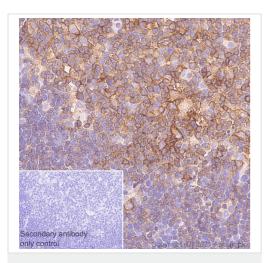
Application notes

Is unsuitable for Flow Cyt (Intra).

Target

Function	Receptor for TNFSF6/FASLG. The adapter molecule FADD recruits caspase-8 to the activated receptor. The resulting death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. FAS-mediated apoptosis may have a role in the induction of peripheral tolerance, in the antigen-stimulated suicide of mature T-cells, or both. The secreted isoforms 2 to 6 block apoptosis (in vitro).
Tissue specificity	lsoform 1 and isoform 6 are expressed at equal levels in resting peripheral blood mononuclear cells. After activation there is an increase in isoform 1 and decrease in the levels of isoform 6.
Involvement in disease	Defects in FAS are the cause of autoimmune lymphoproliferative syndrome type 1A (ALPS1A) [MIM:601859]; also known as Canale-Smith syndrome (CSS). ALPS is a childhood syndrome involving hemolytic anemia and thrombocytopenia with massive lymphadenopathy and splenomegaly.
Sequence similarities	Contains 1 death domain. Contains 3 TNFR-Cys repeats.
Domain	Contains a death domain involved in the binding of FADD, and maybe to other cytosolic adapter proteins.
Cellular localization	Secreted and Cell membrane.

Images

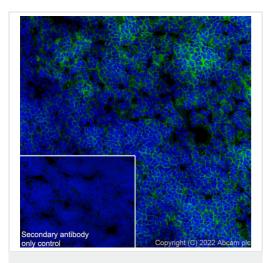


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fas antibody
[EPR24898-74] (ab271016)

Immunohistochemical analysis of paraffin-embedded sections of mouse thymus labelling Fas with ab271016 at 1/100 dilution followed by ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous staining on mouse thymus. Counterstained with Hematoxylin.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. The section was incubated with ab271016 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

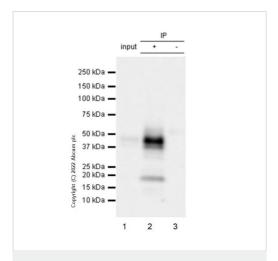
Secondary antibody only control: PBS instead of primary antibody followed by LeicaDS9800 (Bond™ Polymer Refine Detection) as a secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-Fas antibody [EPR24898-74] (ab271016)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse thymus (fresh) tissue labelling Fas with ab 271016 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (**ab150081**) at 1/1000 dilution (Green). Positive staining on mouse thymus. The nuclear counter stain was DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody follwed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) at 1/1000 dilution.



Immunoprecipitation - Anti-Fas antibody [EPR24898-74] (ab271016)

Fas was immunoprecipitated from mouse thymus tissue lysate with ab271016 at 1/30 dilution (2 μ g in 0.35 mg lysates). Western blot was performed from innunoprecipitate using ab271016 at 1/1000 dilution. Secondary antibody VeriBlot for IP secondary antibody(HRP)(ab131366) at 1/5000 dilution.

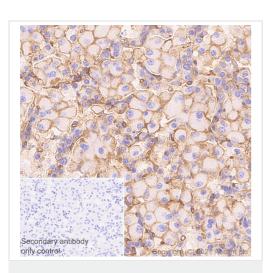
Lane 1: Mouse thymus tissue lysate (Input) 10 μg

Lane 2: ab271016 IP in Mouse thymus tissue lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab271016 in mouse thymus tissue lysate

Exposure time: 5.5 seconds

The 20KDa band could be a degradation or cleavage product, as demonstrated by the use of fresh lysate in the WB data.

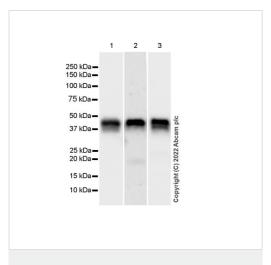


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fas antibody
[EPR24898-74] (ab271016)

Immunohistochemical analysis of paraffin-embedded sections of mouse lung cancer labelling Fas with ab271016 at 1/100 dilution followed by ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous staining on mouse lung cancer tissue. Counterstained with Hematoxylin.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. The section was incubated with ab271016 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: PBS instead of primary antibody followed by LeicaDS9800 (Bond™ Polymer Refine Detection) as a secondary antibody.



Western blot - Anti-Fas antibody [EPR24898-74] (ab271016)

All lanes : Anti-Fas antibody [EPR24898-74] (ab271016) at 1/1000 dilution

Lane 1 : Mouse liver tissue lysate
Lane 2 : Mouse heart tissue lysate
Lane 3 : Mouse lung tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 37 kDa **Observed band size:** 45 kDa

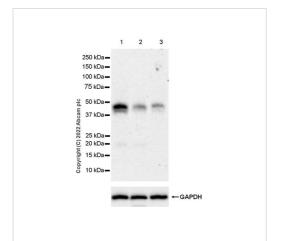
5% NFDM/TBST was used as a blocking and diluting buffer.

Exposure times:

Lane 1, 3: 26 seconds;

Lane 2:136 seconds.

The observed MW are consistent with what has been described in the literature (PMID: 28883393).



Western blot - Anti-Fas antibody [EPR24898-74] (ab271016)

All lanes : Anti-Fas antibody [EPR24898-74] (ab271016) at 1/1000 dilution

Lane 1: Mouse spleen tissue lysate

Lane 2: Mouse stomach tissue lysate

Lane 3: Mouse skin tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 37 kDa **Observed band size:** 45 kDa Exposure time: 3 minutes

5% NFDM/TBST was used as blocking and diluting buffer.

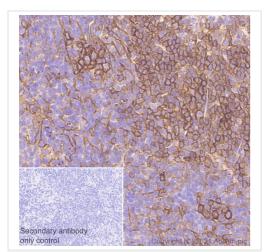
The observed MW are consistent with what has been described in the literature (PMID: 28883393).

Low expression: stomach, skin (PMID: 31582729; PMID: 11106570).

Immunohistochemical analysis of paraffin-embedded sections of mouse spleen labelling Fas with ab271016 at 1/100 dilution followed by ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous staining on mouse spleen. Counterstained with Hematoxylin.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. The section was incubated with ab271016 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: PBS instead of primary antibody followed by LeicaDS9800 (Bond™ Polymer Refine Detection) as a secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fas antibody
[EPR24898-74] (ab271016)

250 kDa 250 kDa 250 kDa 150 kDa = 150 kDa-100 kDa 100 kDa 100 kDa-75 kDa-75 kDa= 75 kDa= 50 kDa 50 kDa= 37 kDa= 37 kDa 37 kDa-25 kDa = 20 kDa = 25 kDa= 25 kDa-20 kDa-15 kDa= 15 kDa-15 kDa= 10 kDa= 10 kDa= 10 kDa

Western blot - Anti-Fas antibody [EPR24898-74] (ab271016)

All lanes : Anti-Fas antibody [EPR24898-74] (ab271016) at 1/1000 dilution

Lane 1: Mouse thymus tissue lysate

Lane 2: Mouse thymus tissue fresh lysate

Lane 3: A20 (mouse reticulum sarcoma B lymphocyte) whole cell fresh lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 37 kDa **Observed band size:** 45 kDa

5% NFDM/TBST was used as a blocking and diluting buffer.

Exposure times:

Lane 1-2:37 seconds;

Lane 3:81 seconds.

Lanes 2-3 lysate was made fresh and used immediately to minimize protein degradation.

The 20 kDa band in lane 1 could be a degradation or cleavage product.

1 2 3

250 kDa - 150 kDa - 150 kDa - 75 kDa - 75 kDa - 75 kDa - 75 kDa - 20 kDa - 20

Western blot - Anti-Fas antibody [EPR24898-74] (ab271016)

All lanes : Anti-Fas antibody [EPR24898-74] (ab271016) at 1/1000 dilution

Lane 1 : J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate

Lane 2 : A20 (mouse reticulum sarcoma B lymphocyte) whole cell lysate

Lane 3: L929 (mouse connective tissue fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 37 kDa **Observed band size:** 45 kDa

5% NFDM/TBST was used as a blocking and diluting buffer.

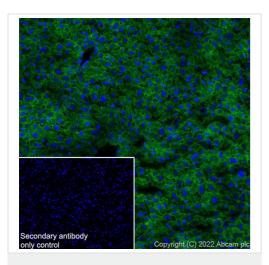
Exposure times:

Lane 1, 3: 92 seconds;

Lane 2:10 seconds.

Low expression: L929 (PMID: 7523113).

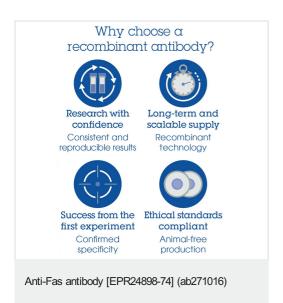
The 20 kDa band in lane 1-2 could be a degradation or cleavage product.



Immunohistochemistry (Frozen sections) - Anti-Fas antibody [EPR24898-74] (ab271016)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse liver (fresh) tissue labelling Fas with ab 271016 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (<u>ab150081</u>) at 1/1000 dilution (Green). Positive staining on mouse liver. The nuclear counter stain was DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) at 1/1000 dilution.



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