abcam

Product datasheet

Anti-Fas antibody [EPR5700] - BSA and Azide free ab178076





RabMAb

7 Images

Overview

Product name Anti-Fas antibody [EPR5700] - BSA and Azide free

Description Rabbit monoclonal [EPR5700] to Fas - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF

Unsuitable for: Flow Cyt (Intra) or IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa cell lysate. IHC-P: Human tonsil tissue. ICC/IF: Raji cells

General notes ab178076 is the carrier-free version of **ab133619**.

Our <u>carrier-free</u> 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.

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. Do Not Freeze.

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Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clone number Monoclonal EPR5700

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab178076 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 at an assay dependent concentration. Detects a band of approximately 45 kDa (predicted molecular weight: 37 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt (Intra) or IP.

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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 Isoform 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 diseaseDefects 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.

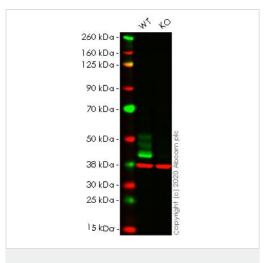
Contains 3 TNFR-Cys repeats.

DomainContains a death domain involved in the binding of FADD, and maybe to other cytosolic adapter

proteins.

Cellular localization Secreted and Cell membrane.

Images



Western blot - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

All lanes : Anti-Fas antibody [EPR5700] (**ab133619**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: FAS knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

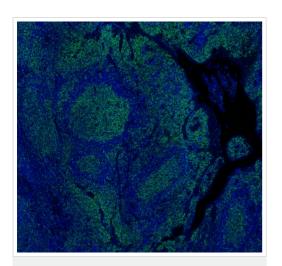
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (ab133619).

Lanes 1-2: Merged signal (red and green). Green - <u>ab133619</u> observed at 37 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

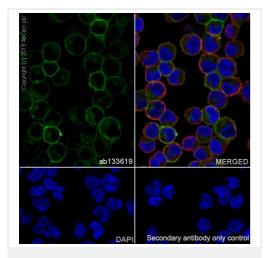
ab133619 Anti-Fas antibody [EPR5700] was shown to specifically react with Fas in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265260 (knockout cell lysate ab256911) was used. Wild-type and Fas knockout samples were subjected to SDS-PAGE. ab133619 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 and 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 paraffinembedded sections) - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

Anti-Fas antibody [EPR5700] (ab133619)

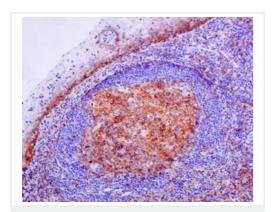
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling Fas with ab133619 at a dilution of 1:500. Heat mediated antigen retrieval was performed using AR9 antigen retrieval solution, and microwave treatment for 15 min at 20% power. Anti-Rabbit/Mouse HRP polymer (PerkinElmer Opal Polymer HRP Ms Plus Rb) was used as secondary antibody. Opal tyramide amplification was performed using Opal 520 fluorophore. Counterstained with DAPI stain. Image scanned with Vectra 3.0 and analyzed via Phenochart software. This image was courteously provided by Dr. Houssein Abdul Sater, Georgia Cancer Center.



Immunocytochemistry/ Immunofluorescence - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab133619</u>).

Immunocytochemistry analysis of Raji (Human Burkitt's lymphoma B lymphocyte) labeling Fas with purified ab133619 at 1/50 dilution. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.31 µg/ml) was used as counterstain. Nuclei were stained blue with DAPI. Negative control: PBS instead of the primary antibody.

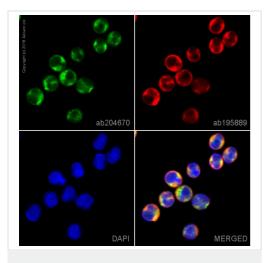


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

Immunohistochemical analysis of paraffin embedded Human tonsil tissue labelling CD95 with <u>ab133619</u> antibody at a dilution of 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133619**).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



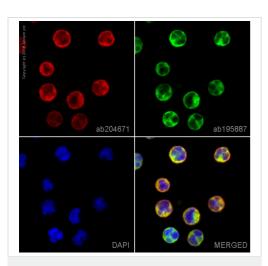
Immunocytochemistry/ Immunofluorescence - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

Clone EPR5700 (ab178076) has been successfully conjugated by Abcam. This image was generated using Anti-Fas antibody [EPR5700] (Alexa Fluor® 488). Please refer to ab204670 for protocol details.

<u>ab204670</u> staining Fas in Raji cells. The cells were fixed with 80% methanol (5 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 overnight at +4°C with <u>ab204670</u> at 1/100 dilution (shown in green) and <u>ab195889</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in Raji cells fixed with 4% formaldehyde (10 min).



Immunocytochemistry/ Immunofluorescence - Anti-Fas antibody [EPR5700] - BSA and Azide free (ab178076)

Clone EPR5700 (ab178076) has been successfully conjugated by Abcam. This image was generated using Anti-Fas antibody [EPR5700] (Alexa Fluor® 647). Please refer to ab204671 for protocol details.

<u>ab204671</u> staining Fas in Raji cells. The cells were fixed with 4% formaldehyde (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 overnight at +4°C with <u>ab204671</u> at 1/50 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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