abcam

Product datasheet

Anti-FADD antibody [EPR4415] ab108601

KO VALIDATED Recombinant RabMAb

8 References 7 Images

Overview

Product name	Anti-FADD antibody [EPR4415]
Description	Rabbit monoclonal [EPR4415] to FADD
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human FADD aa 1-150. The exact sequence is proprietary. Database link: **Q13158**
Positive control	WB: A431, Jurkat, HeLa, and SKBR-3 cell lysates. IHC-P: Human kidney tissue. Flow Cyt (intra): A431 cells. IP: HeLa 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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant

Clonality	Monoclonal
Clone number	EPR4415
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108601 in the following tested applications.

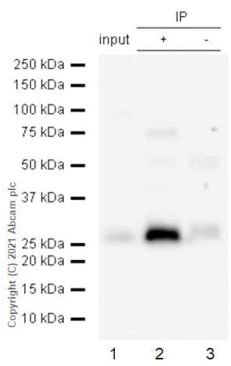
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 28 kDa (predicted molecular weight: 23 kDa).
IP		1/10 - 1/100.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function	Apoptotic adaptor molecule that recruits caspase-8 or caspase-10 to the activated Fas (CD95) or TNFR-1 receptors. The resulting aggregate called the death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation. Active caspase-8 initiates the subsequent cascade of caspases mediating apoptosis.
Tissue specificity	Expressed in a wide variety of tissues, except for peripheral blood mononuclear leukocytes.
Sequence similarities	Contains 1 death domain. Contains 1 DED (death effector) domain.
Domain	Contains a death domain involved in the binding of the corresponding domain within Fas receptor.

Images



Immunoprecipitation - Anti-FADD antibody [EPR4415] (ab108601)

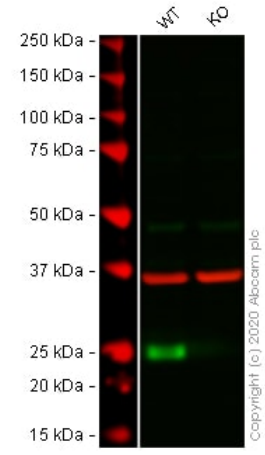
FADD was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with 108601 at 1/120 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab108601 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab108601 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-FADD antibody [EPR4415] (ab108601)

All lanes : Anti-FADD antibody [EPR4415] (ab108601) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : FADD knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

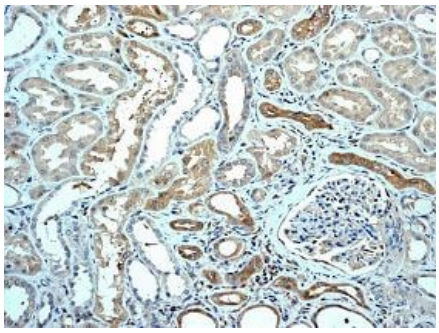
Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 23 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab108601 observed at 23 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108601 was shown to react with FADD in wild-type HeLa cells in western blot with loss of signal observed in FADD knockout cell line

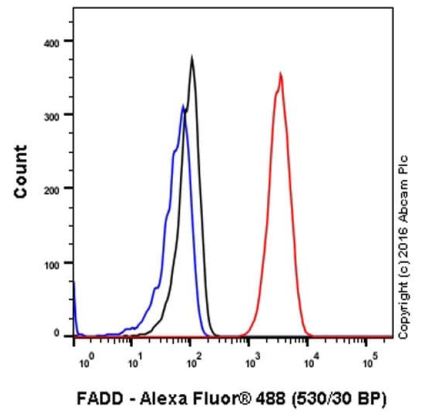
ab261817 (FADD knockout cell lysate **ab257261**). Wild-type and FADD knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab108601 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FADD antibody [EPR4415] (ab108601)

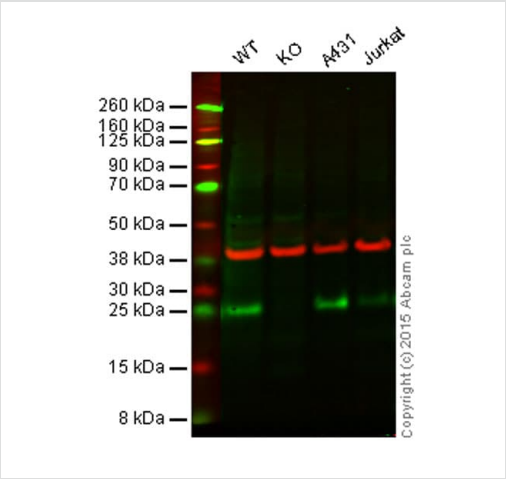
Immunohistochemical staining of paraffin-embedded Human kidney tissue using ab108601 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-FADD antibody [EPR4415] (ab108601)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling FADD with purified ab108601 at 1/140 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Western blot - Anti-FADD antibody [EPR4415] (ab108601)

All lanes : Anti-FADD antibody [EPR4415] (ab108601) at 1/1000 dilution

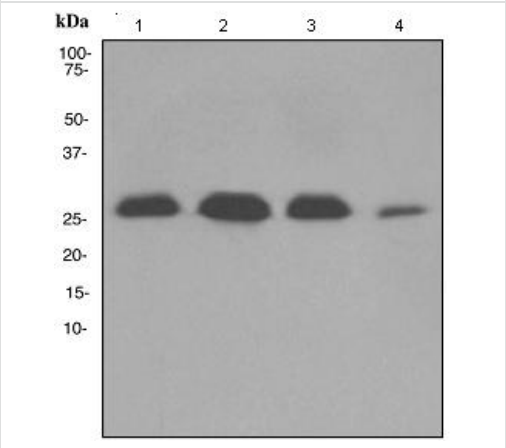
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : FADD knockout HAP1 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 23 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab108601 observed at 25 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab108601 was shown to specifically react with FADD when FADD knockout samples were used. Wild-type and FADD knockout samples were subjected to SDS-PAGE. ab108601 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-FADD antibody [EPR4415] (ab108601)

All lanes : Anti-FADD antibody [EPR4415] (ab108601) at 1/1000 dilution

Lane 1 : A431 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : SKBR-3 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 23 kDa

Anti-FADD antibody [EPR4415] (ab108601)

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