abcam

Product datasheet

Anti-RIG-I/DDX58 antibody [EPR18629] ab180675

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-RIG-I/DDX58 antibody [EPR18629]
Description	Rabbit monoclonal [EPR18629] to RIG-I/DDX58
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A549, 293, HeLa and Jurkat whole cell lysates; Human fetal kidney and stomach lysates. IP: Jurkat whole cell 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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18629
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab180675 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)**	1/1000. Detects a band of approximately 107 kDa (predicted molecular weight: 107 kDa).
IP		1/100.

Target

Function Involved in innate immune defense against viruses. Upon interaction with intracellular dsRNA produced during viral replication, triggers a transduction cascade involving MAVS/IPS1, which results in the activation of NF-kappa-B, IRF3 and IRF7 and the induction of the expression of antiviral cytokines such as IFN-beta and RANTES (CCL5). Detects dsRNA produced from non-self dsDNA by RNA polymerase III, such as Epstein-Barr virus-encoded RNAs (EBERs). Essential for the production of interferons in response to RNA viruses including paramyxoviruses, influenza viruses, Japanese encephalitis virus and HCV.

Tissue specificity Present in vascular smooth cells (at protein level).

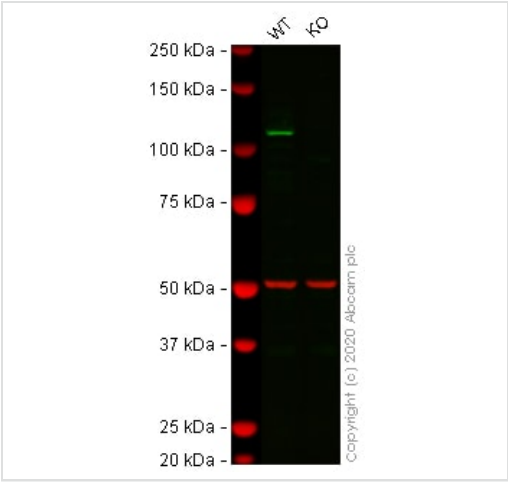
Sequence similarities Belongs to the helicase family.
Contains 2 CARD domains.
Contains 1 helicase ATP-binding domain.
Contains 1 helicase C-terminal domain.

Domain The repressor domain controls homomultimerization and interaction with MAVS.
The helicase domain is responsible for dsRNA recognition.
The 2 CARD domains are responsible for interaction with and signaling through MAVS.
The second CARD domain is the primary site for 'Lys-63'-linked ubiquitination.

Post-translational modifications Isgylated. Conjugated to ubiquitin-like protein ISG15 upon IFN-beta stimulation.
Ubiquitinated. Undergoes 'Lys-63'-linked ubiquitination. Lys-172 is the critical site for TRIM25-mediated ubiquitination, for MAVS binding and to induce anti-viral signal transduction. Lys-154, Lys-164 and Lys-172 are critical sites for RNF135-mediated ubiquitination. Deubiquitinated by CYLD, a protease that selectively cleaves 'Lys-63'-linked ubiquitin chains.

Cellular localization Cytoplasm. Colocalized with TRIM25 at cytoplasmic perinuclear bodies.

Images



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

All lanes : Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : DDX58 knockout A549 cell lysate

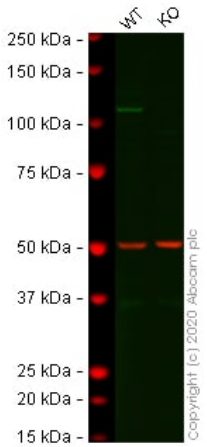
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 107 kDa
Observed band size: 107 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab180675 observed at 107 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab180675 was shown to react with DDX58 in A549 wild-type cells in western blot with loss of signal observed in DDX58 knockout cell line **ab267117** (DDX58 knockout cell lysate **ab257917**). Wild-type and DDX58 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab180675 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) 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.



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

All lanes : Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : DDX58 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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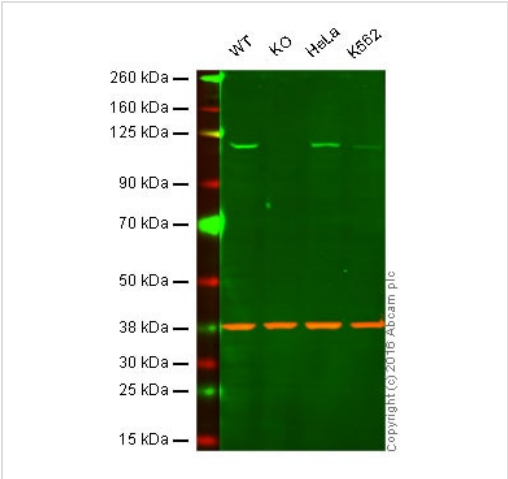
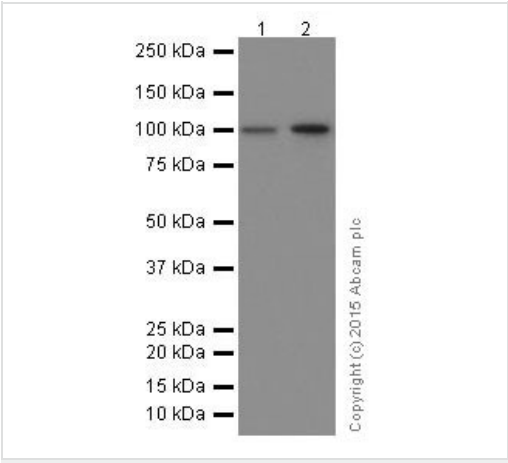
Lanes 1 - 2: Merged signal (red and green). Green - ab180675 observed at 107 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab180675 was shown to react with DDX58 in wild-type A549 cells in western blot with loss of signal observed in DDX58 knockout cell line **ab267116** (DDX58 knockout cell lysate **ab257916**). Wild-type and DDX58 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab180675 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) 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.



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: RIG-I/DDX58 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: K562 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab180675 observed at 107 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab180675 was shown to specifically react with RIG-I/DDX58 when RIG-I/DDX58 knockout samples were used. Wild-type and RIG-I/DDX58 knockout samples were subjected to SDS-PAGE. ab180675 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10 000 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-RIG-I/DDX58 antibody [EPR18629] (ab180675)

All lanes : Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution

Lane 1 : 293 (Human epithelial cells from embryonic kidney) whole cell lysate
Lane 2 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

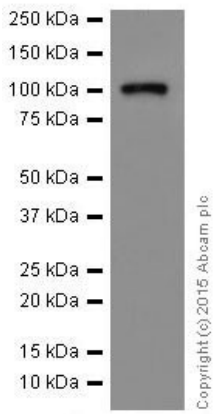
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 107 kDa
Observed band size: 107 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

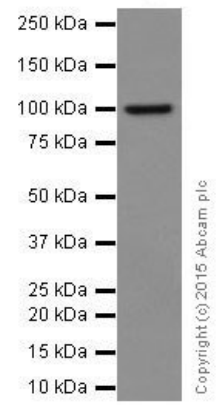
Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution + Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 107 kDa
Observed band size: 107 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

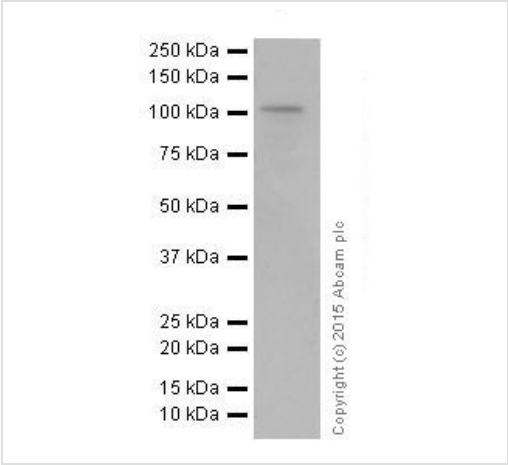
Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

Secondary
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 107 kDa
Observed band size: 107 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675) at 1/1000 dilution + Human stomach lysate at 10 µg

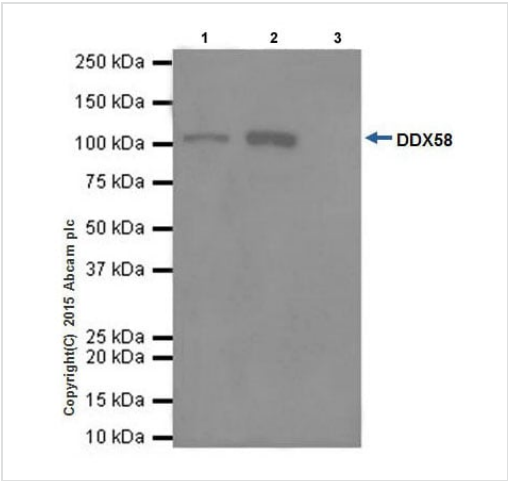
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 107 kDa
Observed band size: 107 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

RIG-I/DDX58 was immunoprecipitated from 1mg of Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate with ab180675 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab180675 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: Jurkat whole cell lysate 10ug (Input). Lane 2: ab180675 IP in Jurkat whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab180675 in Jurkat whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.

Anti-RIG-I/DDX58 antibody [EPR18629] (ab180675)

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