# abcam

# Product datasheet

# Anti-RIG-I/DDX58 antibody [EPR18629] - BSA and Azide free ab240230





RabMAb

# 4 Images

#### Overview

Product name Anti-RIG-I/DDX58 antibody [EPR18629] - BSA and Azide free

**Description** Rabbit monoclonal [EPR18629] to RIG-I/DDX58 - BSA and Azide free

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** ab240230 is the carrier-free version of **ab180675**.

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 <u>conjugation kits</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

1

### **Properties**

**Form** Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

**Carrier free** Yes

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR18629

Isotype ΙgG

### **Applications**

Our Abpromise quarantee covers the use of ab240230 in the following tested applications. The Abpromise guarantee

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 107 kDa (predicted molecular weight: 107 kDa).
IP		Use at an assay dependent concentration.

#### **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 nonself 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.

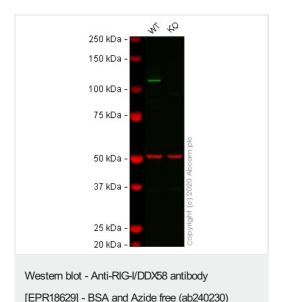
Post-translational lsgylated. Conjugated to ubiquitin-like protein ISG15 upon IFN-beta stimulation.

modifications Ubiquitinated. Undergoes 'Lys-63'-linked ubiquitination. Lys-172 is the critical site for TRIM25mediated 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**



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.

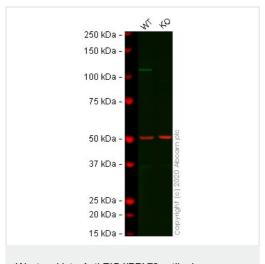
Predicted band size: 107 kDa

Observed band size: 107 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab180675).

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] - BSA and Azide free (ab240230)

**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.

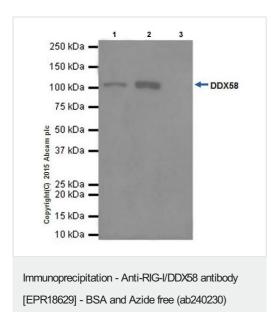
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (<u>ab180675</u>).

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

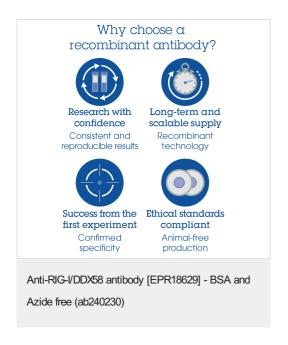
ab180675 was shown to react with DDX58 in A549 wild-type cells in western blot with loss of signal observed in DDX58 knockout cell line ab267116 (DDX58 knockout cell lysate ab257916). Wild-type and DDX58 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (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.



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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab180675).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

#### Our Abpromise to you: Quality guaranteed and expert technical support

• Replacement or refund for products not performing as stated on the datasheet

- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

## Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors