# abcam

# Product datasheet

# Anti-TRIM21/SS-A antibody [EPR20290] ab207728



Recombinant RabMAb

**5 References** 9 Images

Overview

**Product name** Anti-TRIM21/SS-A antibody [EPR20290]

Rabbit monoclonal [EPR20290] to TRIM21/SS-A **Description** 

**Host species** Rabbit

**Specificity** This reagent is not recommended for mouse or rat IHC-P and human ICC/IF.

**Tested applications** Suitable for: WB, IHC-P

Unsuitable for: ICC/IF or IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa whole cell lysate untreated or treated with human IFN gamma; A549, HEK-293T and

> MOLT-4 whole cell lysates; human fetal spleen, fetal kidney and thymus lysates; rat spleen and thymus lysates; mouse thymus lysate. IHC-P: human tonsil tissue and wild-type A549 cell pellet.

**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: 7.2

Preservative: 0.01% Sodium azide

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

**Purity** Protein A purified

ClonalityMonoclonalClone numberEPR20290

**Isotype** IgG

#### **Applications**

# The Abpromise guarantee

Our Abpromise guarantee covers the use of ab207728 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 50 kDa (predicted molecular weight: 54 kDa).
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

**Application notes** 

Is unsuitable for ICC/IF or IP.

#### **Target**

**Function** 

E3 ubiquitin-protein ligase whose activity is dependent on E2 enzymes, UBE2D1, UBE2D2, UBE2E1 and UBE2E2. Forms a ubiquitin ligase complex in cooperation with the E2 UBE2D2 that is used not only for the ubiquitination of USP4 and IKBKB but also for its self-ubiquitination. Component of cullin-RING-based SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complexes such as SCF(SKP2)-like complexes. A TRIM21-containing SCF(SKP2)-like complex is shown to mediate ubiquitination of CDKN1B ('Thr-187' phosphorylated-form), thereby promoting its degradation by the proteasome. Monoubiquitinates IKBKB that will negatively regulates Tax-induced NF-kappa-B signaling. Negatively regulates IFN-beta production postpathogen recognition by polyubiquitin-mediated degradation of IRF3. Mediates the ubiquitinmediated proteasomal degradation of IgG1 heavy chain, which is linked to the VCP-mediated ER-associated degradation (ERAD) pathway. Promotes IRF8 ubiquitination, which enhanced the ability of IRF8 to stimulate cytokine genes transcription in macrophages. Plays a role in the regulation of the cell cycle progression. Enhances the decapping activity of DCP2. Exists as a ribonucleoprotein particle present in all mammalian cells studied and composed of a single polypeptide and one of four small RNA molecules. At least two isoforms are present in nucleated and red blood cells, and tissue specific differences in RO/SSA proteins have been identified. The common feature of these proteins is their ability to bind HY RNAs.2.

Tissue specificity

Isoforms 1 and 2 are expressed in fetal and adult heart and fetal lung.

**Pathway** 

Protein modification; protein ubiquitination.

Sequence similarities

Belongs to the TRIM/RBCC family.

Contains 1 B box-type zinc finger.

Contains 1 B30.2/SPRY domain.

Contains 1 RING-type zinc finger.

Domain

The coiled-coil is necessary for the cytoplasmic localization. The B30.2/SPRY domain is necessary for the cytoplasmic localization, the interaction with IRF3 and for the IRF3-driven interferon beta promoter activity. The RING-type zinc finger is necessary for ubiquitination and for

# Post-translational modifications

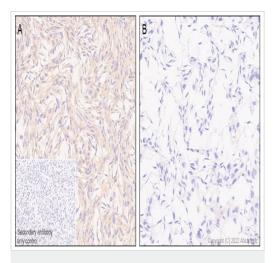
#### **Cellular localization**

the IRF3-driven interferon beta promoter activity. Interacts with SKP2 and CUL1 in a RING finger-independent manner.

Autoubiquitinated; does not lead to its proteasomal degradation. Deubiquitinated by USP4; leading to its stabilization.

Cytoplasm. Nucleus. Cytoplasm > P-body. Enters the nucleus upon exposure to nitric oxide. Localizes to small dot- or rod-like structures in the cytoplasm, called cytoplasmic bodies (P-body) that are located underneath the plasma membrane and also diffusely in the cytoplasm and are highly motil in cells. Cytoplasmic bodies are located along the microtubules and do not share the same cytoplasmic bodies with TRIM5. Colocalizes with DCP2 in P-body.

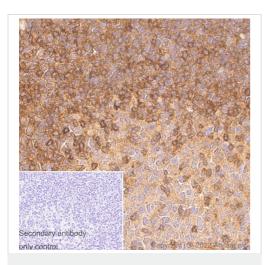
#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

Immunohistochemical analysis of paraffin-embedded A: Wild-type A549 (Human lung carcinoma epithelial cell) cell pellet, B: TRIM21 knockout A549 (ab267080) cell pellet labelling TRIM21/SS-A with ab207728 at 1/500 dilution (1.212 µg/ml) followed by LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody at a ready to use concentration. Positive staining on (A) wild-type A549 cell pellet, no staining on (B) TRIM21 knockout A549 (ab267080) cell pellet. The section was incubated with ab207728 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

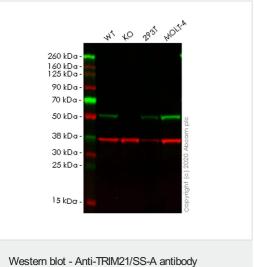
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

Immunohistochemistry analysis of paraffin-embedded human tonsil tissue sections labelling TRIM21/SS-A with ab207728 at 1/100 dilution. The section was incubated with ab207728 for 10 mins at room temperature. Ready to use Leica DS9800 (Bond™ Polymer Refine Detection) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 minutes.

Positive staining on human tonsil. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument.



Western blot - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate

**Lane 2 :** TRIM21 knockout A549 (Human lung carcinoma cell line) whole cell lysate

**Lane 3**: HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 4 : MOLT-4 (Human lymphoblastic leukemia cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

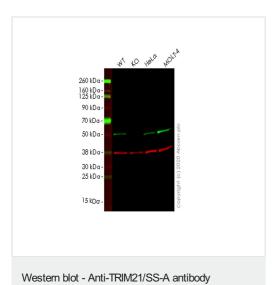
## **Secondary**

**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 54 kDa Observed band size: 50 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab207728 observed at 50 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab207728 Anti-TRIM21/SS-A antibody [EPR20290] was shown to specifically react with TRIM21/SS-A in wild-type A549 cells. Loss of signal was observed when knockout cell line <a href="mailto:ab267024">ab267024</a> (knockout cell line <a href="mailto:ab267024">ab267024</a> (knockout cell lysate <a href="mailto:ab257766">ab257766</a>) was used. Wild-type and TRIM21/SS-A knockout samples were subjected to SDS-PAGE. ab207728 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab245">ab207728</a> and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab245">ab26728</a> ) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



[EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/500 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: TRIM21 knockout A549 cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

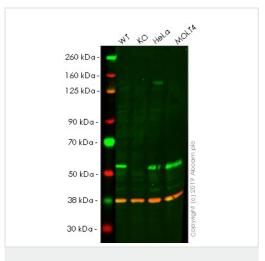
## **Secondary**

**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 54 kDa
Observed band size: 50 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab207728 observed at 50 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

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Western blot - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: TRIM21 knockout HAP1 whole cell lysate

Lane 3: HeLa whole cell lysate

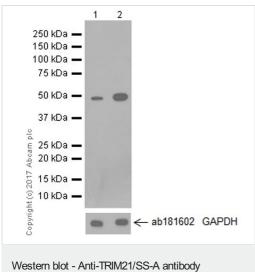
Lane 4: MOLT-4 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab207728 observed at 50 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab207728 was shown to specifically react with in wild-type HAP1 cells as signal was lost in TRIM21 knockout cells. Wild-type and TRIM21 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab207728 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/1000 dilution

**Lane 1 :** Untreated HeLa (human epithelial cell line from cervix adenocarcinoma), whole cell lysate

Lane 2: HeLa (human epithelial cell line from cervix adenocarcinoma) treated with 10 ng/ml human interferon-a (ab48750) for 16 hours

Lysates/proteins at 10 µg per lane.

# **Secondary**

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

Developed using the ECL technique.

**Predicted band size:** 54 kDa **Observed band size:** 50 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The level of TRIM21 expression can be elevated by IFN alpha treatment (PMID: 18071879).

1 2 3 4 5 250 kDa — 150 kDa — 100 kDa —

100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa —

Western blot - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/1000 dilution

**Lane 1**: HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen), whole cell lysate

Lane 2 : MOLT-4 (human lymphoblastic leukemia cell line), whole cell lysate

Lane 3: Human fetal spleen lysate

Lane 4: Human fetal kidney lysate

Lane 5: Human thymus lysate

Lysates/proteins at 20 µg per lane.

## Secondary

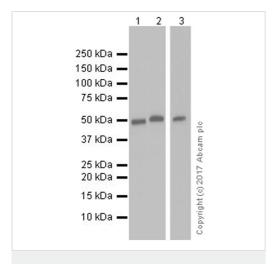
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

**Predicted band size:** 54 kDa **Observed band size:** 50 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-TRIM21/SS-A antibody [EPR20290] (ab207728)

**All lanes :** Anti-TRIM21/SS-A antibody [EPR20290] (ab207728) at 1/1000 dilution

Lane 1 : Rat spleen lysate

Lane 2 : Rat thymus lysate

Lane 3 : Mouse thymus 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

Developed using the ECL technique.

**Predicted band size:** 54 kDa **Observed band size:** 50 kDa

Exposure times: Lane 1-2: 30 seconds; Lane 3: 3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.



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

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