# Anti-TRIM29 Antibody [PSH02-99] HA721918

Product Type: Species reactivity: Applications: Molecular Wt: Clone number:	Recombinant Rabbit monoclonal IgG, primary antibodies Human, Mouse, Rat WB, IHC-P, IF-Cell, FC Predicted band size: 66 kDa PSH02-99
Description:	Tripartite motif-containing protein 29 (TRIM29, ATDC) was isolated as a candidate gene by its ability to complement the radiosensitivity defect of an ataxia-telangiectasia (AT) cell line. This putative transcription regulator belongs to the TRIM (tripartite motif) protein family that is characterized by highly conserved amino-terminal RING finger, B-box, and coiled-coil domains. The TRIM29 protein binds and sequesters cytosolic p53, repressing expression of p53 target genes including p21 and Noxa by preventing p53 from entering the nucleus. Expression of TRIM29 inhibits p53 function and results in increased cell proliferation. TRIM29 enhances tumor growth and metastasis in vivo and high TRIM29 levels are seen in most invasive pancreatic cancers. The oncogenic effect of TRIM29 appears to require $\beta$ -catenin as expression of both proteins is elevated in pancreatic cancer cell lines and tissues .
lmmunogen:	Synthetic peptide within human TRIM29 aa 1-588 / 588.
Positive control:	A431 cell lysate, SiHa cell lysate, SW1990 cell lysate, HT-29 cell lysate, A431, human prostate tissue, human skin tissue, mouse skin tissue, rat skin tissue.
Subcellular location:	Cytoplasm.
Database links:	SwissProt: Q14134 Human   Q8R2Q0 Mouse Entrez Gene: 300656 Rat
Recommended Dilutions: WB IHC-P IF-Cell FC	1:2,000 1:500-1:2,000 1:100 1:1,000
Storage Buffer:	PBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\!{\rm C}$ after thawing. Aliquot store at -20 $^\circ\!\!{\rm C}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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#### Images

**Fig1:** Western blot analysis of TRIM29 on different lysates with Rabbit anti-TRIM29 antibody (HA721918) at 1/1,000 dilution.

Lane 1: A431 cell lysate Lane 2: SiHa cell lysate Lane 3: SW1990 cell lysate Lane 4: HT-29 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 66 kDa Observed band size: 70 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721918) at 1/2,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



kDa PAS SHEN

TRIM29

-70kDa

- HSP90

250-150-

100 72-55-42-35-25-

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**Fig2:** Immunohistochemical analysis of paraffin-embedded human prostate tissue with Rabbit anti-TRIM29 antibody (HA721918) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721918) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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**Fig3:** Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-TRIM29 antibody (HA721918) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721918) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-TRIM29 antibody (HA721918) at 1/2000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721918) at 1/2000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-TRIM29 antibody (HA721918) at 1/2000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721918) at 1/2000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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**Fig6:** Immunocytochemistry analysis of A431 cells labeling TRIM29 with Rabbit anti-TRIM29 antibody (HA721918) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-TRIM29 antibody (HA721918) at 1/100 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor<sup>TM</sup> 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor = 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



Fig7: Flow cytometric analysis of A431 cells labeling TRIM29.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721918, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor<sup>TM</sup> 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- Wang L, Heidt DG, Lee CJ, Yang H, Logsdon CD, Zhang L, Fearon ER, Ljungman M, Simeone DM. Oncogenic function of ATDC in pancreatic cancer through Wnt pathway activation and beta-catenin stabilization. Cancer Cell. 2009 Mar 3;15(3):207-19.
- 2. Yuan Z, Villagra A, Peng L, Coppola D, Glozak M, Sotomayor EM, Chen J, Lane WS, Seto E. The ATDC (TRIM29) protein binds p53 and antagonizes p53-mediated functions. Mol Cell Biol. 2010 Jun;30(12):3004-15.
- 3. Kapp LN, Painter RB, Yu LC, van Loon N, Richard CW 3rd, James MR, Cox DR, Murnane JP. Cloning of a candidate gene for ataxia-telangiectasia group D. Am J Hum Genet. 1992 Jul;51(1):45-54.

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