

Anti-SAMD9 Antibody [PSH02-75]

HA721853



| | |
|----------------------------|---|
| Product Type: | Recombinant Rabbit monoclonal IgG, primary antibodies |
| Species reactivity: | Human |
| Applications: | WB, IF-Cell, IHC-P, FC |
| Molecular Wt: | Predicted band size: 184 kDa |
| Clone number: | PSH02-75 |

Description: Double-stranded nucleic acid binding that acts as a an antiviral factor by playing an essential role in the formation of cytoplasmic antiviral granules. May play a role in the inflammatory response to tissue injury and the control of extra-osseous calcification, acting as a downstream target of TNF-alpha signaling. Involved in the regulation of EGR1, in coordination with RGL2. May be involved in endosome fusion. Widely expressed. Very low levels are detected in skeletal muscle. Not detected in brain. Down-regulated in aggressive fibromatosis, as well as in breast and colon cancers. Up-regulated in fibroblasts from patients with normophosphatemic tumoral calcinosis (NFTC). Up-regulated by TNF-alpha through p38 MAPKs and NF-kappa-B. Up-regulated by osmotic shock. Induced by IFNG.

Immunogen: Recombinant protein within human SMAD9 aa 1-250 / 1,589.

Positive control: A549 cell lysate, HeLa cell lysate, A431 cell lysate, Raji cell lysate, MCF7 cell lysate, HepG2 cell lysate, HCT 116 cell lysate, U-87 MG cell lysate, A431, human skin tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: Q5K651 Human

Recommended Dilutions:

| | |
|----------------|---------|
| WB | 1:1,000 |
| IF-Cell | 1:100 |
| IHC-P | 1:200 |
| FC | 1:1,000 |

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

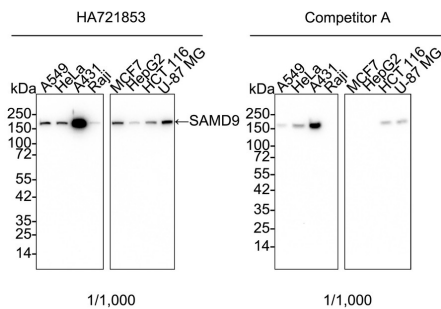
Technical:0086-571-89986345

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Images

Fig1: Western blot analysis of SAMD9 on different lysates with Rabbit anti-SAMD9 antibody (HA721853) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.



Lane 1: A549 cell lysate
 Lane 2: HeLa cell lysate
 Lane 3: A431 cell lysate
 Lane 4: Raji cell lysate
 Lane 5: MCF7 cell lysate
 Lane 6: HepG2 cell lysate
 Lane 7: HCT 116 cell lysate
 Lane 8: U-87 MG cell lysate

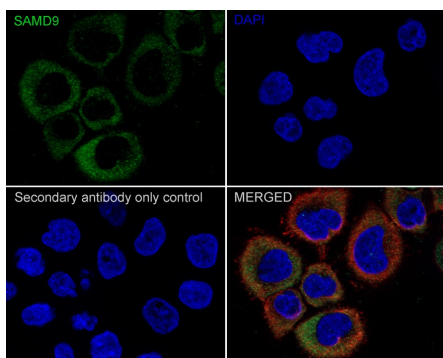
Lysates/proteins at 20 µg/Lane.

Predicted band size: 184 kDa
 Observed band size: 184 kDa

Exposure time: 2 minutes; ECL: K1801;
 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 (HA721853) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of A431 cells labeling SAMD9 with Rabbit anti-SAMD9 antibody (HA721853) 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-SAMD9 antibody (HA721853) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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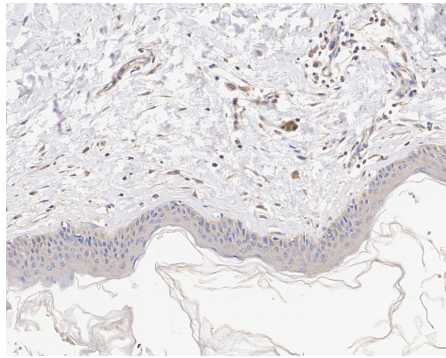


Fig3: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-SAMD9 antibody (HA721853) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA721853) at 1/200 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.

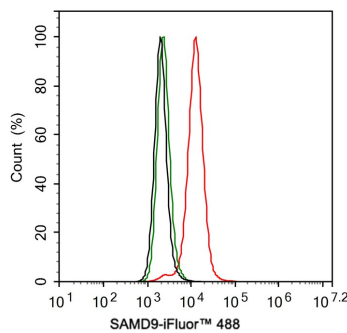


Fig4: Flow cytometric analysis of A431 cells labeling SAMD9.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721853, 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™ 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).

Fig5: Western blot analysis of SAMD9 on different lysates with Rabbit anti-SAMD9 antibody (HA721853) at 1/1,000 dilution.

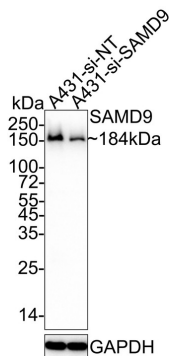
Lane 1: A431-si NT cell lysate
Lane 2: A431-si SAMD9 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 184 kDa
Observed band size: 184 kDa

Exposure time: 24 seconds;

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 (HA721853) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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

Background References

1. Liu J., McFadden G. SAMD9 is an innate antiviral host factor with stress response properties that can be antagonized by poxviruses. *J. Virol.* 89:1925-1931 (2015)
2. Nounamo B., Li Y., O'Byrne P., Kearney A.M., Khan A., Liu J. An interaction domain in human SAMD9 is essential for myxoma virus host- range determinant M062 antagonism of host anti-viral function. *Virology* 503:94-102 (2017)

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