

Anti-Sonic Hedgehog Protein/SHH(C-Product) Antibody [SC05-28]

ET1610-6



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	SC05-28

Description: The Drosophila segment polarity gene hedgehog (hh) encodes a precursor protein which undergoes autocleavage to generate amino- and carboxy-terminal peptides. Both proteins are secreted and appear to function in embryonic and imaginal disc patterning. Several vertebrate homologs of Drosophila hh have been identified. These include Sonic hedgehog (Shh) (alternatively designated Vhh-1), Desert hedgehog (Dhh) and Indian hedgehog (Ihh). Each contain amino-terminal signal peptides and apparently function as secreted proteins involved in the mediation of various cell-cell interactions. Shh resembles Drosophila hh in that it is processed to generate an amino-terminal secreted peptide that is retained at or near the cell surface and a carboxy-terminal glycosylated more diffusible peptide.

Immunogen: Synthetic peptide within Human Sonic Hedgehog Protein aa 258-307 / 462.

Positive control: Mouse kidney tissue lysate, Mouse brain tissue lysate, Mouse liver tissue lysate, H22 cell lysate, Neuro-2a cell lysate, NIH/3T3 cell lysate, Rat heart tissue lysate, Rat liver tissue lysate, Rat brain tissue lysate, Rat lung tissue lysate, Neuro-2a, Hela, human liver tissue, mouse liver tissue, rat kidney tissue, rat liver tissue.

Subcellular location: Secreted, Cell membrane.

Database links: SwissProt: Q15465 Human | Q62226 Mouse | Q63673 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
FC	1:50-1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

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Images

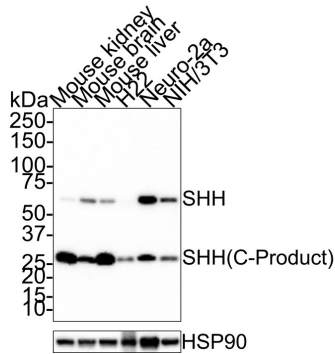


Fig1: Western blot analysis of Sonic Hedgehog Protein/SHH(C-Product) on different lysates with Rabbit anti-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) at 1/1,000 dilution.

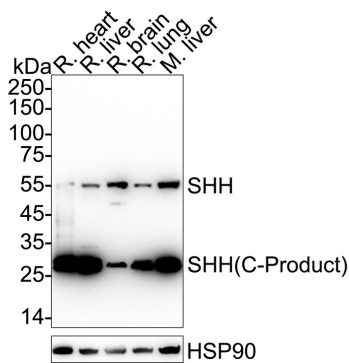
Lane 1: Mouse kidney tissue lysate (30 µg/Lane)
 Lane 2: Mouse brain tissue lysate (30 µg/Lane)
 Lane 3: Mouse liver tissue lysate (30 µg/Lane)
 Lane 4: H22 cell lysate (15 µg/Lane)
 Lane 5: Neuro-2a cell lysate (15 µg/Lane)
 Lane 6: NIH/3T3 cell lysate (15 µg/Lane)

Predicted band size: 50 kDa
 Observed band size: 28/55 kDa

Exposure time: 30 seconds;
 4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFD/MTBST for 1 hour at room temperature. The primary antibody (ET1610-6) at 1/1,000 dilution was used in 5% NFD/MTBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of Sonic Hedgehog Protein/SHH(C-Product) on different lysates with Rabbit anti-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) at 1/1,000 dilution.



Lane 1: Rat heart tissue lysate
 Lane 2: Rat liver tissue lysate
 Lane 3: Rat brain tissue lysate
 Lane 4: Rat lung tissue lysate
 Lane 5: Mouse liver tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 50 kDa
 Observed band size: 28/55 kDa

Exposure time: 59 seconds; ECL: K1801;
 4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFD/MTBST for 1 hour at room temperature. The primary antibody (ET1610-6) at 1/1,000 dilution was used in primary antibody dilution (K1803) 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.

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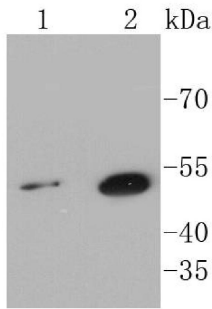


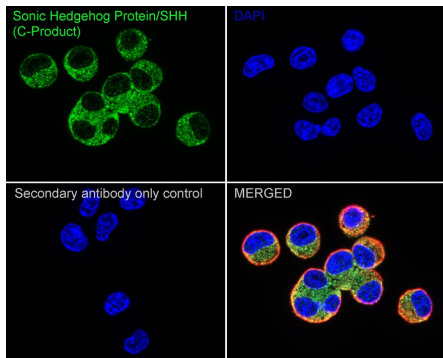
Fig3: Western blot analysis of Sonic Hedgehog Protein on different lysates using anti-Sonic Hedgehog Protein antibody at 1/1,000 dilution.

Positive control:

Lane 1: HeLa

Lane 2: HepG2

Fig4: Immunocytochemistry analysis of Neuro-2a cells labeling Sonic Hedgehog Protein/SHH(C-Product) with Rabbit anti-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) 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.

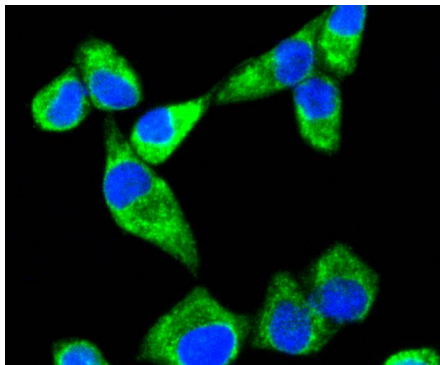


Fig5: ICC staining Sonic Hedgehog Protein in HeLa cells (green). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

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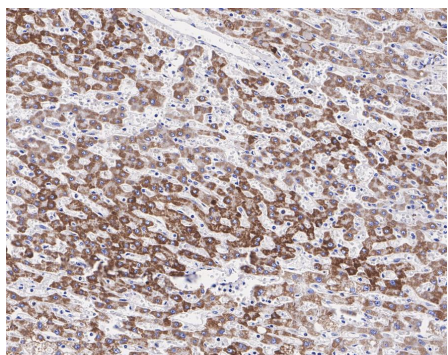


Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) at 1/1,000 dilution.

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 (ET1610-6) at 1/1,000 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.

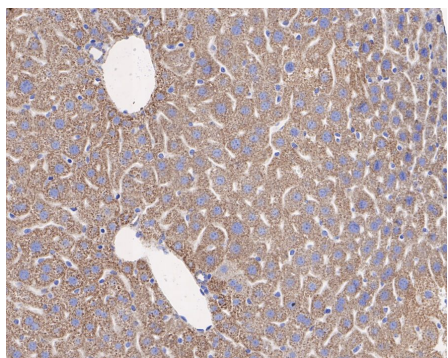


Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Sonic Hedgehog Protein/SHH(C-Product) antibody (ET1610-6) at 1/1,000 dilution.

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 (ET1610-6) at 1/1,000 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.

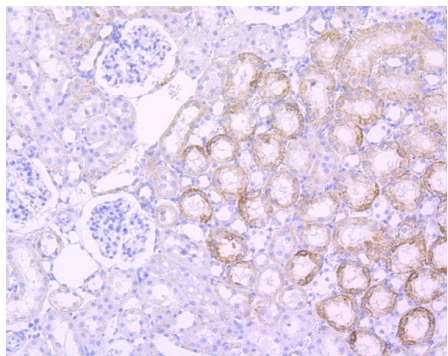


Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-Sonic Hedgehog Protein antibody. Counter stained with hematoxylin.

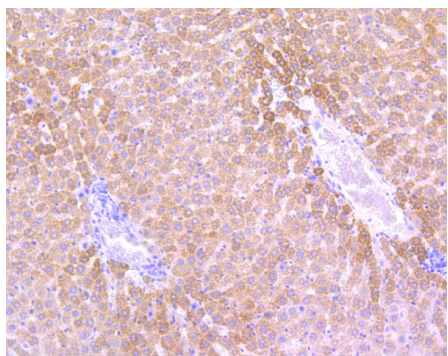


Fig9: Immunohistochemical analysis of paraffin-embedded rat liver tissue using anti-Sonic Hedgehog Protein antibody. Counter stained with hematoxylin.

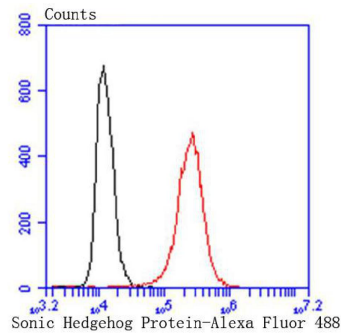


Fig10: Flow cytometric analysis of HeLa cells with Sonic Hedgehog Protein antibody at 1/50 dilution (red) compared with an unlabelled control (cells without incubation with primary antibody; black). Alexa Fluor 488-conjugated goat anti rabbit IgG was used as the secondary antibody.

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

Background References

1. Zhang X et al. Expression of SOX9 and CDX2 in nongoblet columnar-lined esophagus predicts the detection of Barrett's esophagus during follow-up. *Mod Pathol* 28:654-61 (2015).
2. Li H et al. Olfactomedin 4 deficiency promotes prostate neoplastic progression and is associated with upregulation of the hedgehog-signaling pathway. *Sci Rep* 5:16974 (2015).

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