

Anti-Sonic Hedgehog antibody [EP1190Y] ab53281

重组 RabMAb

★★★★★ **6 Abreviews** **79 References** **13 图像**

概述

产品名称	Anti-Sonic Hedgehog抗体[EP1190Y]
描述	兔单克隆抗体[EP1190Y] to Sonic Hedgehog
宿主	Rabbit
特异性	Specific for both full length (51kDa) and c-product subunit (27kDa) of human Sonic Hedgehog protein
经测试应用	适用于: Flow Cyt (Intra), ICC/IF, WB, IHC-P
种属反应性	与反应: Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	Human fetal liver and kidney lysates, human kidney, fetal membrane and human pancreatic carcinoma tissue and HeLa cells.
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
存储溶液	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol, 0.05% BSA</p>
纯度	Protein A purified
克隆	单克隆

克隆编号 EP1190Y

同种型 IgG

应用

The Abpromise guarantee **Abpromise™** 承诺保证使用 ab53281 于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度；实际最佳的稀释度/浓度应由使用者检定。

应用	Ab 评论	说明
Flow Cyt (Intra)		1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	1/250 - 1/500.
WB	★★★★★ (2)	1/1000 - 1/10000. Detects a band of approximately 51 kDa (predicted molecular weight: 51 kDa).
IHC-P	★★★★★ (3)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/100.

靶标

功能 Binds to the patched (PTC) receptor, which functions in association with smoothened (SMO), to activate the transcription of target genes. In the absence of SHH, PTC represses the constitutive signaling activity of SMO. Also regulates another target, the gli oncogene. Intercellular signal essential for a variety of patterning events during development: signal produced by the notochord that induces ventral cell fate in the neural tube and somites, and the polarizing signal for patterning of the anterior-posterior axis of the developing limb bud. Displays both floor plate- and motor neuron-inducing activity. The threshold concentration of N-product required for motor neuron induction is 5-fold lower than that required for floor plate induction.

组织特异性 Expressed in fetal intestine, liver, lung, and kidney. Not expressed in adult tissues.

疾病相关 Defects in SHH are the cause of microphthalmia isolated with coloboma type 5 (MCOPCB5) [MIM:611638]. Microphthalmia is a clinically heterogeneous disorder of eye formation, ranging from small size of a single eye to complete bilateral absence of ocular tissues. Ocular abnormalities like opacities of the cornea and lens, scarring of the retina and choroid, cataract and other abnormalities like cataract may also be present. Ocular colobomas are a set of malformations resulting from abnormal morphogenesis of the optic cup and stalk, and the fusion of the fetal fissure (optic fissure). Defects in SHH are the cause of holoprosencephaly type 3 (HPE3) [MIM:142945]. Holoprosencephaly (HPE) [MIM:236100] is the most common structural anomaly of the brain, in which the developing forebrain fails to correctly separate into right and left hemispheres. Holoprosencephaly is genetically heterogeneous and associated with several distinct facies and phenotypic variability. The majority of HPE3 cases are apparently sporadic, although clear examples of autosomal dominant inheritance have been described. Interestingly, up to 30% of

obligate carriers of HPE3 gene in autosomal dominant pedigrees are clinically unaffected. Defects in SHH are a cause of solitary median maxillary central incisor (SMMCI) [MIM:147250]. SMMCI is a rare dental anomaly characterized by the congenital absence of one maxillary central incisor.

Defects in SHH are the cause of triphalangeal thumb-polysyndactyly syndrome (TPTPS) [MIM:174500]. TPTPS is an autosomal dominant syndrome characterized by a wide spectrum of pre- and post-axial abnormalities due to altered SHH expression pattern during limb development. TPTPS mutations have been mapped to the 7q36 locus in the LMBR1 gene which contains in its intron 5 a long-range cis-regulatory element of SHH expression.

序列相似性

Belongs to the hedgehog family.

翻译后修饰

The C-terminal domain displays an autoproteolysis activity and a cholesterol transferase activity. Both activities result in the cleavage of the full-length protein and covalent attachment of a cholesterol moiety to the C-terminal of the newly generated N-terminal fragment (N-product). The N-product is the active species in both local and long-range signaling, whereas the C-product has no signaling activity.

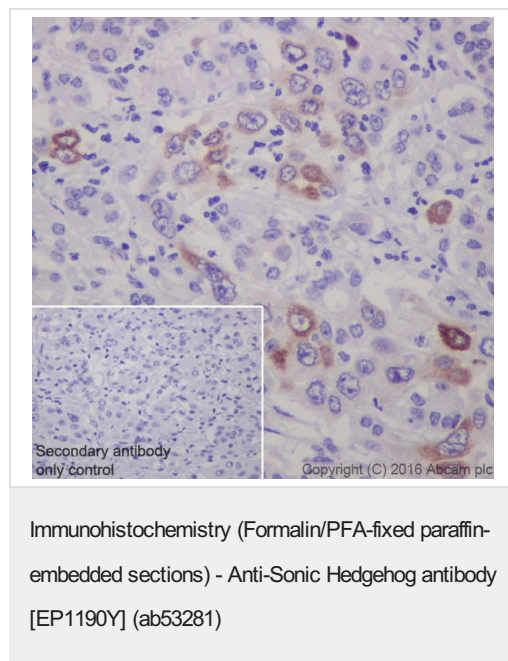
Cholesterylation is required for N-product targeting to lipid rafts and multimerization.

N-palmitoylation of Cys-24 by HHAT is required for N-product multimerization and full activity.

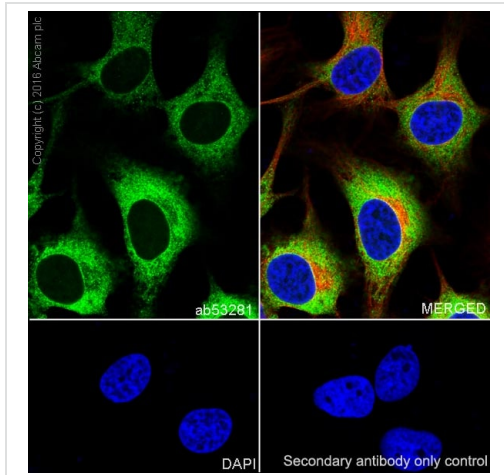
细胞定位

Cell membrane. The N-product either remains associated with lipid rafts at the cell surface, or forms freely diffusible active multimers with its hydrophobic lipid-modified N- and C-termini buried inside and Secreted > extracellular space. The C-terminal peptide diffuses from the cell.

图片

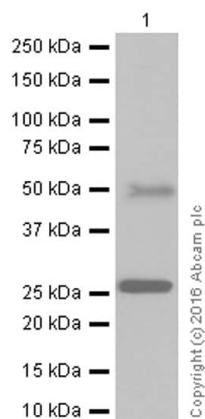


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue sections labeling Sonic Hedgehog with purified ab53281 at 1/2000 dilution (0.097 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Tissue was counterstained with Hematoxylin. **ab97051** Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunocytochemistry/ Immunofluorescence - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Sonic Hedgehog with purified ab53281 at 1/250 dilution (0.8µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1/1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281) at 1/2000 dilution (purified) + Human fetal liver lysate at 15 µg

Secondary

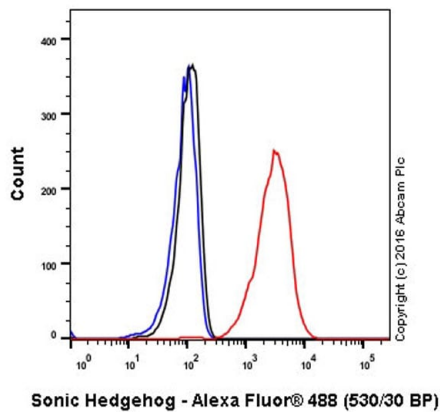
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 51 kDa

Observed band size: 27,50 kDa

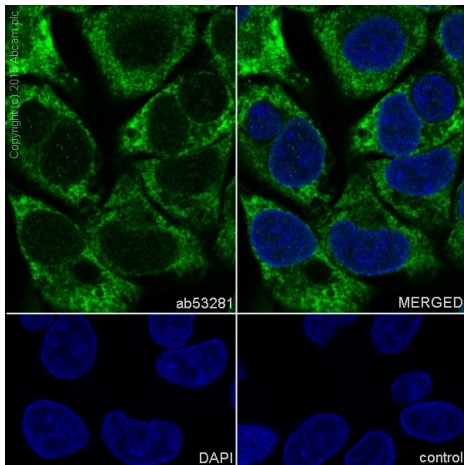
Blocking and diluting buffer: 5% NFDM/TBST.

50kDa: Full-length SHH; 27kDa: C-terminal product



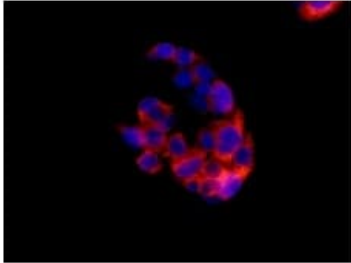
Flow Cytometry (Intracellular) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Intracellular Flow Cytometry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Sonic Hedgehog with purified ab53281 at 1/20 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - cells without incubation with primary antibody and secondary antibody (Blue).



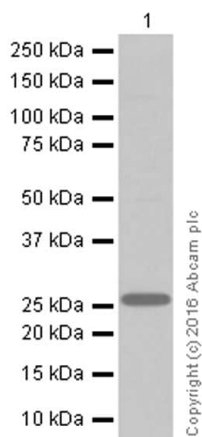
Immunocytochemistry/ Immunofluorescence - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling Sonic Hedgehog with purified ab53281 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised by 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).



Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Sonic Hedgehog with unpurified ab53281.

Immunocytochemistry/ Immunofluorescence - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)



Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281) at 1/1000 dilution (purified) + Human fetal kidney lysate at 15 µg

Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

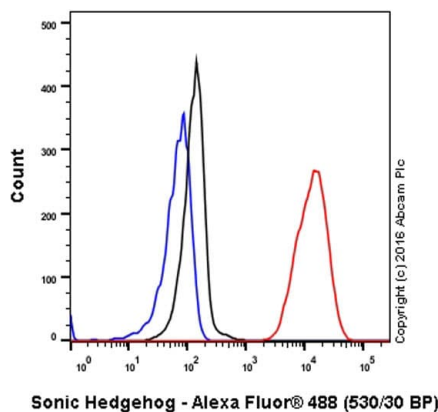
Predicted band size: 51 kDa

Observed band size: 27 kDa

Western blot - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

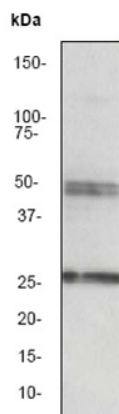
Blocking and diluting buffer: 5% NFDM /TBST .

27KDa: C-terminal product.



Flow Cytometry (Intracellular) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Sonic Hedgehog with unpurified ab53281 at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281) at 1/2000 dilution (unpurified) + Fetal liver membrane at 10 µg

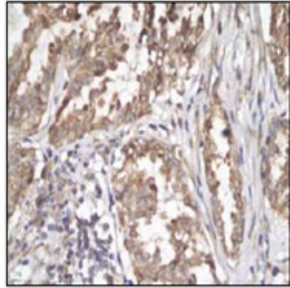
Secondary

Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 51 kDa

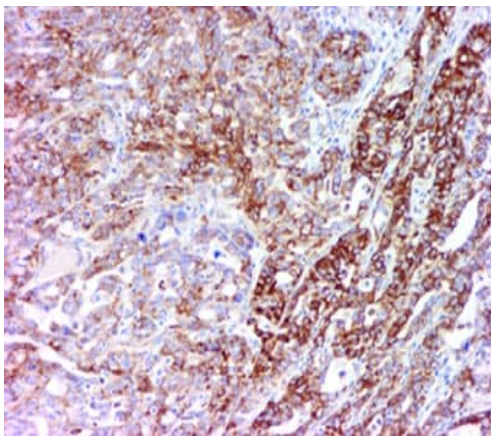
Observed band size: 51 kDa

Additional bands at: 27 kDa (possible isoform), 49 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemical analysis of Formalin fixed paraffin-embedded human kidney cancerous tissue labeling Sonic Hedgehog with unpurified ab53281 at 1/100 dilution.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

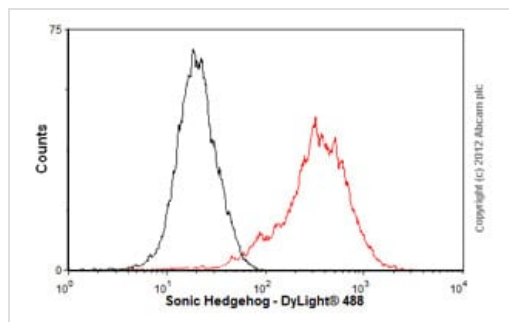


Immunohistochemical analysis of Formalin fixed paraffin embedded human ovarian cancer tissue, staining Sonic Hedgehog with unpurified ab53281.

Antigen retrieval was carried out in citrate buffer using a pressure cooker for 40 minutes. Sections were blocked with blocking agent before incubating with primary antibody (1/2000) for 90 minutes at room temperature. Staining was detected using DAB.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Image from McCann CK et al., PLoS One. 2011;6(11):e28077. Epub 2011 Nov 29. Fig 1.; doi:10.1371/journal.pone.0028077; November 29, 2011, PLoS ONE 6(11): e28077.



Flow Cytometry (Intracellular) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

Overlay histogram showing HepG2 cells stained with unpurified ab53281 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab53281, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) ([ab96899](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Why choose a recombinant antibody?



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Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

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