

### Anti-Sonic Hedgehog antibody [EP1190Y] ab53281

リコンビナント **RabMAb**

★★★★★ **6 Abreviews** **79 References** 画像数 13

#### 製品の概要

|              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 製品名          | Anti-Sonic Hedgehog antibody [EP1190Y]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 製品の詳細        | Rabbit monoclonal [EP1190Y] to Sonic Hedgehog                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 由来種          | Rabbit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 特異性          | Specific for both full length (51kDa) and c-product subunit (27kDa) of human Sonic Hedgehog protein                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| アプリケーション     | <b>適用あり:</b> Flow Cyt (Intra), ICC/IF, WB, IHC-P                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 種交差性         | <b>交差種:</b> Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 免疫原          | Synthetic peptide within Human Sonic Hedgehog. The exact sequence is proprietary. (Peptide available as <b>ab203144</b> )                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| ポジティブ・コントロール | Human fetal liver and kidney lysates, human kidney, fetal membrane and human pancreatic carcinoma tissue and HeLa cells.                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 特記事項         | <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <b>see here</b>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <b>RabMAb<sup>®</sup> patents</b>.</p> |

#### 製品の特性

|       |                                                                                                                                                        |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| 製品の状態 | Liquid                                                                                                                                                 |
| 保存方法  | 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. |
| バッファー | <p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol, 0.05% BSA</p>                                          |
| 精製度   | Protein A purified                                                                                                                                     |
| ポリ/モノ | モノクローナル                                                                                                                                                |

|        |         |
|--------|---------|
| クローン名  | EP1190Y |
| アイソタイプ | IgG     |

## アプリケーション

**The Abpromise guarantee**      **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab53281の使用に適用されます  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション         | Abreviews | 特記事項                                                                                                                                                                                                              |
|------------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Flow Cyt (Intra) |           | 1/10 - 1/100.<br><b>ab172730</b> - 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.<br>See <b>IHC antigen retrieval protocols</b> .<br><b>For unpurified use at 1/100.</b> |

## ターゲット情報

|       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 機能    | 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.                                                                                                                                                                                                                                                                                                                                                                                             |
| 組織特異性 | Expressed in fetal intestine, liver, lung, and kidney. Not expressed in adult tissues.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 関連疾患  | 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).<br>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.

#### 配列類似性

Belongs to the hedgehog family.

#### 翻訳後修飾

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.

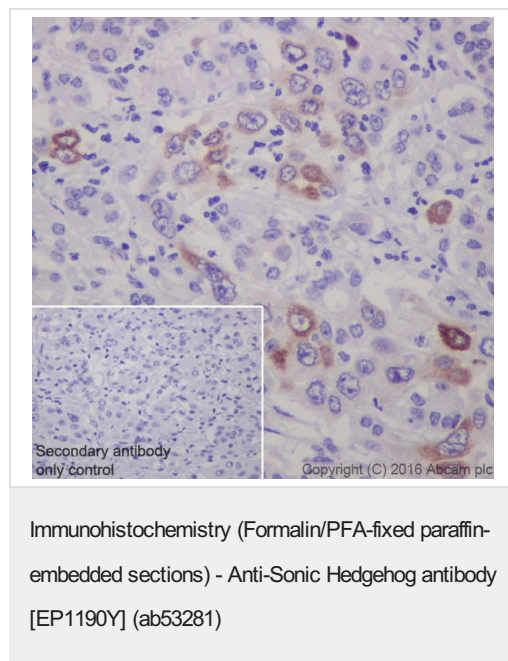
Cholesterylation is required for N-product targeting to lipid rafts and multimerization.

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

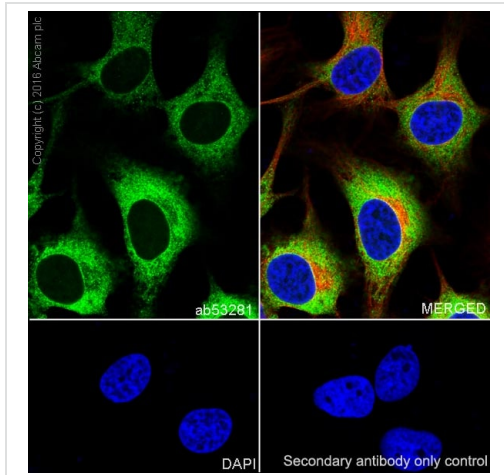
#### 細胞内局在

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.

#### 画像

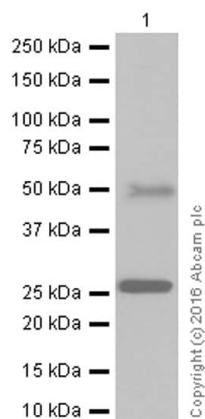


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue sections labeling Sonic Hedgehog with purified ab53281 at 1/2000 dilution (0.097 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Tissue was counterstained with 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

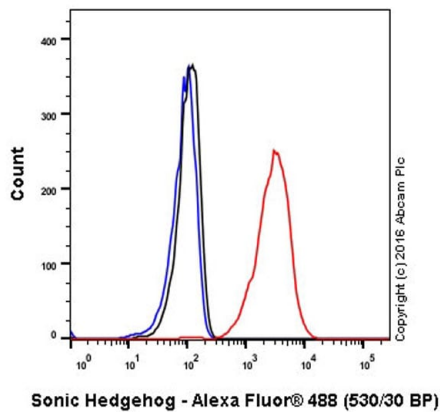
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 51 kDa

**Observed band size:** 27,50 kDa

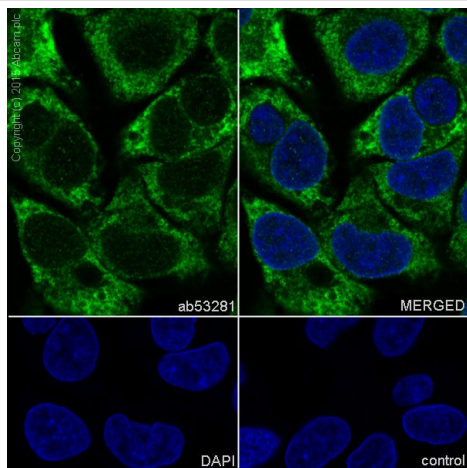
Blocking and diluting buffer: 5% NFDM/TBST.

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



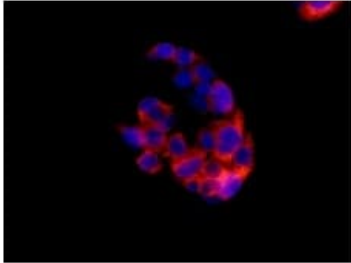
Flow Cytometry (Intracellular) - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

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).



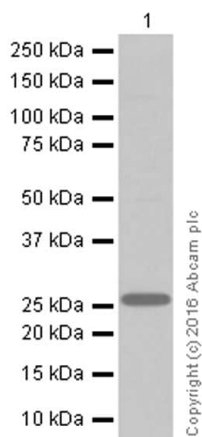
Immunocytochemistry/ Immunofluorescence - Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

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

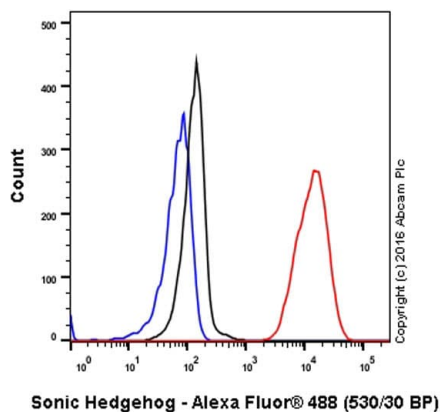
**Predicted band size:** 51 kDa

**Observed band size:** 27 kDa

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

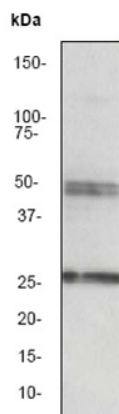
Blocking and diluting buffer: 5% NFDM /TBST .

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

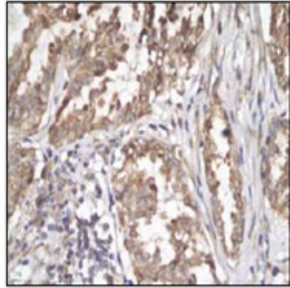
Goat anti-rabbit HRP at 1/2000 dilution

**Predicted band size:** 51 kDa

**Observed band size:** 51 kDa

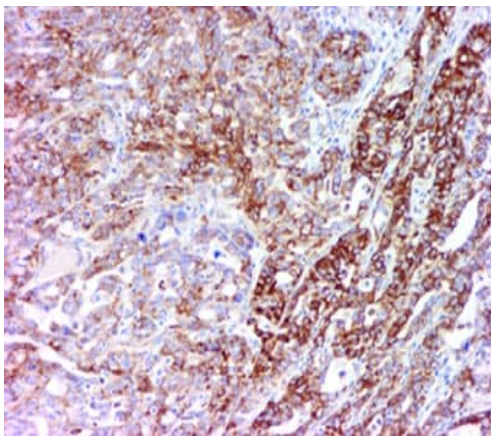
**Additional bands at:** 27 kDa (possible isoform), 49 kDa. We are unsure as to the identity of these extra bands.





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)



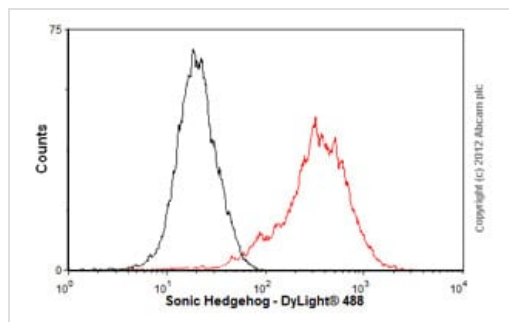
Immunohistochemical analysis of Formalin fixed paraffin embedded human ovarian cancer tissue, staining Sonic Hedgehog with unpurified ab53281.

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/1x10<sup>6</sup> 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?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Sonic Hedgehog antibody [EP1190Y] (ab53281)

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