### Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-NOTCH4 antibody [EPR18049]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [EPR18049] to NOTCH4</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: Flow Cyt, ICC/IF, IP, WB</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Recombinant fragment within Human NOTCH4 aa 1100-1300. The exact sequence is proprietary. Database link: Q99466</td>
</tr>
<tr>
<td>Positive control</td>
<td>WB: Human NOTCH4 fragment recombinant protein; Human fetal lung, placenta, fetal brain, fetal heart and fetal kidney lysates; HeLa, HepG2, A549, Jurkat, C6, RAW 264.7, PC-12, NIH/3T3 and F9 whole cell lysates; Mouse brain, heart, spleen and placenta lysates; Rat brain and heart lysates. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt: HeLa and NIH/3T3 cells. IP: HeLa whole cell lysate.</td>
</tr>
<tr>
<td>General notes</td>
<td>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.</td>
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</table>

### Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage buffer</td>
<td>Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA</td>
</tr>
<tr>
<td>Purity</td>
<td>Protein A purified</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone number</td>
<td>EPR18049</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
</tbody>
</table>
**Application**

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td>1/200.</td>
<td></td>
</tr>
<tr>
<td>ICC/IF</td>
<td>1/200.</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>1/60.</td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>1/1000.</td>
<td>Detects a band of approximately 150 kDa (predicted molecular weight: 210 kDa).</td>
</tr>
</tbody>
</table>

**Function**

Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination. Upon ligand activation through the released notch intracellular domain (NICD) it forms a transcriptional activator complex with RBPJ/RBPSUH and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs. May regulate branching morphogenesis in the developing vascular system.

**Tissue specificity**

Highly expressed in the heart, moderately in the lung and placenta and at low levels in the liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, bone marrow and fetal liver. No expression was seen in adult brain or peripheral blood leukocytes.

**Sequence similarities**

Belongs to the NOTCH family.
Contains 5 ANK repeats.
Contains 28 EGF-like domains.
Contains 3 LNR (Lin/Notch) repeats.

**Post-translational modifications**

Synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase in the trans-Golgi network before it reaches the plasma membrane to yield an active, ligand-accessible form. Cleavage results in a C-terminal fragment N(TM) and a N-terminal fragment N(EC). Following ligand binding, it is cleaved by TNF-alpha converting enzyme (TACE) to yield a membrane-associated intermediate fragment called notch extracellular truncation (NEXT). This fragment is then cleaved by presenilin dependent gamma-secretase to release a notch-derived peptide containing the intracellular domain (NICD) from the membrane.

**Cellular localization**

Cell membrane and Nucleus. Following proteolytical processing NICD is translocated to the nucleus.

**Target**

Our Abpromise guarantee covers the use of ab184742 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
All lanes: Anti-NOTCH4 antibody [EPR18049] (ab184742) at 1/2000 dilution

Lane 1: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2: HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 3: A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (Agarose) (ab97052) at 1/100000 dilution

Predicted band size: 210 kDa
Observed band size: 150 kDa
Why is the actual band size different from the predicted?

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1, 2 and 3: 15 seconds; Lane 4: 3 minute.
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling NOTCH4 with ab184742 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 at 1/1000 dilution.

Flow cytometric analysis of 2% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labelling NOTCH4 with ab184742 at 1/200 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A]-Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.

**All lanes**: Anti-NOTCH4 antibody [EPR18049] (ab184742) at 1/2000 dilution

**Lane 1**: Human fetal lung lysate  
**Lane 2**: Human placenta lysate  
**Lane 3**: Human fetal brain lysate  
**Lane 4**: Human fetal heart lysate  
**Lane 5**: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary  
**All lanes**: Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution
Predicted band size: 210 kDa
Observed band size: 150 kDa

**why is the actual band size different from the predicted?**

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: Lane 1, 3, 4 and 5: 3 minutes; Lane 2: 30 seconds.
The bands beneath the 150 kD NECD band are likely to be the degradation fragments.

Anti-NOTCH4 antibody [EPR18049] (ab184742) at 1/100 dilution
+ Human NOTCH4 fragment recombinant protein at 0.01 µg

**Secondary**
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 210 kDa

**Exposure time:** 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

**All lanes:** Anti-NOTCH4 antibody [EPR18049] (ab184742) at 1/2000 dilution

Lane 1: Mouse brain lysate
Lane 2: Mouse heart lysate
Lane 3: Mouse spleen lysate
Lane 4: Rat brain lysate
Lane 5: Rat heart lysate
Lane 6: C6 (Rat glioblastoma cell line) whole cell lysate
Lane 7: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 8: PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 9: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 10: Mouse placenta lysate
Lane 11: F9 (Mouse embryonic testicular cancer cell line) whole cell lysate
Lysates/proteins at 10 µg per lane.

**Secondary**

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 210 kDa

**Observed band size:** 150 kDa

*why is the actual band size different from the predicted?*

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1,2,3,4 and 5: 1 minute; Lane 6, 7, 8 and 9: 15 seconds; Lane 10 and 11: 30 seconds.

The bands beneath the 150 kD NECD band are likely to be the degradation fragments.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling NOTCH4 with ab184742 at 1/200 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on NIH/3T3 cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.
Flow cytometric analysis of 2% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling NOTCH4 with ab184742 at 1/200 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A]-Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.

NOTCH4 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab184742 at 1/60 dilution.

Western blot was performed from the immunoprecipitate using ab184742 at 1/5000 dilution.

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.

Lane 1: HeLa whole cell lysate, 10µg (Input).
Lane 2: ab184742 IP in HeLa whole cell lysate.
Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab184742 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 1 second.

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