# abcam

### Product datasheet

## Anti-Nicastrin antibody [EPR2575Y] ab68145





#### 1 References 4 Images

#### Overview

**Product name** Anti-Nicastrin antibody [EPR2575Y]

**Description** Rabbit monoclonal [EPR2575Y] to Nicastrin

**Host species** Rabbit

Suitable for: WB **Tested applications** 

Unsuitable for: ICC/IF or IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Synthetic peptide within Human Nicastrin aa 650-750 (C terminal). The exact sequence is **Immunogen** 

proprietary.

Positive control WB: HEK293T, HeLa, Jurkat and HL-60 cell lysates; C6 cell lysate; Neuro-2a cell lysate; U-87 MG

and SH-SY5Y cell lysates.

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### **Properties**

**Form** 

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.2

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

**Purity** Protein A purified

Clonality Monoclonal Clone number **EPR2575Y** 

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab68145 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000 - 1/2000. Detects a band of approximately 100-120, 130-160 kDa (predicted molecular weight: 78 kDa).

#### **Application notes**

Is unsuitable for ICC/IF or IHC-P.

#### **Target**

-u			

Essential subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (beta-amyloid precursor protein). It probably represents a stabilizing cofactor required for the assembly of the gamma-secretase complex.

**Tissue specificity** 

Widely expressed.

Sequence similarities

Belongs to the nicastrin family.

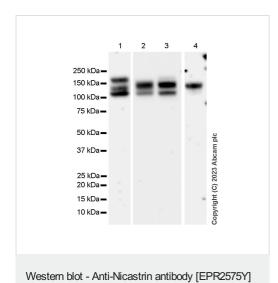
**Cellular localization** 

Membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I

to stage IV.

#### **Images**

(ab68145)



**All lanes :** Anti-Nicastrin antibody [EPR2575Y] (ab68145) at 1/1000 dilution

Lane 1: C6 (rat glial tumor glial cell) whole cell lysate

Lane 2 : Neuro-2a (mouse neuroblastoma neuroblast) whole cell

lysate

Lane 3: U-87 MG (human glioblastoma-astrocytoma epithelial cell)

whole cell lysate

Lane 4: SH-SY5Y (human neuroblastoma epithelial cell) whole cell

lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 78 kDa

Observed band size: 100-140 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: Lane 1, 4: 180 seconds; Lane 2, 3: 15 seconds.

The bands observed is consistency with literature (PMID: 15322084).

The lysates were freshly made and used for Western Blotting immediately to minimize protein degradation.

**All lanes :** Anti-Nicastrin antibody [EPR2575Y] (ab68145) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: NCSTN knockout HEK293T cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : HL-60 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

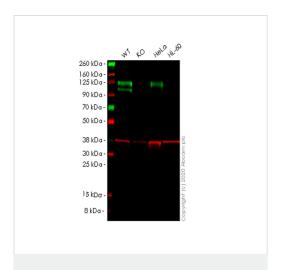
All lanes: Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 78 kDa

Observed band size: 100-125 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab68145 observed at 100-125 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab68145 Anti-Nicastrin antibody [EPR2575Y] was shown to specifically react with Nicastrin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266602">ab266602</a> (knockout cell lysate <a href="mailto:ab258061">ab258061</a>) was used. Wild-type and Nicastrin knockout samples were subjected to SDS-PAGE. ab68145 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8245</a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse



Western blot - Anti-Nicastrin antibody [EPR2575Y] (ab68145)

 $lgG~H\&L~(IRDye^{®}~680RD)$  preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

kDa 1 2 3
2501501007550372520-

Western blot - Anti-Nicastrin antibody [EPR2575Y] (ab68145)

**All lanes :** Anti-Nicastrin antibody [EPR2575Y] (ab68145) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : HL-60 cell lysate
Lane 3 : Jurkat cell lysate

Lysates/proteins at 10 µg per lane.

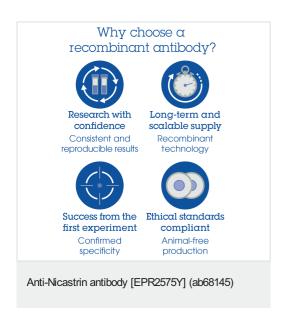
#### **Secondary**

All lanes: HRP conjugated Goat anti-Rabbit at 1/2000 dilution

Predicted band size: 78 kDa

Additional bands at: 100-120 kDa (possible isoform), 130-160

kDa (possible isoform)



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