abcam

Product datasheet

Anti-Doublecortin antibody [EPR19997] ab207175



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Overview

Product name Anti-Doublecortin antibody [EPR19997]

Description Rabbit monoclonal [EPR19997] to Doublecortin

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, IHC-Fr, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human fetal brain and brain lysates; Mouse and rat brain lysates; SH-SY5Y whole cell lysate.

IHC-P: Mouse and rat adult hippocampus tissues. IHC-Fr: Rat adult hippocampus tissue. ICC/IF:

SH-SY5Y cells.

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 Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR19997

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab207175 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
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 40 kDa (predicted molecular weight: 40 kDa).
IHC-Fr		1/100. Antigen retrieval: Heated citrate solution (10mM citrate PH 6.0 + 0.05% Tween-20). IHC-Fr is only recommended for rat species.
ICC/IF		1/250.

Target

Function

Seems to be required for initial steps of neuronal dispersion and cortex lamination during cerebral cortex development. May act by competing with the putative neuronal protein kinase DCAMKL1 in binding to a target protein. May in that way participate in a signaling pathway that is crucial for neuronal interaction before and during migration, possibly as part of a calcium ion-dependent signal transduction pathway. May be part with LIS-1 of an overlapping, but distinct, signaling pathways that promote neuronal migration.

Tissue specificity

Highly expressed in neuronal cells of fetal brain (in the majority of cells of the cortical plate, intermediate zone and ventricular zone), but not expressed in other fetal tissues. In the adult, highly expressed in the brain frontal lobe, but very low expression in other regions of brain, and not detected in heart, placenta, lung, liver, skeletal muscles, kidney and pancreas.

Involvement in disease

Defects in DCX are the cause of lissencephaly X-linked type 1 (LISX1) [MIM:300067]; also called X-LIS or LIS. LISX1 is a classic lissencephaly characterized by mental retardation and seizures that are more severe in male patients. Affected boys show an abnormally thick cortex with absent or severely reduced gyri. Clinical manifestations include feeding problems, abnormal muscular tone, seizures and severe to profound psychomotor retardation. Female patients display a less severe phenotype referred to as 'doublecortex'.

Defects in DCX are the cause of subcortical band heterotopia X-linked (SBHX) [MIM:300067]; also known as double cortex or subcortical laminar heterotopia (SCLH). SBHX is a mild brain malformation of the lissencephaly spectrum. It is characterized by bilateral and symmetric plates or bands of gray matter found in the central white matter between the cortex and cerebral ventricles, cerebral convolutions usually appearing normal.

Note=A chromosomal aberration involving DCX is found in lissencephaly. Translocation t(X;2) (q22.3;p25.1).

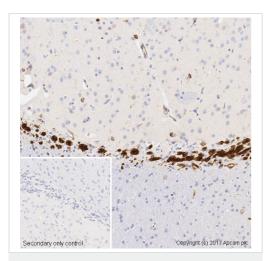
Sequence similarities

Contains 2 doublecortin domains.

Cellular localization

Cytoplasm.

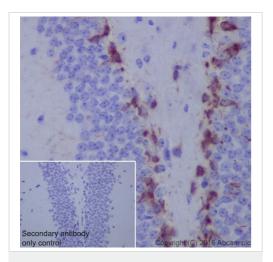
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Doublecortin antibody
[EPR19997] (ab207175)

IHC image of Doublecortin staining in a formalin fixed, paraffin embedded normal rat hippocampus (SVZ region) tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab207175 at 1/500 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. As a negative control (inset), an identical assay was performed without adding the primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

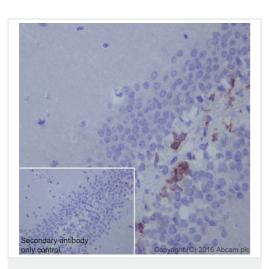


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Doublecortin antibody
[EPR19997] (ab207175)

Immunohistochemical analysis of paraffin-embedded mouse adult hippocampus tissue labeling Doublecortin with ab207175 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on mouse hippocampal dentate gyrus is observed [PMID:23690918] [PMID:16814555]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

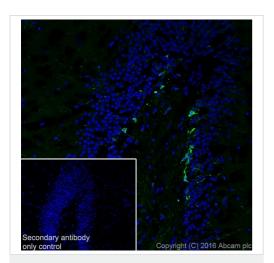


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Doublecortin antibody
[EPR19997] (ab207175)

Immunohistochemical analysis of paraffin-embedded rat adult hippocampus tissue labeling Doublecortin with ab207175 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on rat hippocampal dentate gyrus is observed [PMID:23690918] [PMID:16814555]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

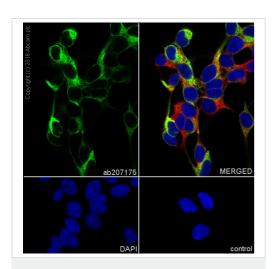
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Frozen sections) - Anti-Doublecortin antibody [EPR19997] (ab207175)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen rat adult hippocampus tissue labeling Doublecortin with ab207175 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining on rat hippocampal dentate gyrus is observed [PMID:23690918] [PMID:16814555]. The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

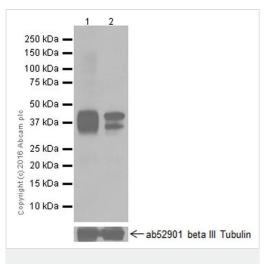


Immunocytochemistry/ Immunofluorescence - Anti-Doublecortin antibody [EPR19997] (ab207175)

Immunofluorescent analysis of 100% Methanol-fixed SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling Doublecortin with ab207175 at 1/250 dilution, followed by Goat antirabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on SH-SY5Y cell line.

The nuclear counter stain 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 lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



Western blot - Anti-Doublecortin antibody [EPR19997] (ab207175)

All lanes : Anti-Doublecortin antibody [EPR19997] (ab207175) at 1/10000 dilution

Lane 1: Human fetal brain lysate

Lane 2: Human brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 40 kDa **Observed band size:** 40 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The higher molecular weight band is phosphorylated form and the lower molecular weight band is non-phosphorylated form. (PMID:

17178868).

Doublecortin is highly expressed during embryonic development and downregulated in adult tissues. (PMID: 10399933).

All lanes : Anti-Doublecortin antibody [EPR19997] (ab207175) at 1/10000 dilution

Lane 1: SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

Lane 2: Mouse brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 40 kDa **Observed band size:** 40 kDa

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

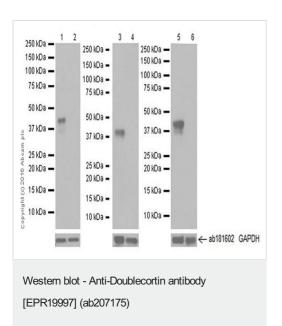
[EPR19997] (ab207175)

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 15 seconds; Lane 2: 3 minutes.

The higher molecular weight band is phosphorylated form and the lower molecular weight band is non-phosphorylated form. (PMID: 17178868).

Doublecortin is highly expressed during embryonic development and downregulated in adult tissues. (PMID: 10399933).



All lanes : Anti-Doublecortin antibody [EPR19997] (ab207175) at 1/1000 dilution

Lane 1: Human fetal brain lysate

Lane 2: Human fetal heart lysate

Lane 3: Mouse brain lysate

Lane 4: Mouse heart lysate

Lane 5: Rat brain lysate

Lane 6: Rat heart lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 40 kDa **Observed band size:** 40 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1-4: 3 minutes; Lane 5/6: 10 seconds.

The observed expression profile is consistent with what has been described in the literature (PMID: 10550327).



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