abcam

Product datasheet

Anti-DGCR8 antibody [EPR18757] ab191875

Recombinant RabMAb

★★★★★ 2 Abreviews 25 References 9 Images

Overview

Product name Anti-DGCR8 antibody [EPR18757]

Description Rabbit monoclonal [EPR18757] to DGCR8

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa, HEK-293, WEHI-3, Neuro-2a, PC-12 and NIH/3T3 whole cell lysates; Human fetal

kidney lysate; Mouse brain, mouse testis and rat brain lysates. ICC/IF: HeLa and Jurkat cells. IP:

HEK-293 whole cell lysate.

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 (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR18757

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab191875 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
Flow Cyt (Intra)		1/1000.
WB	*** <u>*</u>	1/1000. Detects a band of approximately 100 kDa (predicted molecular weight: 86 kDa).
ICC/IF		1/1000.
IP		1/60.

Target

Function

Component of the microprocessor complex that acts as a RNA- and heme-binding protein that is involved in the initial step of microRNA (miRNA) biogenesis. Component of the microprocessor complex that is required to process primary miRNA transcripts (pri-miRNAs) to release precursor miRNA (pre-miRNA) in the nucleus. Within the microprocessor complex, DGCR8 function as a molecular anchor necessary for the recognition of pri-miRNA at dsRNA-ssRNA junction and directs DROSHA to cleave 11 bp away form the junction to release hairpin-shaped pre-miRNAs that are subsequently cut by the cytoplasmic DICER to generate mature miRNAs. The hemebound DGCR8 dimer binds pri-miRNAs as a cooperative trimer (of dimers) and is active in triggering pri-miRNA cleavage, whereas the heme-free DGCR8 monomer binds pri-miRNAs as a dimer and is much less active. Both double-stranded and single-stranded regions of a pri-miRNA are required for its binding. Involved in the silencing of embryonic stem cells self-renewal.

Tissue specificity

Ubiquitously expressed.

Sequence similarities

 ${\it Contains 2 DRBM (double-stranded RNA-binding) domains.}$

Contains 1 WW domain.

Domain

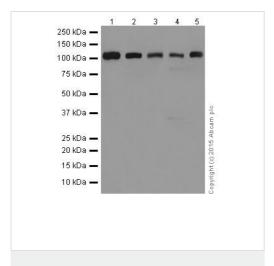
Both DRBM domains are required for efficient binding to pri-miRNA. The region between residues 276 and 498 has an autoinhibitory function on pri-miRNA processing activity.

Cellular localization

Nucleus. Nucleus > nucleolus. Colocalizes with nucleolin and DROSHA in the nucleolus. Mostly detected in the nucleolus as electron-dense granular patches around the fibrillar center (FC) and granular component (GC). Also detected in the nucleoplasm as small foci adjacent to splicing speckles near the chromatin structure. Localized with DROSHA in GW bodies (GWBs), also

known as P-bodies.

Images



Western blot - Anti-DGCR8 antibody [EPR18757] (ab191875)

All lanes : Anti-DGCR8 antibody [EPR18757] (ab191875) at 1/1000 dilution

Lane 1: HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lane 3 : WEHI-3 (Mouse leukemia cell line) whole cell lysate

Lane 4 : Neuro-2a (Mouse neuroblastoma cells) whole cell lysate

Lane 5: Mouse testis lysate

Lysates/proteins at 20 µg per lane.

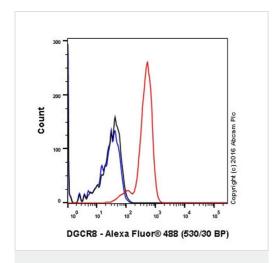
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 86 kDa **Observed band size:** 100 kDa

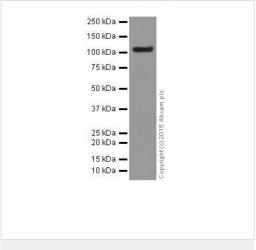
Exposure time: 30 seconds

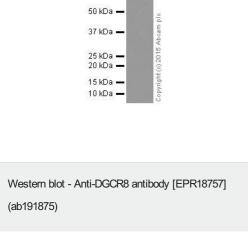
Blocking/Dilution buffer: 5% NFDM/TBST.

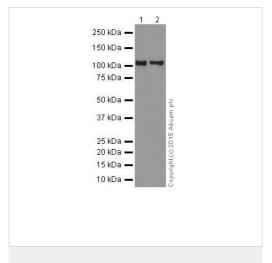


Flow Cytometry (Intracellular) - Anti-DGCR8 antibody [EPR18757] (ab191875)

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) labelling DGCR8 with purified ab191875 at 1/1000 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Alexa Fluor[®] 488 goat anti-rabbit lgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.







Western blot - Anti-DGCR8 antibody [EPR18757] (ab191875)

Anti-DGCR8 antibody [EPR18757] (ab191875) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/50000 dilution

Predicted band size: 86 kDa Observed band size: 100 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes: Anti-DGCR8 antibody [EPR18757] (ab191875) at 1/1000 dilution

Lane 1: Mouse brain lysate Lane 2: Rat brain lysate

Lysates/proteins at 10 µg per lane.

Secondary

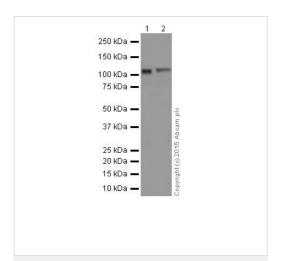
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 86 kDa Observed band size: 100 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-DGCR8 antibody [EPR18757] (ab191875)

All lanes : Anti-DGCR8 antibody [EPR18757] (ab191875) at 1/1000 dilution

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lane 2: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 86 kDa

Observed band size: 100 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

ab191875 MERGED

DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-DGCR8 antibody [EPR18757] (ab191875)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling DGCR8 with ab191875 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on HeLa cell line.

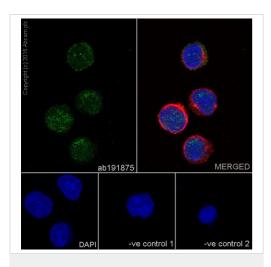
The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191875 at 1/500 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit lgG

H&L) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-DGCR8 antibody [EPR18757] (ab191875)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling DGCR8 with ab191875 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

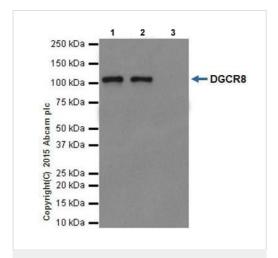
Confocal image showing nuclear and weakly cytoplasmic staining on Jurkat cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191875 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Immunoprecipitation - Anti-DGCR8 antibody [EPR18757] (ab191875)

DGCR8 was immunoprecipitated from 1mg of HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate with ab191875 at 1/60 dilution.

Western blot was performed from the immunoprecipitate using ab191875 at 1/1000 dilution.

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

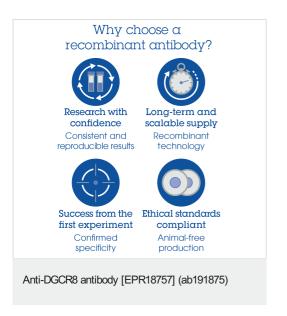
Lane 1: HEK-293 whole cell lysate 10ug (Input).

Lane 2: ab191875 IP in HEK-293 whole cell lysate.

Lane 3: Rabbit monoclonal IgG ($\underline{ab172730}$) instead of ab191875 in HEK-293 whole cell lysate.

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

Exposure time: 30 seconds.



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