abcam

Product datasheet

Anti-Claudin 5 antibody [EPR7583] ab131259

Recombinant RabMAb

★★★★ 7 Abreviews 66 References 14 Images

Overview

Product name Anti-Claudin 5 antibody [EPR7583]

Description Rabbit monoclonal [EPR7583] to Claudin 5

Host species Rahhit

Specificity ab131259 failed to detect target band in mouse brain tissue.

Mouse species is recommended based on WB result. This antibody is unsuitable to be used in

IHC on mouse tissues.

This antibody failed to get signal in cell lines when performing western blot, so we do not

recommend to use it when testing cell lines in westen blot.

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

Unsuitable for: IHC-Fr

Reacts with: Mouse, Human Species reactivity

Immunogen Synthetic peptide within Human Claudin 5 aa 200 to the C-terminus (intracellular). The exact

> sequence is proprietary. Database link: **000501**

Positive control WB: Mouse lung lysates, Human fetal brain, lung squamous cell cancer and small cell lung cancer,

IHC-P: Human colon, tonsil, placenta, uterus, kidney and lung tissues. ICC/IF: HUVEC 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

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.

Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clone number Monoclonal EPR7583

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab131259 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
ICC/IF	★★★★ <u>(1)</u>	Use a concentration of 0.008 μg/ml.
WB	****(1)	1/1000 - 1/10000. Detects a band of approximately 23 kDa (predicted molecular weight: 23 kDa). ab131259 failed to detect target band in mouse brain tissue.
		This antibody failed to get signal in cell lines when performing western blot, so we do not recommend to use it when testing cell lines in westen blot.
IHC-P	* * * in in (4)	1/100 - 1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Application notes Is unsuitable for IHC-Fr.

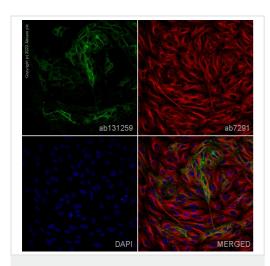
Target

Function Plays a major role in tight junction-specific obliteration of the intercellular space.

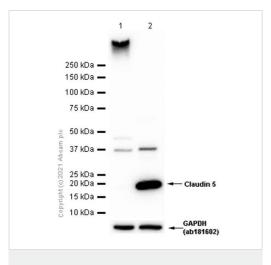
Sequence similarities Belongs to the claudin family.

Cellular localization Cell junction > tight junction. Cell membrane.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Claudin 5 antibody [EPR7583] (ab131259)



Western blot - Anti-Claudin 5 antibody [EPR7583] (ab131259)

ab131259 staining Claudin 5 in HUV-EC cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab131259 at 0.008µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

All lanes : Anti-Claudin 5 antibody [EPR7583] (ab131259) at 1/1000 dilution

Lane 1 : Mouse lung lysates, boiled
Lane 2 : Mouse lung lysates, unboiled

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: 23 kDa Observed band size: 20 kDa

Exposure time: 20 seconds

We recommend not to boil the samples after lysis to get desired WB results.

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

All lanes: Anti-Claudin 5 antibody [EPR7583] (ab131259) at

250 kDa —

150 kDa —

100 kDa —

75 kDa —

Lane 1: Mouse lung tissue lysates with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051)

Lane 2: Rat lung tissue lysates with 5% NFDM/TBST

Predicted band size: 23 kDa **Observed band size:** 20 kDa

Exposure time: 3 minutes

We didn't recommend rat for that ab131259 failed to detect target band in rat lung in which Claudin 5 is highly expressed.

All lanes : Anti-Claudin 5 antibody [EPR7583] (ab131259) at 1/1000 dilution (unpurified)

Lane 1: Human fetal brain lysate

Lane 2: Human lung squamous cell cancer lysate

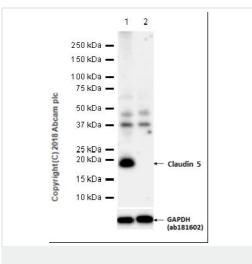
Lane 3: Human small cell lung cancer lysate

Lysates/proteins at 10 µg per lane.

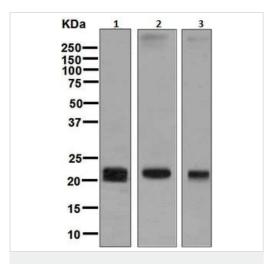
Secondary

All lanes: HRP labelled Goat anti Rabbit lgG at 1/2000 dilution

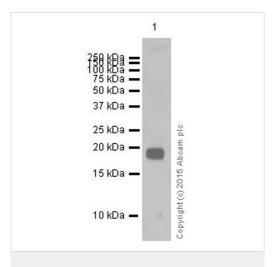
Predicted band size: 23 kDa



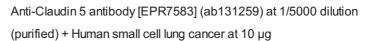
Western blot - Anti-Claudin 5 antibody [EPR7583] (ab131259)



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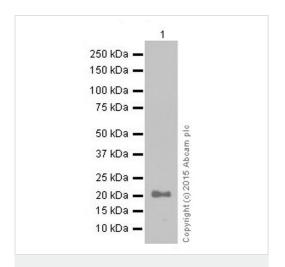


Secondary

HRP goat anti-rabbit lgG (H+L) at 1/5000 dilution

Predicted band size: 23 kDa **Observed band size:** 20 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Claudin 5 antibody [EPR7583] (ab131259)

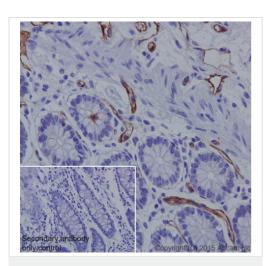
Anti-Claudin 5 antibody [EPR7583] (ab131259) at 1/20000 dilution (purified) + Human lung squamous cell cancer lysate at 10 µg

Secondary

HRP goat anti-rabbit lgG (H+L) at 1/5000 dilution

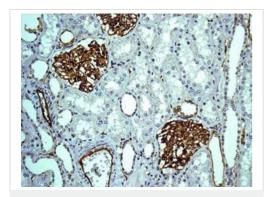
Predicted band size: 23 kDa **Observed band size:** 20 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

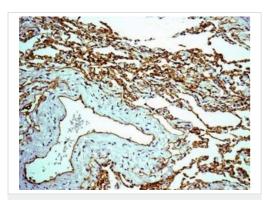
Immunohistochemical staining of paraffin embedded human colon with purified ab131259 at a working dilution of 1/1000. The secondary antibody used is HRP goat anti-rabbit lgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Immunohistochemical analysis of paraffin embedded Human kidney tissue labelling Claudin 5 with unpurified ab131259 antibody at 1/100 dilution.

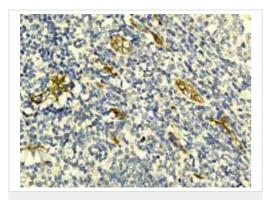
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Immunohistochemical analysis of paraffin embedded Human lung tissue labelling Claudin 5 with unpurified ab131259 antibody at 1/100 dilution.

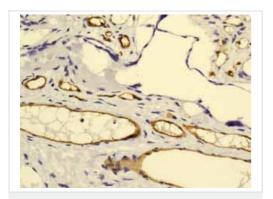
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Unpurified ab131259 showing positive staining in Normal tonsil tissue.

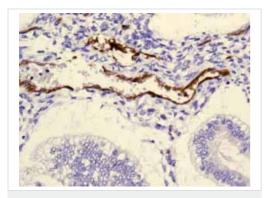
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Unpurified ab131259 showing positive staining in Normal placenta tissue.

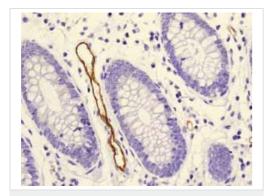
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Unpurified ab131259 showing positive staining in Normal uterus tissue.

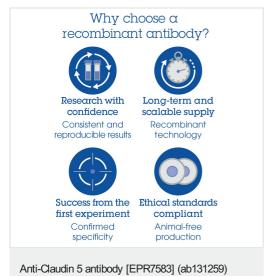
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 5 antibody
[EPR7583] (ab131259)

Unpurified ab131259 showing positive staining in Normal colon tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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