

# **Product datasheet**

# Anti-Pax2 antibody [EP3251] ab79389

Recombinant RabMAb

\*\*\*\*\* 11 Abreviews 36 References 12 Images

# Overview

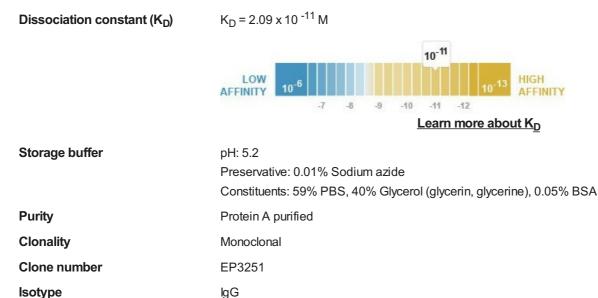
| Product name        | Anti-Pax2 antibody [EP3251]  |  |  |
|---------------------|--|--|--|
| Description         | Rabbit monoclonal [EP3251] to Pax2   |  |  |
| Host species        | Rabbit   |  |  |
| Tested applications | Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF  |  |  |
| Species reactivity  | Reacts with: Mouse, Rat, Human   |  |  |
| Immunogen           | Synthetic peptide within Human Pax2 aa 1-100. The exact sequence is proprietary.<br>Database link: <u>Q02962</u><br>(Peptide available as <u>ab188213</u> )  |  |  |
| Positive control    | WB: HEK-293 transfected with his tagged human full-length PAX2. IHC-P: Human, rat and mouse kidney tissues, Human kidney cancer tissues. ICC/IF: HepG2 cells. Flow Cyt (intra): K562 cells.  |  |  |
| General notes       | <ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> <li>The Life Science industry has been in the grips of a reproducibility crisis for a number of years.</li> <li>Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</li> <li>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&amp;As</li> </ul> |  |  |

# Properties

Form Storage instructions Liquid

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.



# Applications

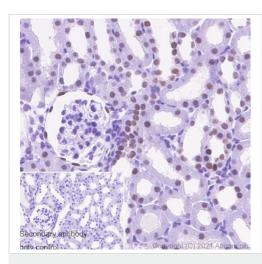
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab79389 in the following tested applications.

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

| Application      | Abreviews        | Notes   |
|------------------|------------------|---|
| Flow Cyt (Intra) |                  | 1/50.<br><u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an<br>isotype control with this antibody.  |
| WB               | ★★★★☆(1)         | 1/1000 - 1/10000. Detects a band of approximately 45 kDa<br>(predicted molecular weight: 45 kDa).Can be blocked with <u>Pax2</u><br><u>peptide (ab188213)</u> .                 |
| IHC-P            | ★★★★★ <u>(2)</u> | 1/500 - 1/2500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <b>IHC antigen retrieval protocols</b> . |
| ICC/IF           | ★★★★ ( <u>3)</u> | 1/50.   |

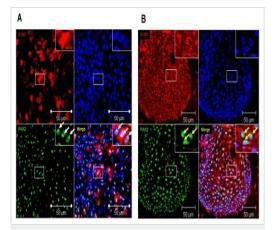
| Target                |  |
|-----------------------|--|
| Relevance             | Pax2 is a transcription factor critically required during the development of the nervous and excretory systems, including the midbrain, hindbrain, spinal cord, eye, ear and urogenital tract. Like other products of the Pax gene family, Pax2 encodes a conserved 128 amino acid paired box DNA-binding domain in the N-terminal portion of the molecule. Function: Probable transcription factor that may have a role in kidney cell differentiation. Has a critical role in the development of the urogenital tract, the eyes, and the CNS. Tissue specificity: Expressed in primitive cells of the kidney, ureter, eye, ear and central nervous system. |
| Cellular localization | Nuclear  |

#### Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labelling Pax2 with ab79389 at 1/100 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, aRabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin. Positive staining on mouse kidney cancer. The section was incubated with ab79389 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



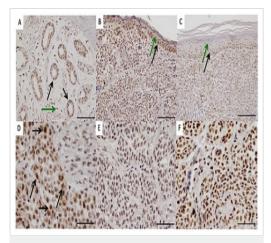
#### Immunocytochemistry/ Immunofluorescence - Anti-

#### Pax2 antibody [EP3251] (ab79389)

Lee SB et al. PAX2 regulates ADAM10 expression and mediates anchorage-independent cell growth of melanoma cells. PLoS One 6:e22312 (2011). Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/ Pax2 is expressed in melanocytes of benign nevi **(A)** and melanoma cells of patients with malignant melanoma **(B)**. Cells were grown on coverslips and fixed with 4% paraformaldehyde/PBS. After washing the cells with PBS, cells were permeabilized and blocked with 0.1% Triton X-100/PBS containing 5% BSA. Pax2 (green) was examined by immunofluorescent analysis using ab79389 at 1/100 dilution (incubated for 1 hour at room temperature). Following 3 times washing, bound antibodies were deteced by Alexa 488 conjugated goat anti-mouse or Cy3 conjugated goat anti-mouse secondary antibodies.

Following PBS-washing nuclei were stained with 4′6-diamidino-2phenylindole (DAPI, Blue) and cells were mounted in Fluoromount-G<sup>™</sup> and examined by fluorescence microscopy.

White arrows in the higher magnified insets indicate PAX2 expression in nucleoli of melanocytes of benign nevi (**A**), yellow arrows in the higher magnified insets specify PAX2 expression in nucleoli of melanoma cells (**B**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389)

Lee SB et al. PAX2 regulates ADAM10 expression and mediates anchorage-independent cell growth of melanoma cells. PLoS One 6:e22312 (2011).

Immunohistochemical analysis of Pax2 expression in tissue sections of human benign nevi and malignant melanoma using ab79389 at 1/100 dilution.

All specimens were fixed in 4% formaline (pH 7.4), embedded in paraffin followed by cutting with a microtome (3  $\mu$ m thickness). The slides were deparaffinized in xylol for 20 minutes and then rehydrated in descending series of ethanol (100%, 100%, 96%, 96%, 70%, and 70%). For antigen retrieval the slides were boiled in citrate buffer (pH 6.0) for 40 min, and then allowed to cool down for 15 min. After washing with PBS buffer the endogenous peroxidase was blocked with H<sub>2</sub>O<sub>2</sub> for 15 min at room temperature. After washing in PBS the slides were incubated with the antibody against PAX2 (dilution 1 $\Box$ 100) for 60 min at room temperature and washed in PBS again.

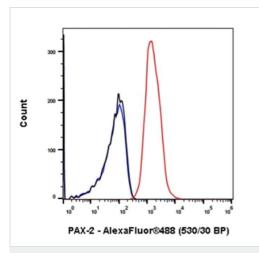
The secondary antibody was incubated for 20 min at room temperature and after washing the slides in PBS the biotin streptavidine label was incubated for 20 min at room temperature. A detection kit including horseradish peroxidase and diaminobenzidine as chromogene was applied for 5 min. Counterstaining was performed with hematoxilin for 6 min.

(**A**) In normal sweat glands, Pax2 is expressed in gland epithelial cells (black arrows) while intermingled stromal cells only show very weak or absent nuclear Pax2 expression (green arrow) Bar represents 100 μm.

(**B**, **C**) Normal appearing epidermal cell layers adjacent to (**B**, bar represent 100  $\mu$ m) nevi or (**C**, bar represent 200  $\mu$ m) malignant melanoma show a differentially Pax2 expression with strongest Pax2 levels in germinal basal cell layers (black arrows) decreasing in higher differentiated keratinocytes and finally being absent in corneocytes (green arrows).

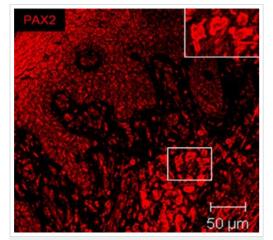
**(D)** Malignant melanoma cells - heterogeneous nuclear Pax2 expression. Strongest expression in large atypical nuclei with prominent nucleoli (black arrows).

(E, F) Pax2 expression in intradermal nevi was heterogeneous and did not correlate with histological features.



Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast)labelling Pax2 with ab79389 at 1/30. Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (**ab150077**) at 1/2000 was used as the secondary antibody (red). Rabbit monoclonal IgG (**ab172730**) was used as the isotype control (black). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

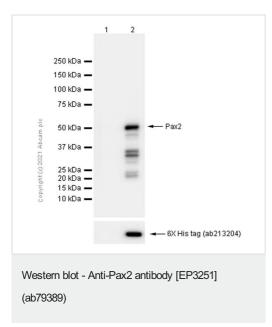
Flow Cytometry (Intracellular) - Anti-Pax2 antibody [EP3251] (ab79389)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251]

#### (ab79389)

Image from Lee SB et al., PLoS One. 2011;6(8):e22312. Epub 2011 Aug 18. Fig 5.; doi:10.1371/journal.pone.0022312; August 18, 2011, PLoS ONE 6(8): e22312. Immunohistochemical analysis of Human malignant melanoma tissue, staining Pax2 with unpurified ab79389. Antigen retrieval was performed via heat mediation in a citrate buffer (pH 6). Sections were blocked with 0.1% Triton X-100/PBS containing 1% BSA and 10% horse serum for 1 hour. Samples were incubated with primary antibody overnight at 4°C. A Cy3<sup>®</sup>conjugated goat anti-rabbit IgG was used as the secondary antibody.



All lanes : Anti-Pax2 antibody [EP3251] (ab79389) at 1/10000 dilution

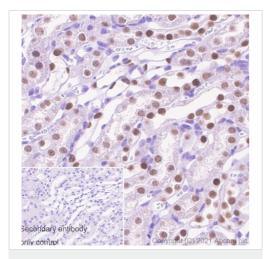
Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) transfected with empty vector whole cell lysate
Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) transfected with his tagged human full-length PAX2

Lysates/proteins at 15 µg per lane.

#### Secondary

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

### Predicted band size: 45 kDa



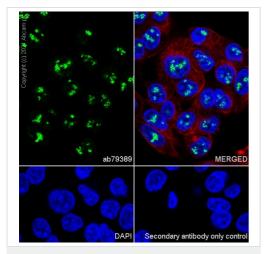
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389)

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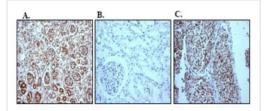
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney cancer tissue labelling Pax2 with ab79389 at 1/100 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, aRabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin. Positive staining on human kidney cancer. The section was incubated with ab79389 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389)

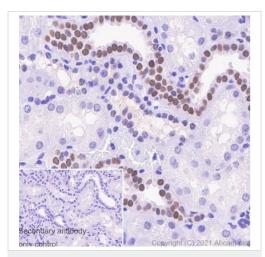


Immunocytochemistry/ Immunofluorescence - Anti-Pax2 antibody [EP3251] (ab79389)

Immunocytochemistry analysis of HepG2 (human hepatocellular carcinoma epithelial cell) labelling Pax2 with ab79389 at 1/200 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 at room temperature for 4 minutes. . Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 was used as the secondary antibody (green). Cells were counterstained with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red). Nuclear DNA was labelled with DAPI (blue). Confocal image showing nucleolar staining in HepG2 cell line.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of (A) human fetal kidney, (B) human normal kidney and (C) human renal cell carcinoma tissues labelled Pax2 with unpurified ab79389 at a dilution of 1/1000.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pax2 antibody [EP3251] (ab79389) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling Pax2 with ab79389 at 1/100 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <u>ab97051</u>, aRabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin. Positive staining on human kidney. The section was incubated with ab79389 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



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