# abcam

#### Product datasheet

## Anti-Otx2 antibody [EPR20375] ab183951

Recombinant RabMAb

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#### Overview

**Product name** Anti-Otx2 antibody [EPR20375]

**Description** Rabbit monoclonal [EPR20375] to Otx2

**Host species** Rahhit

Specificity This antibody recognizes isoform 1 and isoform 2 of Otx2.

**Tested applications** Suitable for: WB, IHC-P, IP

Species reactivity Reacts with: Mouse, Rat, Human

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control WB: Y79 whole cell lysate. IHC-P: Human retina and retinoblastoma tissues; Mouse E14.5

choroid plexus and retina tissues; Rat E14.5 choroid plexus tissue. Mouse e14 whole foetus. IP:

Y79 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** 

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, 0.05% BSA, 40% Glycerol

**Purity** Protein A purified

Clonality Monoclonal

Clone number EPR20375

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab183951 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		1/5000. Detects a band of approximately 32, 34 kDa (predicted molecular weight: 32 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

#### **Target**

Function Probably plays a role in the development of the brain and the sense organs. Can bind to the BCD

target sequence (BTS): 5'-TCTAATCCC-3'.

**Tissue specificity** Expressed in brain.

**Involvement in disease** Defects in OTX2 are the cause of microphthalmia syndromic type 5 (MCOPS5) [MIM:610125].

Microphthalmia is a clinically heterogeneous disorder of eye formation, ranging from small size of a single eye to complete bilateral absence of ocular tissues. Up to 80% of cases of microphthalia occur in association with syndromes that include non-ocular abnormalities. MCOPS5 patients manifest unilateral or bilateral microphthalmia/clinical anophthalmia and variable additional features including coloboma, microcornea, cataract, retinal dystrophy, hypoplasia or agenesis of the optic nerve, agenesis of the corpus callosum, developmental delay, joint laxity, hypotonia, and

seizures.

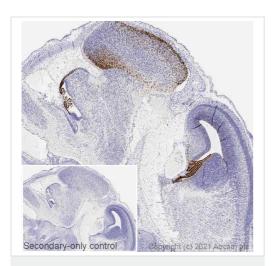
**Sequence similarities**Belongs to the paired homeobox family. Bicoid subfamily.

Contains 1 homeobox DNA-binding domain.

**Developmental stage** Embryo.

Cellular localization Nucleus.

#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

IHC image of Otx2 staining in a section of formalin-fixed paraffinembedded normal mouse e14 foetal brain 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 20mins. The section was then incubated with ab183951, 0.5ug/ml, 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. The inset secondary-only control image is taken from an identical assay without 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-Otx2 antibody
[EPR20375] (ab183951)

IHC image of Otx2 staining in a section of formalin-fixed paraffinembedded normal mouse e14 foetus 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 20mins. The section was then incubated with ab183951, 0.5ug/ml, 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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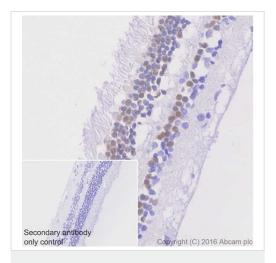


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

Immunohistochemical analysis of paraffin-embedded rat E14.5 choroid plexus tissue labeling Otx2 with ab183951 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on choroid plexus of E14.5 rat is observed [PMID: 23364326]. 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.

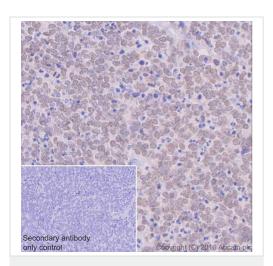


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

Immunohistochemical analysis of paraffin-embedded human retina tissue labeling Otx2 with ab183951 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on inner nuclear layer and outer nuclear layer of the human retina is observed [PMID: 19686387]. 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.

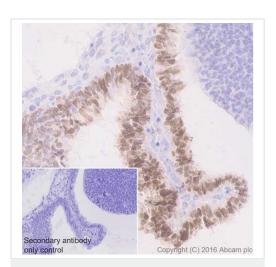


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

Immunohistochemical analysis of paraffin-embedded human retinoblastoma tissue labeling Otx2 with ab183951 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on tumor cells of human retinoblastoma is observed [PMID: 19686387]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.

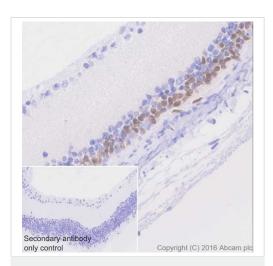


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

Immunohistochemical analysis of paraffin-embedded mouse E14.5 choroid plexus tissue labeling Otx2 with ab183951 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on choroid plexus of E14.5 mouse is observed [PMID: 23364326]. 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.

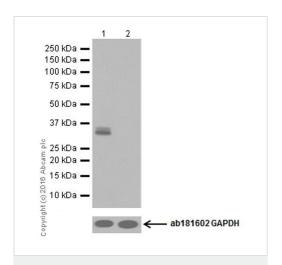
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody
[EPR20375] (ab183951)

Immunohistochemical analysis of paraffin-embedded mouse retina tissue labeling Otx2 with ab183951 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on nuclear layer of the mouse retina is observed [PMID: 19686387]. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.



Western blot - Anti-Otx2 antibody [EPR20375] (ab183951)

**All lanes :** Anti-Otx2 antibody [EPR20375] (ab183951) at 1/5000 dilution

Lane 1 : Y79 (Human retinoblastoma cell line) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix

adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

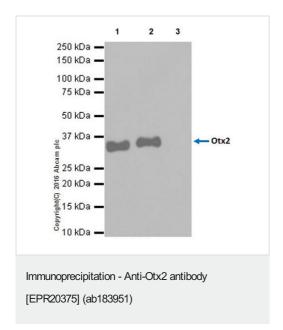
**Predicted band size:** 32 kDa **Observed band size:** 32,34 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control: HeLa (PMID: 12559959).

This antibody recognizes isoform 1 and isoform 2 of Otx2.



Otx2 was immunoprecipitated from 0.35 mg of Y79 (Human retinoblastoma cell line) whole cell lysate with ab183951 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab183951 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

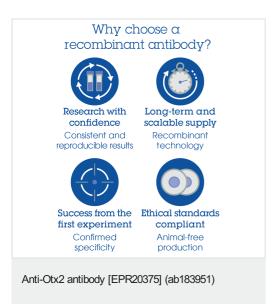
Lane 1: Y79 whole cell lysate, 10 µg (Input).

Lane 2: ab183951 IP in Y79 whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of ab183951 in Y79 whole cell lysate.

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

Exposure time: 30 seconds.



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