abcam

Product datasheet

Anti-Otx2 antibody ab114138

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Overview

Product name Anti-Otx2 antibody

Description Rabbit polyclonal to Otx2

Host species Rabbit

Tested applications Suitable for: IHC-P. WB

Species reactivity Reacts with: Mouse, Rat, Human, Recombinant fragment

Predicted to work with: Horse, Chimpanzee, Macaque monkey, Gorilla, Chinese hamster,

Orangutan 4

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

Positive control This antibody gave a positive signal in Otx2 recombinant protein as well as Y79 and WERI whole

cell lysates and the following tissue lysates: Mouse Embryonic Brain E10; Mouse Embryonic

Brain E14; Mouse Embryonic Brain E16; Mouse Eye. FFPE: Mouse e14 whole foetus

General notes The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

> 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.

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&As

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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab114138 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	★★★★☆ (3)	1/500 - 1/600. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 37 kDa (predicted molecular weight: 32 kDa).

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.

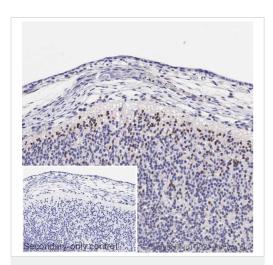
Sequence similarities Belongs to the paired homeobox family. Bicoid subfamily.

Contains 1 homeobox DNA-binding domain.

Developmental stage Embryo.

Cellular localization Nucleus.

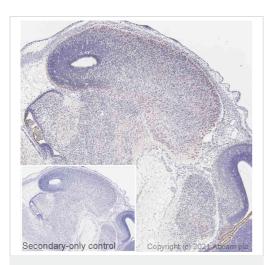
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody (ab114138)

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 ab114138, 2.5ug/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.

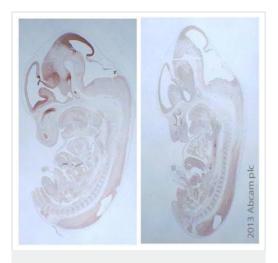
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody (ab114138)

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 ab114138, 2.5ug/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.

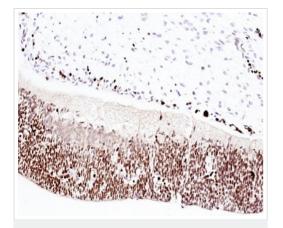
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



IHC-P image of Otx2 antibody (ab114138) on paraffin-embedded mouse embryo sections. The image on the left is of a WT embryo, whilst on the right is an embryo where Otx2 has been replaced with human Otx1 (hOtx1^{Ob2/Ob2}). The sections underwent heat mediated antigen retrieval using Citrate Buffer, primary antibody was used at 1:500 dilution and Biocare Mach4 Kit was used to detect the primary antibody.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody (ab114138)

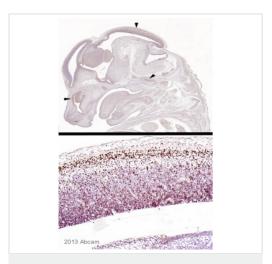
This image is courtesy of Prof. Antonio Simeone, Institute of Genetics and Biophysics, Naples, Italy.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody (ab114138)

This image is courtesy of Carl Hobbs (Kings College London, United Kingdom)

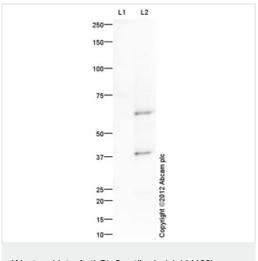
IHC-P image of Otx2 staining on Rat E12 embryo using ab114138 (1:1500). The sections were departfinized and subjected to heat mediated antigen retrieval using citric acid. The section were then blocked using 1% BSA for 10 mins at 21°C. ab114138 was diluted 1:1500 using TBS/BSA/Azide and incubated for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to Biotin (1:250).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Otx2 antibody (ab114138)

This image is courtesy of Carl Hobbs (Kings College London, United Kingdom)

IHC-P image of Otx2 staining on mouse E14 embryo using ab114138 (1:1000). The sections were deparffinized and subjected to heat mediated antigen retrieval using citric acid. The section were then blocked using 1% BSA for 10 mins at 21°C. ab114138 was diluted 1:1000 using TBS/BSA/Azide and incubated for 2 hours at 21°C. The secondary antibody used was goat polyclonal to rabbit IgG conjugated to Biotin (1:250).



Western blot - Anti-Otx2 antibody (ab114138)

All lanes: Anti-Otx2 antibody (ab114138) at 1 µg/ml

Lane 1 : OTX1 (Human) - Recombinant Protein

Lane 2 : OTX2 Transient Overexpression Lysate

Lysates/proteins at 0.1 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

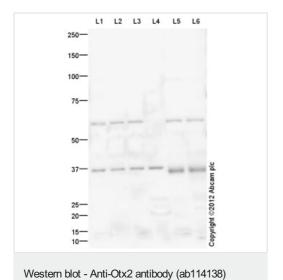
Predicted band size: 32 kDa **Observed band size:** 37 kDa

Additional bands at: 62 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 8 minutes

Lane 1 represents the Otx1 Recombinat protein which was used as a negative control for ab114138. This blot was produced using a 10% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab114138 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.



All lanes: Anti-Otx2 antibody (ab114138) at 1 µg/ml

Lane 1: E10 Mouse Embryo Brain Tissue Lysate

Lane 2: E14 Mouse Embryo Brain Tissue Lysate

Lane 3: E16 Ms Embryo Brain Tissue Lysate

Lane 4: Mouse eye tissue lysate - total protein (ab4029)

Lane 5: Y79 (Human retinoblastoma cell line) Whole Cell Lysate

Lane 6: WERI (Human Retinoblastoma) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 32 kDa **Observed band size:** 37 kDa

Additional bands at: 62 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 12 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being

transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab114138 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.

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