

Product datasheet

Anti-Otx1 + Otx2 antibody - ChIP Grade ab21990

★★★★★ 9 Abreviews 49 References 7 Images

Overview

Product name	Anti-Otx1 + Otx2 antibody - ChIP Grade
Description	Rabbit polyclonal to Otx1 + Otx2 - ChIP Grade
Host species	Rabbit
Specificity	ab21990 is raised against the C-terminal region of the Human Otx2 protein. This product is batch tested in Y79 cell lysate, recombinant Otx1 and overexpressed Otx2. All batches should detect endogenous Otx2, overexpressed Otx2 and recombinant or overexpressed Otx1.
Tested applications	Suitable for: IHC-Fr, ICC/IF, ChIP, WB, IHC-P, IP
Species reactivity	Reacts with: Mouse, Chicken, Cow, Human, Xenopus laevis Predicted to work with: Zebrafish 
Immunogen	Synthetic peptide corresponding to Human Otx2 aa 250 to the C-terminus (C terminal) conjugated to Keyhole Limpet Haemocyanin (KLH). Database link: P32243 (Peptide available as ab24347 , ab24348)
Positive control	This antibody gave a positive signal in the following whole cell lysates: Y79. This antibody gave a positive signal in the following cell lines: Human Embryonic Stem cells. This antibody gave a positive signal in the following tissues: Formalin Fixed Paraffin Embedded Mouse E6 Embryo Normal; Formalin Fixed Paraffin Embedded Mouse E14.5 Embryo Normal

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.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab21990** 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-Fr	★★★★☆	Use a concentration of 0.8 µg/ml.
ICC/IF	★★★★★	Use a concentration of 0.8 - 4 µg/ml.
ChIP		Use at an assay dependent concentration. PubMed: 18057103
WB	★★★★★	Use a concentration of 1 µg/ml. Detects a band of approximately 37 kDa (predicted molecular weight: 31.6 kDa).
IHC-P	★★★★★	Use at an assay dependent concentration. PubMed: 20150232
IP		Use a concentration of 5 µg/ml.

Target

Relevance

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'.

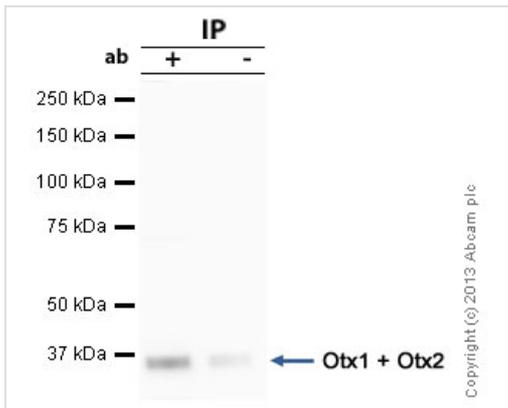
Tissue specificity: Expressed in brain.

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 microphthalmia 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.

Similarity: Belongs to the paired homeobox family. Bicoid subfamily. Contains 1 homeobox DNA-binding domain.

Developmental stage: Embryo.

Images



Immunoprecipitation - Anti-Otx1 + Otx2 antibody -
ChIP Grade (ab21990)

Otx1 +2 was immunoprecipitated using 0.5mg Y79 whole cell extract, 5µg of Rabbit polyclonal to Otx1 +2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Y79 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab21990.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

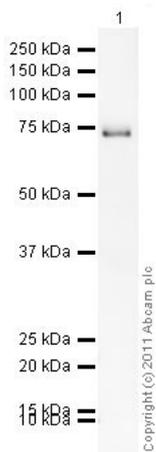
Band: 37kDa; Otx1 +2, non specific - as present in control (lane 2);
37kDa: We are confident this was due to slight lane contamination and the band seen in the IP lane is our target of interest.



Immunohistochemistry (Frozen sections) - Anti-Otx1
+ Otx2 antibody - ChIP Grade (ab21990)

This image is courtesy of an anonymous abreview.

Immunohistochemistry (Frozen sections) analysis of E8.5 mouse embryo brain section labeling Otx1 + Otx2 with ab21990 at 1/300 dilution. The tissue was fixed with paraformaldehyde and permeabilized with PBS / 0.5% v/v Triton X-100. An undiluted donkey anti-rabbit Alexa Fluor[®] 555 secondary antibody was used.



Western blot - Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990)

Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990) at 1 µg/ml + OTX1 (Human) - Recombinant Protein at 0.1 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

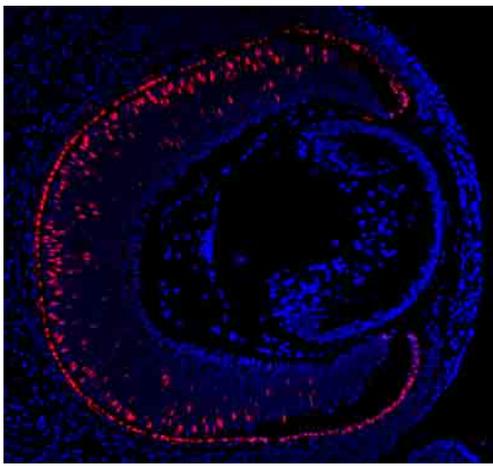
Performed under reducing conditions.

Predicted band size: 31.6 kDa

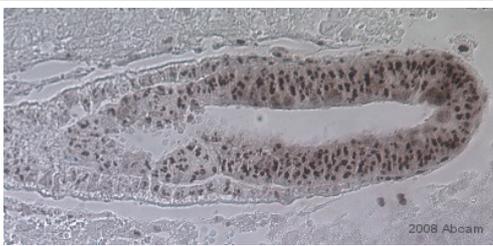
Observed band size: 74 kDa

[why is the actual band size different from the predicted?](#)

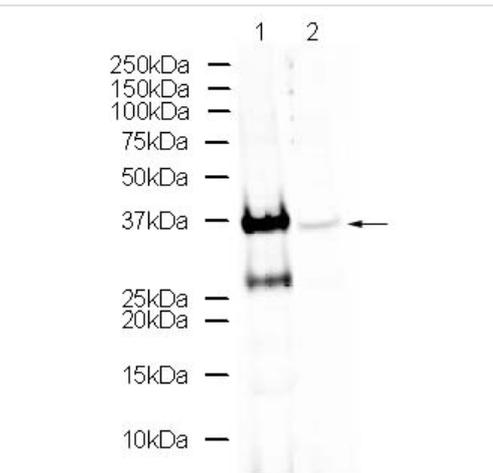
This Otx1 recombinant protein has a GST tag causing it to run at ~74kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990)
This image is courtesy of Erin Bassett



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990)



Western blot - Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990)

ab21990 staining Otx2 in embryonic day 14.5 (E14.5) mouse eye by Immunohistochemistry (formalin fixed, paraffin embedded sections).

The embryo was fixed in 10% neutral buffered formalin overnight at room temperature, then paraffin-embedded and sectioned at 4µm. Following deparaffinization and antigen retrieval in a rice steamer in 10mM sodium citrate (pH 6.0), the sections were blocked with 5% normal goat serum for half an hour at room temperature. ab21990 was diluted in PBS at 1/100, and incubated overnight at 4°C. The secondary was goat anti-rabbit Alexa[®]568, diluted in PBS with 1.5% normal goat serum. The mounting medium contained the nuclear stain DAPI (blue).

Mouse E6 embryos were sectioned and then fixed in paraformaldehyde. Antigen retrieval was performed using citric acid and the sections permeabilized and blocked for 1 hour in serum. Embryos were stained with ab21990 (1/2500) diluted in 10% serum, 1% ovalbumin in PBSTween for 14 hours at 4°C. They were then washed and stained with a goat anti-rabbit biotin conjugated antibody. After using a biotinylated secondary, ABC and then DAB was used for color development.

All lanes : Anti-Otx1 + Otx2 antibody - ChIP Grade (ab21990) at 1 µg/ml

Lane 1 : Y79 Lysate

Lane 2 : Y79 Lysate with Otx2 peptide ([ab24347](#)) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

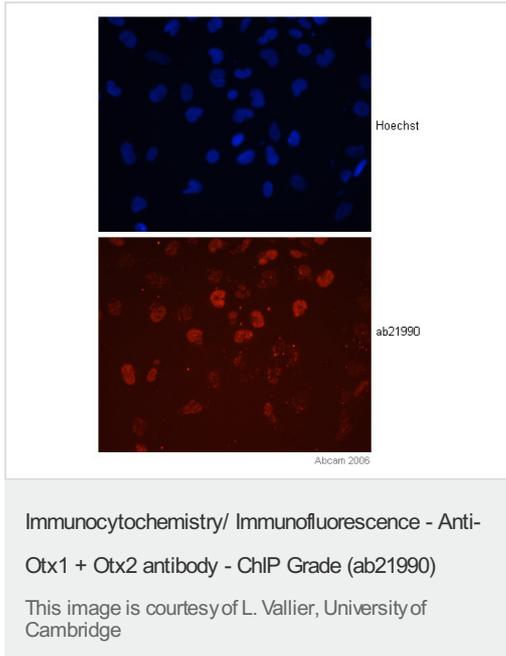
Secondary

All lanes : Alexa Fluor Goat polyclonal to Rabbit IgG at 1/10000 dilution

Predicted band size: 31.6 kDa

Observed band size: 37 kDa [why is the actual band size different from the predicted?](#)

Additional bands at: 28 kDa (possible cleavage fragment), 28 kDa (possible isoform), 28 kDa (possible non-specific binding)



The image shows staining of Otx2 in human embryonic stem cells differentiated into neuroectoderm. As would be expected, staining is nuclear.

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