abcam

Product datasheet

Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] ab108311





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Overview

Product name Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)]

Description Rabbit monoclonal [EPR2688(2)] to Transcription factor AP-2-alpha

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IHC-P, Flow Cyt (Intra), IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control IP: HeLa whole cell lysate; Flow Cyt (intra): JAR cells; ICC/IF: JAR cells; IHC-P: Human breast

carcinoma, and mouse and rat breast tissue; WB: HeLa, C6, Mouse skin and HAP1 cell lysates.

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 Liquid

Storage instructions Shipped at 4°C. 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 (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR2688(2)

Isotype ΙgG

Applications

The Abpromise quarantee

Our Abpromise guarantee covers the use of ab108311 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>*</u>	1/50.
WB		1/1000 - 1/10000. Predicted molecular weight: 48 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20.
IP		1/20.

Target

Function

Sequence-specific DNA-binding protein that interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes. AP-2 factors bind to the consensus sequence 5'-GCCNNNGGC-3' and activate genes involved in a large spectrum of important biological functions including proper eye, face, body wall, limb and neural tube development. They also suppress a number of genes including MCAM/MUC18, C/EBP alpha and MYC. AP-2-alpha is the only AP-2 protein required for early morphogenesis of the lens vesicle.

Involvement in disease

Defects in TFAP2A are the cause of branchiooculofacial syndrome (BOFS) [MIM:113620]; also known as branchial clefts with characteristic facies, growth retardation, imperforate nasolacrimal duct, and premature aging or lip pseudocleft-hemangiomatous branchial cyst syndrome. BOFS is a rare autosomal dominant cleft palate craniofacial disorder with variable expressivity. The major features include cutaneous anomalies, ocular anomalies, characteristic facial appearance (malformed pinnae, oral clefts), and, less commonly, renal and ectodermal (dental and hair) anomalies.

Sequence similarities

Belongs to the AP-2 family.

Domain

The WW-binding motif mediates interaction with WWOX.

Post-translational

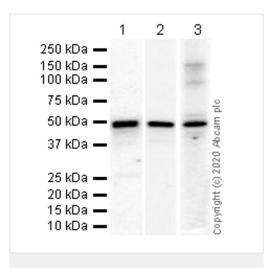
modifications

Sumoylated on Lys-10; which inhibits transcriptional activity.

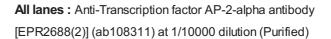
Cellular localization

Nucleus.

Images



Western blot - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)



Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2: C6 (Rat glial tumor glial cell) whole cell lysate

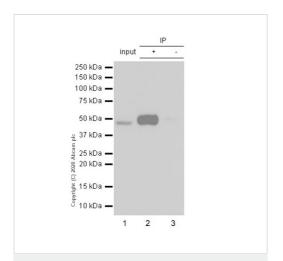
Lane 3: Mouse skin lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 48 kDa



Immunoprecipitation - Anti-Transcription factor AP-2alpha antibody [EPR2688(2)] (ab108311) Purified ab108311 at 1/20 dilution (0.5µg) immunoprecipitating Transcription factor AP-2-alpha in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab108311 + HeLa whole cell lysate.

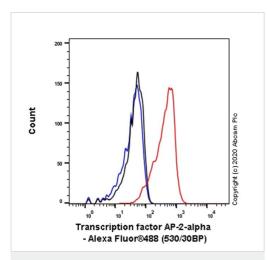
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab108311 in HeLa whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/10,000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

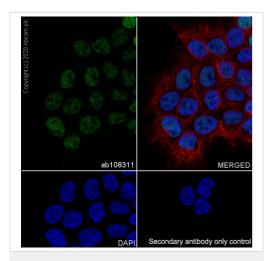
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 48 kDa



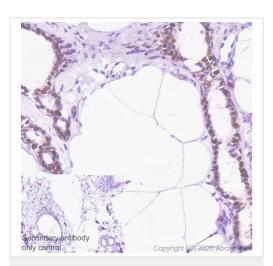
Flow Cytometry (Intracellular) - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

Intracellular Flow Cytometry analysis of JAR (Human placenta choriocarcinoma epithelial cell) cells labeling Transcription factor AP-2-alpha with Purified ab108311 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488, <u>ab150077</u>) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



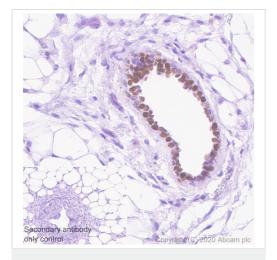
Immunocytochemistry/ Immunofluorescence - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

Immunocytochemistry analysis of JAR (Human placenta choriocarcinoma epithelial cell) cells labeling Transcription factor AP-2-alpha with Purified ab108311 at 1/50 dilution (3.4 μ g/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Antialpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 dilution (2.5 μ g/mL). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 dilution (2 μ g/mL). DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat breast tissue sections labeling
Transcription factor AP-2-alpha with Purified ab108311 at 1/100
dilution (1.07 µg/mL). Heat mediated antigen retrieval using Bond™
Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody.
Hematoxylin was used as a counterstain.

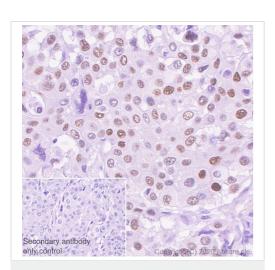


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse breast tissue sections labeling

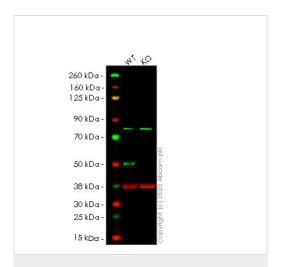
Transcription factor AP-2-alpha with Purified ab108311 at 1/100
dilution (1.07 µg/mL). Heat mediated antigen retrieval using Bond™
Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody.

Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling Transcription factor AP-2-alpha with Purified ab108311 at 1/100 dilution (1.07 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

All lanes : Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: TFAP2A knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

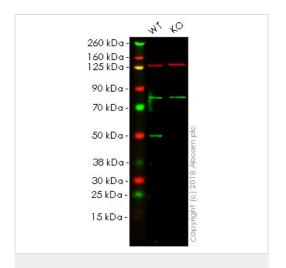
Performed under reducing conditions.

Predicted band size: 48 kDa **Observed band size:** 48 kDa

Lanes 1-2: Merged signal (red and green). Green - ab108311 observed at 48 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab108311 was shown to react with Transcription factor AP-2-alpha in wild-type HeLa cells in western blot. Loss of signal was observed

when knockout cell line <u>ab265122</u> (knockout cell lysate <u>ab257736</u>) was used. Wild-type HeLa and TFAP2A knockout HeLa cell lysates were subjected to SDS-PAGE. ab108311 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311)

All lanes : Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (ab108311) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : TFAP2A (Transcription factor AP-2-alpha) knockout

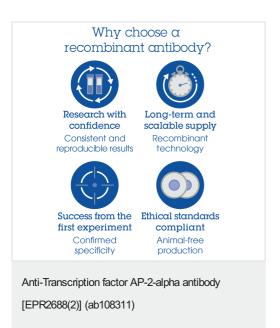
Lysates/proteins at 20 µg per lane.

HAP1 whole cell lysate

Predicted band size: 48 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab108311 observed at 48 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab108311 was shown to recognize Transcription factor AP-2-alpha in wild-type HAP1 cells as signal was lost at the expected MW in TFAP2A (Transcription factor AP-2-alpha) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and TFAP2A (Transcription factor AP-2-alpha) knockout samples were subjected to SDS-PAGE. Ab108311 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



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