abcam

Product datasheet

Anti-FOXC1 antibody [EPR20685] - BSA and Azide free ab230159

KO VALIDATED Recombinant RabMAb

11 Images

Overview

Product name	Anti-FOXC1 antibody [EPR20685] - BSA and Azide free
Description	Rabbit monoclonal [EPR20685] to FOXC1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-Fr, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human gastric cancer tissue. ICC/IF: HEK293 cells (HEK293-FOXC1 KO cells used as negative control) Flow cyto (intra): HEK293 cells
General notes	ab230159 is the carrier-free version of **ab227977**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20685
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab230159 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)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70 kDa (predicted molecular weight: 57 kDa). WB works for some mouse lysates.
IHC-Fr		Use at an assay dependent concentration. Perform heat-mediated antigen retrieval by using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 12 µg/ml. Signal can be observed in both methanol and paraformaldehyde fixed cells.

Target

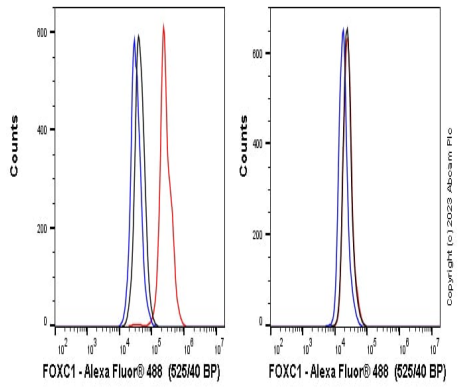
Function	Binding of FREAC-3 and FREAC-4 to their cognate sites results in bending of the DNA at an angle of 80-90 degrees.
Tissue specificity	Expressed in all tissues and cell lines examined.
Involvement in disease	Defects in FOXC1 are the cause of Axenfeld-Rieger syndrome type 3 (RIEG3) [MIM:602482]; also known as Axenfeld-Rieger syndrome (ARS) or Axenfeld syndrome or Axenfeld anomaly. It is

characterized by posterior corneal embryotoxon, prominent Schwalbe line and iris adhesion to the Schwalbe line. Other features may be hypertelorism (wide spacing of the eyes), hypoplasia of the malar bones, congenital absence of some teeth and mental retardation. When associated with tooth anomalies, the disorder is known as Rieger syndrome. Glaucoma is a progressive blinding condition that occurs in approximately half of patients with Axenfeld-Rieger malformations.
Defects in FOXC1 are the cause of iridogoniodysgenesis anomaly (IGDA) [MIM:601631]. IGDA is an autosomal dominant phenotype characterized by iris hypoplasia, goniodysgenesis, and juvenile glaucoma.
Defects in FOXC1 are a cause of Peters anomaly (PAN) [MIM:604229]. Peters anomaly consists of a central corneal leukoma, absence of the posterior corneal stroma and Descemet membrane, and a variable degree of iris and lenticular attachments to the central aspect of the posterior cornea.

Sequence similarities Contains 1 fork-head DNA-binding domain.

Cellular localization Nucleus.

Images



Flow Cytometry (Intracellular) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).

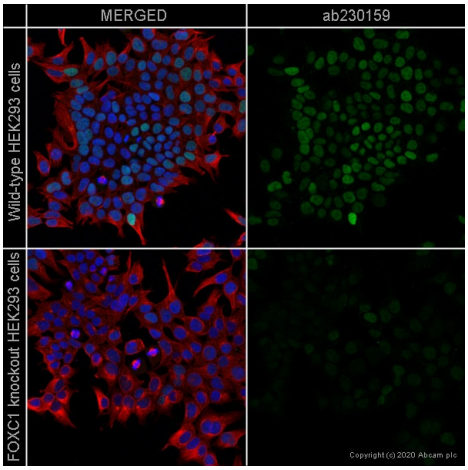
Flow cytometry overlay histogram showing left HEK293 positive cells and right negative Jurkat stained with **ab227977** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab227977**) (1x 10^6 in 100µl at 1.0µg/ml (1/2110)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

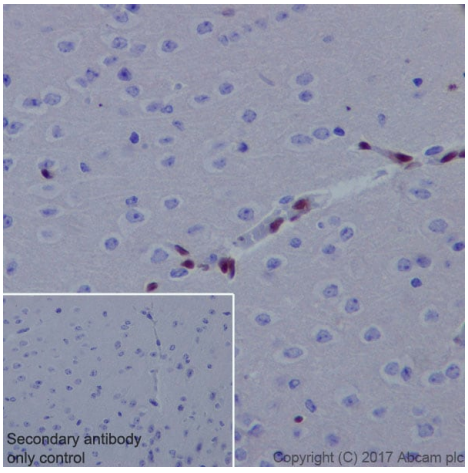
This antibody gave a positive signal in HEK293 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

ab230159 staining FOXC1 in wild-type HEK293 cells (top panel) and FOXC1 knockout HEK293 cells (bottom panel). The cells were fixed with paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab230159 at 12 µg/ml concentration and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



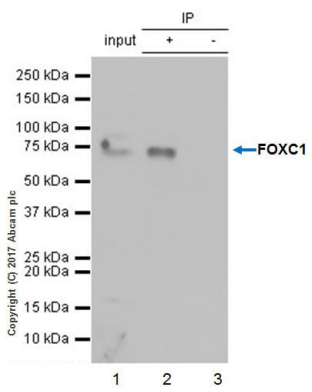
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling FOXC1 with **ab227977** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in the pericytes and endothelium of blood vessels in mouse cerebrum is observed (PMID:25733312; PMID: 23862012). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

FOXC1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with **ab227977** at 1/50 dilution. Western blot was performed from the immunoprecipitate using **ab227977** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

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

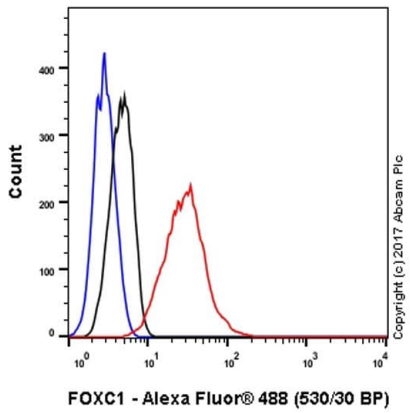
Lane 2: **ab227977** IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab227977** in HeLa whole cell lysate.

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

Exposure time: 10 seconds.

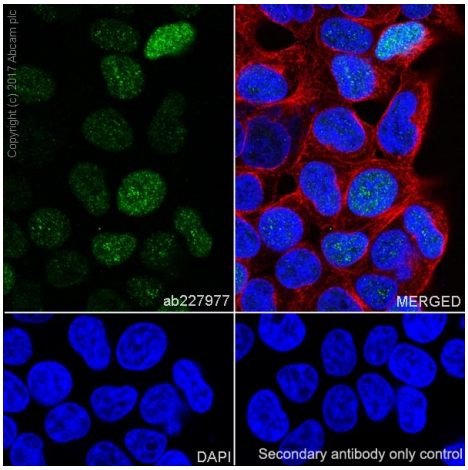
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).



Flow Cytometry (Intracellular) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized ï¿½HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell line labeling ï¿½FOXC1 with **ab227977** at 1/100 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluorï¿½ 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).



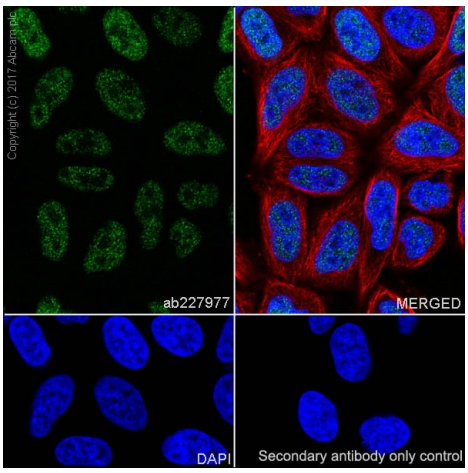
Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells labeling FOXC1 with **ab227977** at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HEK-293T cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).



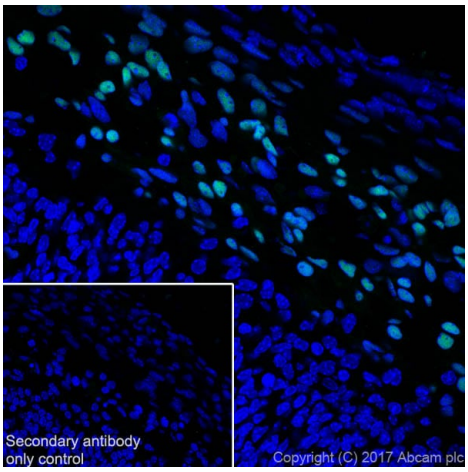
Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling FOXC1 with **ab227977** at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HEK-293T cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).



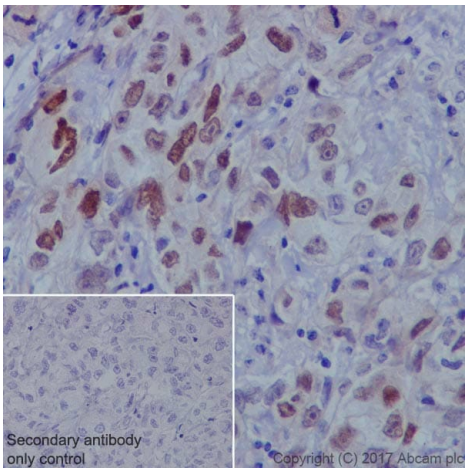
Immunohistochemistry (Frozen sections) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse fetal brain E14.5 tissue labeling FOXC1 with **ab227977** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Positive nuclear staining localized in the meninges and adjacent cortex region on mouse fetal brain (PMID: 23862012).

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).



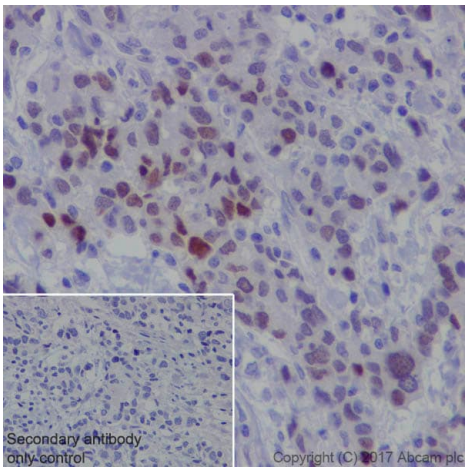
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunohistochemical analysis of paraffin-embedded human basal-like breast cancer tissue labeling FOXC1 with **ab227977** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human basal-like breast cancer (PMID:27708239; PMID:20406990). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



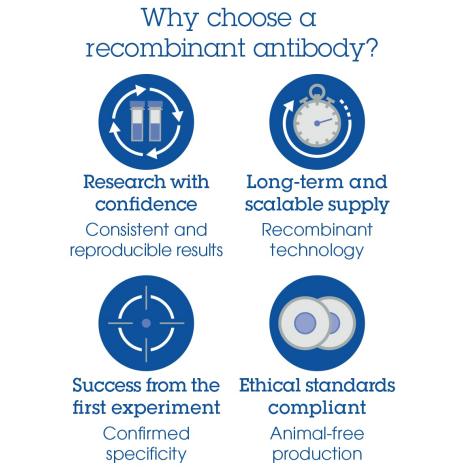
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling FOXC1 with **ab227977** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human gastric cancer (PMID:24329718). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227977**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-FOXC1 antibody [EPR20685] - BSA and Azide free (ab230159)

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