abcam

# Product datasheet

# Anti-FOXC1 antibody [EPR20685] ab227977

KO VALIDATED Recombinant RabMAb

10 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-FOXC1 antibody [EPR20685] |
| **Description** | Rabbit monoclonal [EPR20685] to FOXC1 |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** ICC/IF, IP, Flow Cyt, IHC-P, IHC-Fr, WB |
| **Species reactivity** | **Reacts with:** Mouse, Human |
| **Immunogen** | Synthetic peptide within Human FOXC1 aa 1-100. The exact sequence is proprietary.<br>Database link: Q12948 |
| **Positive control** | WB: MDA-MB-231, HEK-293T and HeLa whole cell lysates; Human fetal kidney lysate. IHC-P: Human gastric cancer and basal-like breast cancer tissues; Mouse cerebrum tissue. IHC-Fr: Mouse fetal brain E14.5 tissue. ICC/IF: HeLa and HEK-293T cells (HEK293-FOXC1 KO cells used as a negative control). Flow Cyt: HEK-293T cells. IP: HeLa whole cell lysate. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information see here.<br><br>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

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| **Form** | Liquid |
| **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** | Preservative: 0.01% Sodium azide<br>Constituents: 0.05% BSA, 40% Glycerol, PBS |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |

| | |
|---|---|
| **Clone number** | EPR20685 |
| **Isotype** | IgG |

## Applications

Our Abpromise guarantee covers the use of **ab227977** in the following tested applications.

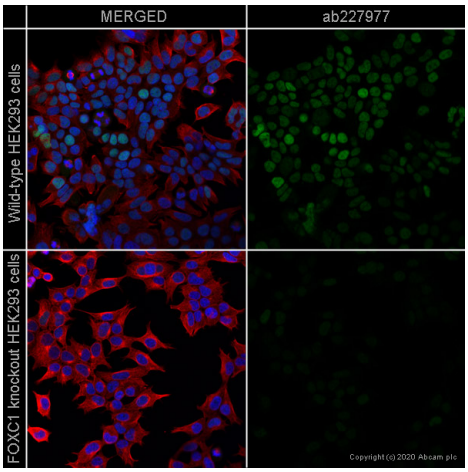
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| ICC/IF | | 1/100. |
| IP | | 1/50. |
| Flow Cyt | | 1/100. |
| IHC-P | | 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| IHC-Fr | | 1/1000.<br>Perform heat-mediated antigen retrieval by using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). |
| WB | | 1/1000. Detects a band of approximately 70 kDa (predicted molecular weight: 57 kDa).<br>WB works for some mouse lysates. |

## 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.<br>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.<br>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. |

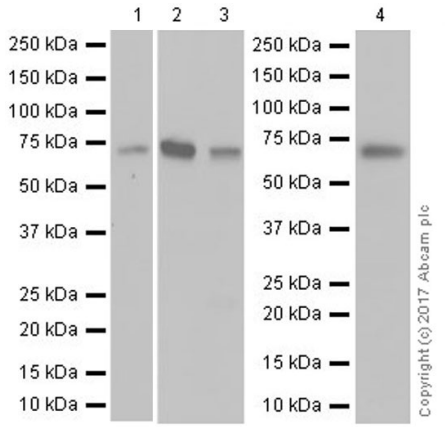
## Images




Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] (ab227977)

ab227977 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 ab227977 at 1/100 dilution 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).




Western blot - Anti-FOXC1 antibody [EPR20685] (ab227977)

**All lanes :** Anti-FOXC1 antibody [EPR20685] (ab227977) at 1/1000 dilution

**Lane 1 :** MDA-MB-231 (human breast adenocarcinoma cell line) whole cell lysate at 20 µg
**Lane 2 :** HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 20 µg
**Lane 3 :** HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg
**Lane 4 :** Human fetal kidney lysate at 10 µg

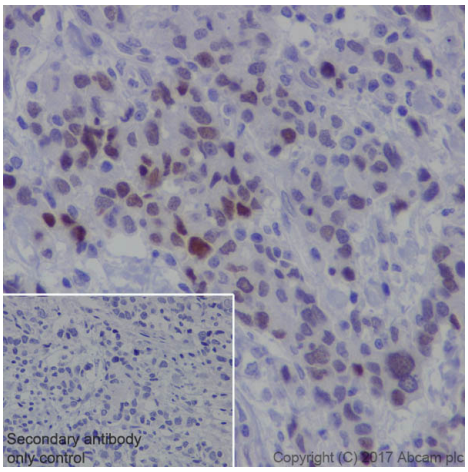
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

**Predicted band size:** 57 kDa
**Observed band size:** 70 kDa
why is the actual band size different from the predicted?

**Exposure time :** Lanes 1 & 4: 3 minutes; Lanes 2-3: 5 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

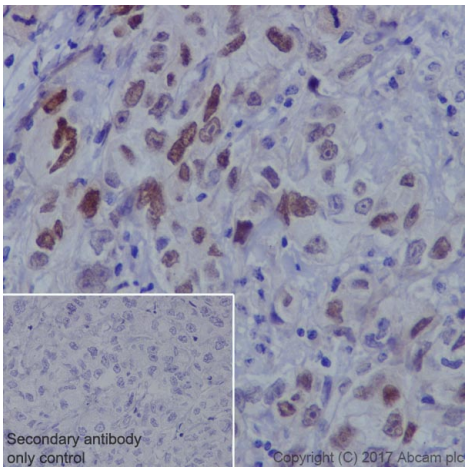
The molecular mass is consistent with what has been described in the literature (PMID: 27708239).




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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

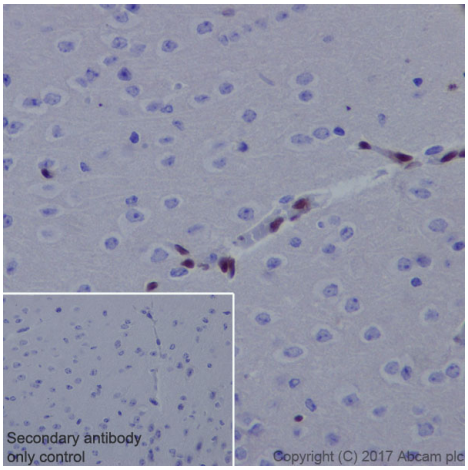
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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

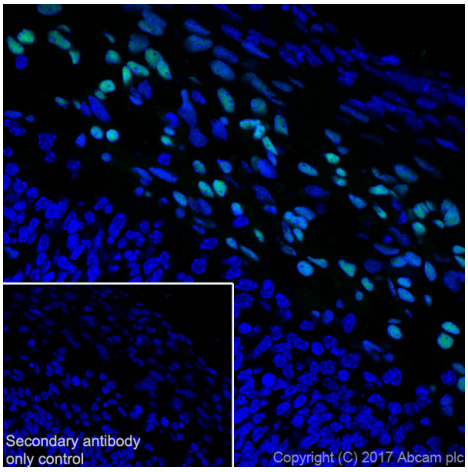
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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

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

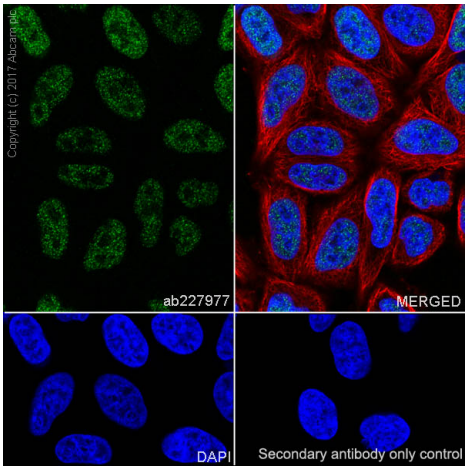



Immunohistochemistry (Frozen sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

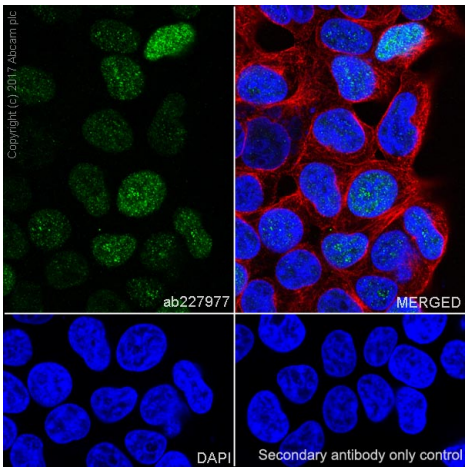



Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

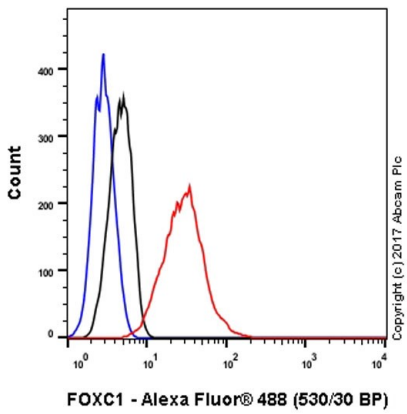



Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

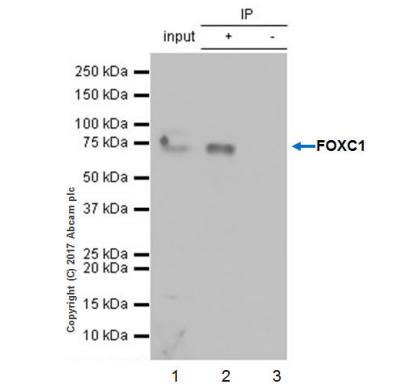
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

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.




Flow Cytometry - Anti-FOXC1 antibody [EPR20685] (ab227977)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell line labeling FOXC1 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 Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.




Immunoprecipitation - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors