

### Anti-FOXC1 antibody [EPR20685] ab227977

KO 評価済 リコンビナント RabMAb

10 References 画像数 12

#### 製品の概要

製品名	Anti-FOXC1 antibody [EPR20685]
製品の詳細	Rabbit monoclonal [EPR20685] to FOXC1
由来種	Rabbit
アプリケーション	適用あり: ICC/IF, Flow Cyt (Intra), IP, IHC-P, IHC-Fr, WB
種交差性	交差種: Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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 (intra): HEK-293T cells. IP: HeLa whole cell lysate.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS</p>
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR20685

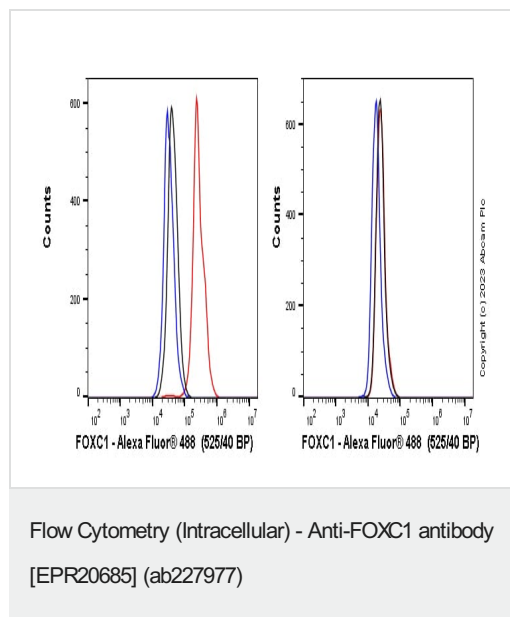
## アプリケーション

**The Abpromise guarantee** Abpromise保証は、 次のテスト済みアプリケーションにおけるab227977の使用に適用されます  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		Use a concentration of 12 µg/ml.
Flow Cyt (Intra)		1/100.
IP		1/50.
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. 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). WB works for some mouse lysates.

## ターゲット情報

機能	Binding of FREAC-3 and FREAC-4 to their cognate sites results in bending of the DNA at an angle of 80-90 degrees.
組織特異性	Expressed in all tissues and cell lines examined.
関連疾患	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.
配列類似性	Contains 1 fork-head DNA-binding domain.
細胞内局在	Nucleus.



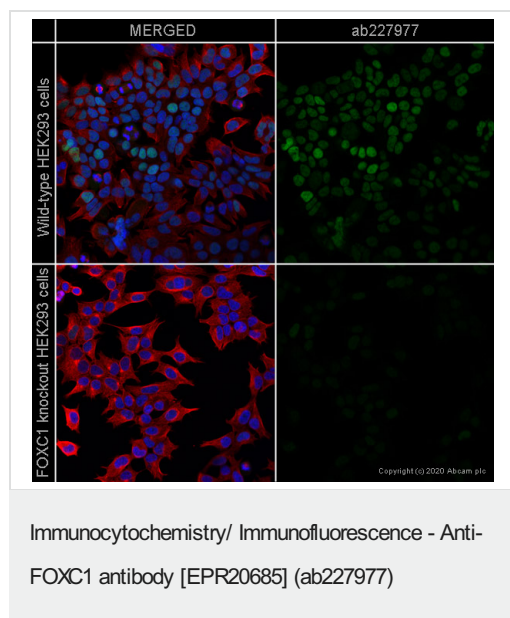
Flow cytometry overlay histogram showing left HEK293 positive cells and right negative Jurkat stained with ab227977 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat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

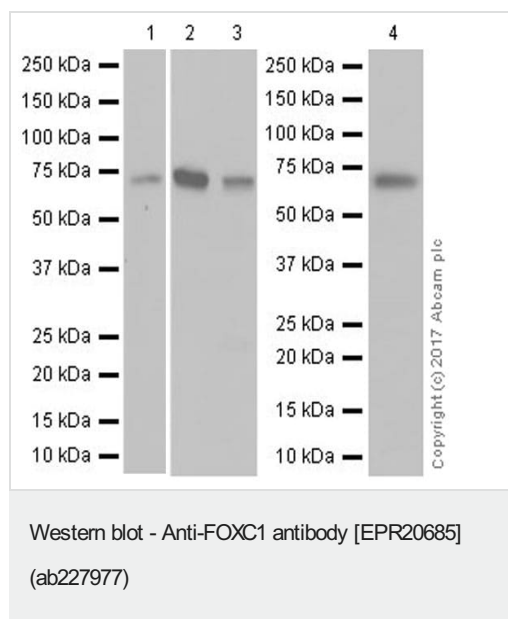
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

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.



ab227977 staining FOXC1 in wild-type HEK293 cells (top panel) and FOXCl 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 12 µg/ml 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).



**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)

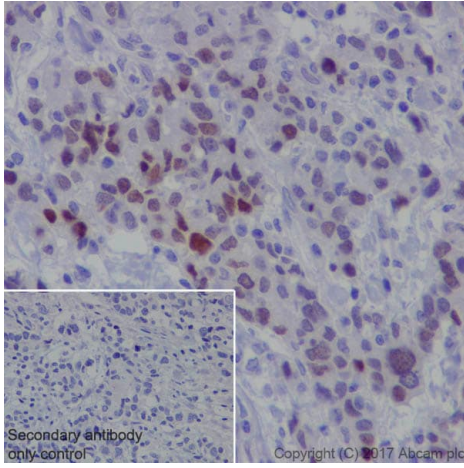
**Predicted band size:** 57 kDa

**Observed band size:** 70 kDa

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

Blocking/Dilution buffer: 5% NFDm/TBST.

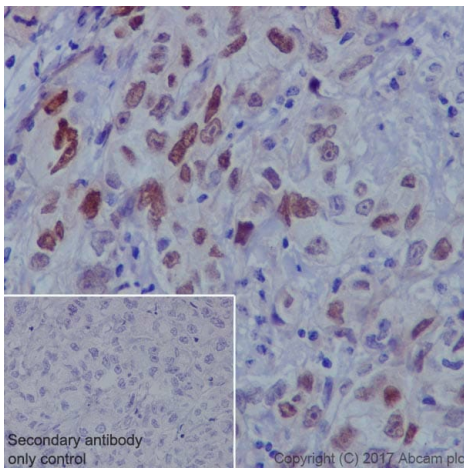
The molecular mass is consistent with what has been described in the literature (PMID: 27708239).



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.

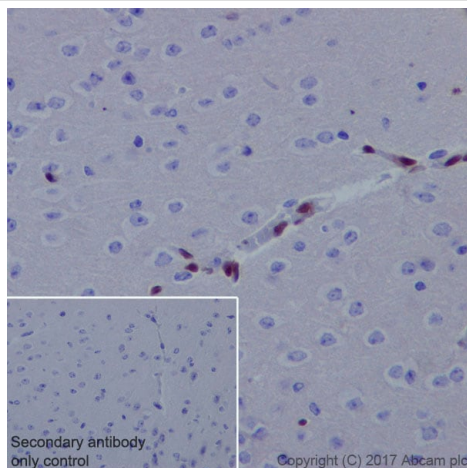


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

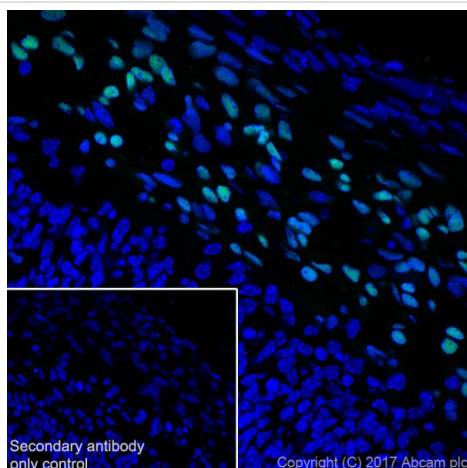




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

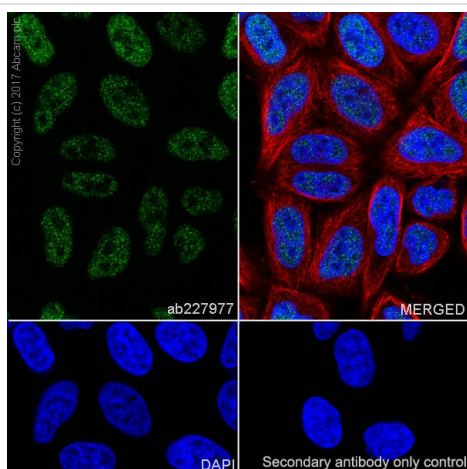


Immunohistochemistry (Frozen sections) - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

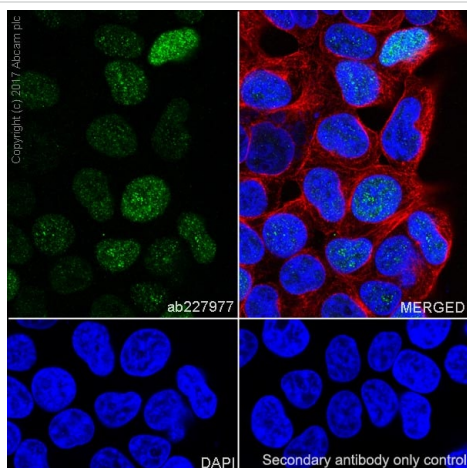


Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

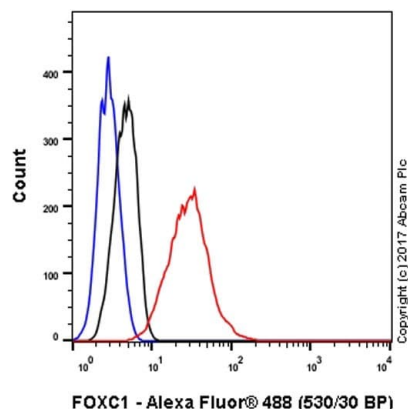


Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20685] (ab227977)

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.

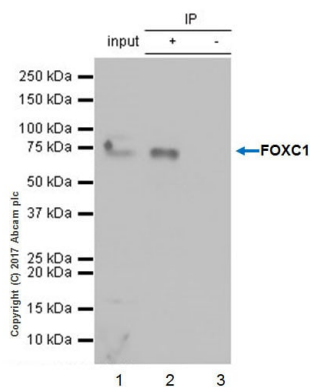
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

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

Intracellular 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).

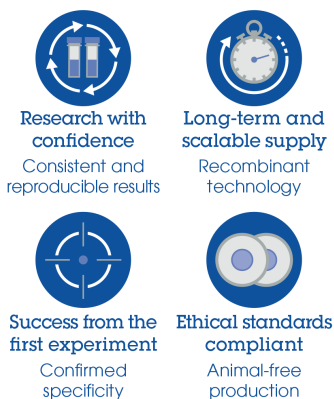
**Lane 2:** ab227977 IP in HeLa whole cell lysate.

**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.

### Why choose a recombinant antibody?



Anti-FOXC1 antibody [EPR20685] (ab227977)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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