abcam

Product datasheet

Anti-FOXC1 antibody [EPR20678] ab223850

Recombinant RabMAb

8 Images

Overview

Product name	Anti-FOXC1 antibody [EPR20678]
Description	Rabbit monoclonal [EPR20678] to FOXC1
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, WB, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment within Human FOXC1 aa 1 to the C-terminus. The exact sequence is proprietary. Database link: Q12948
Positive control	WB: HEK-293T, HeLa, 4T1, MDA-MB-231, MDA-MB-435S, PC-12 and NIH/3T3 whole cell lysates; Human fetal spleen lysate. IHC-P: Human gastric cancer, pancreatic cancer and basal-like breast cancer tissues. ICC/IF: HeLa and HEK-293T cells. Flow Cyt: HEK-293T cells. IP: HeLa whole cell lysate.
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 +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 Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR20678
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab223850** in the following tested applications.

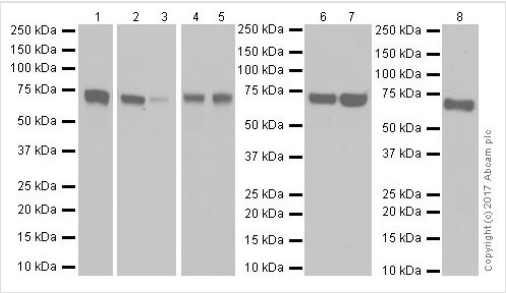
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		1/30.
ICC/IF		1/500.
WB		1/1000. Detects a band of approximately 70 kDa (predicted molecular weight: 56 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC not suitable for mouse & rat tissues.
Flow Cyt		1/500.

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



Western blot - Anti-FOXC1 antibody [EPR20678] (ab223850)

All lanes : Anti-FOXC1 antibody [EPR20678] (ab223850) at 1/1000 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 20 µg
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg
Lane 3 : 4T1 (mouse mammary gland carcinoma epithelial cell line) whole cell lysate at 10 µg
Lane 4 : MDA-MB-231 (human breast adenocarcinoma cell line) whole cell lysate at 20 µg
Lane 5 : MDA-MB-435S (human ductal carcinoma cell line) whole cell lysate at 20 µg
Lane 6 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate at 10 µg
Lane 7 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate at 10 µg
Lane 8 : Human fetal spleen lysate at 10 µg

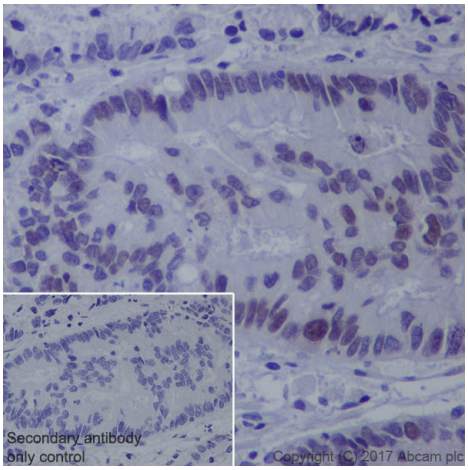
Secondary
Lanes 1-7 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L) Peroxidase conjugated)
Lane 8 : VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/4000 dilution (Goat Anti-Rabbit IgG, (H+L) Peroxidase conjugated)

Predicted band size: 56 kDa
Observed band size: 70 kDa
why is the actual band size different from the predicted?

Exposure time : Lane 1: 15 seconds; Lanes 2-3 & 6-8: 3 minutes; Lanes 4-5: 1 minute.

Blocking/Dilution buffer: 5% NFDM/TBST.

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

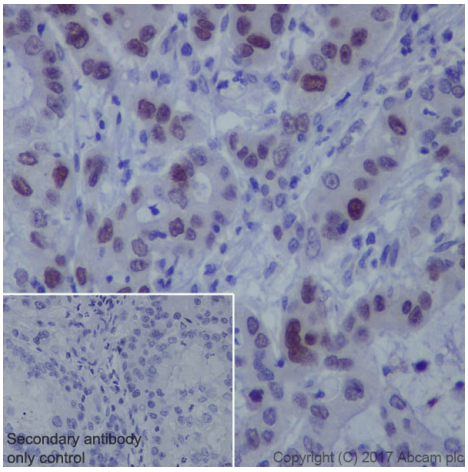


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20678] (ab223850)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling FOXC1 with ab223850 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human gastric cancer is observed(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.

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

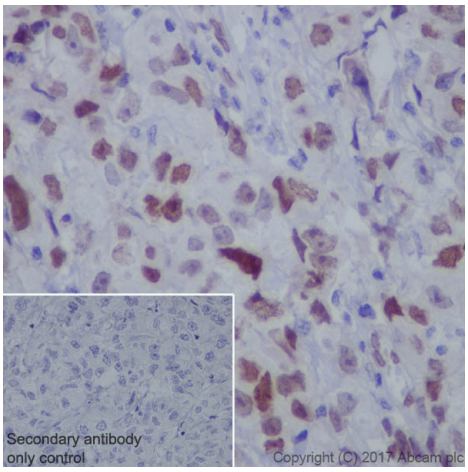


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20678] (ab223850)

Immunohistochemical analysis of paraffin-embedded human pancreatic cancer tissue labeling FOXC1 with ab223850 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human pancreatic cancer is observed(PMID:23242609). 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.

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

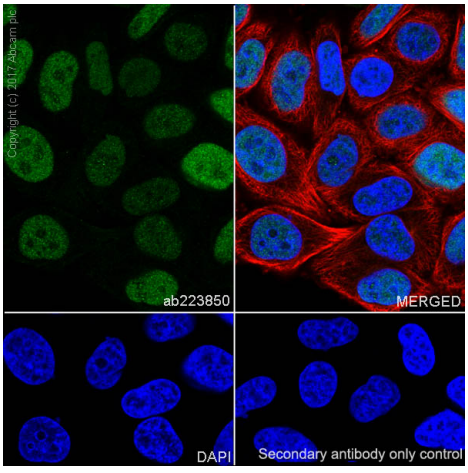


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXC1 antibody [EPR20678] (ab223850)

Immunohistochemical analysis of paraffin-embedded human basal-like breast cancer tissue labeling FOXC1 with ab223850 at 1/1000 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.

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

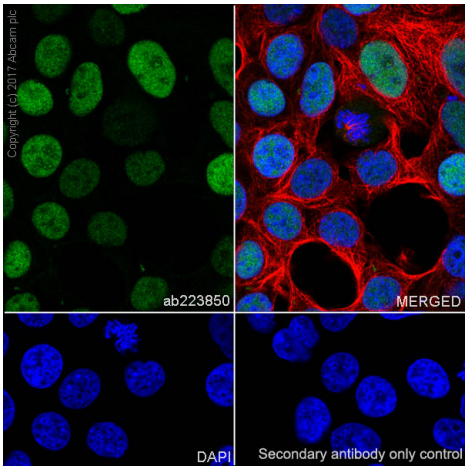


Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20678] (ab223850)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling FOXC1 with ab223850 at 1/500 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.

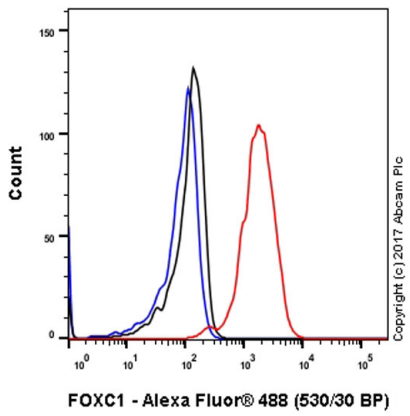


Immunocytochemistry/ Immunofluorescence - Anti-FOXC1 antibody [EPR20678] (ab223850)

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 ab223850 at 1/500 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.

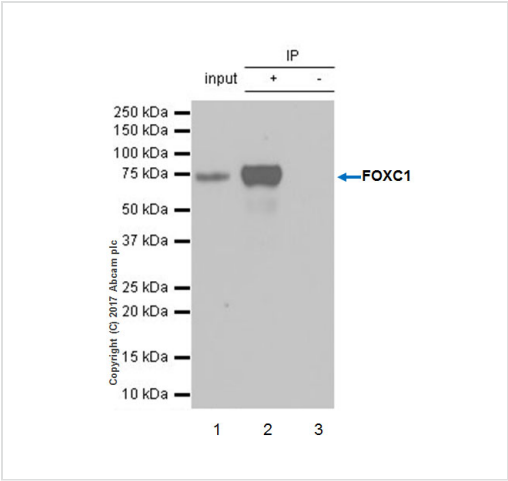
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

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 [EPR20678] (ab223850)

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 ab223850 at 1/500 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 [EPR20678] (ab223850)

FOXC1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab223850 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab223850 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

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

Lane 2: ab223850 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab223850 in HeLa whole cell lysate.

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

Exposure time: 3 minutes.

The molecular mass is consistent with what has been described in the literature (PMID: 22493429).

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