# abcam

## **Product datasheet**

# Anti-ZEB1 antibody [EPR17375] ab203829

KO VALIDATED Recombinant RabMAb

### ★★★★★ 3 Abreviews 87 References 9 Images

#### Overview

Product name	Anti-ZEB1 antibody [EPR17375]
Description	Rabbit monoclonal [EPR17375] to ZEB1
Host species	Rabbit
Specificity	Expression levels of the target protein vary with sample type and some optimisation may be required.
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293, HAP1 and HeLa, MDA-MB231, SKOV3, MCF7, A549 cell lysates. Human Ovary lysates. IHC-P: Human cervix carcinoma and breast carcinoma tissues. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <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> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR17375
lsotype	lgG

#### Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab203829 in the following tested applications.

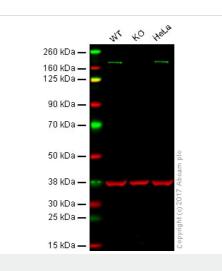
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50. For unpurified use at 1/1000 dilution. <u><b>ab172730</b></u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/500. Detects a band of approximately 200 kDa (predicted molecular weight: 124 kDa).
IHC-P	★ ★ ★ ★ ☆ <u>(2)</u>	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/250.

#### Target

Function	Inhibits interleukin-2 (IL-2) gene expression. May be responsible for transcriptional repression of the IL-2 gene. Enhances or represses the promoter activity of the ATP1A1 gene depending on the quantity of cDNA and on the cell type. Represses E-cadherin promoter and induces an epithelial-mesenchymal transition (EMT) by recruiting SMARCA4/BRG1. Represses BCL6 transcription in the presence of the corepressor CTBP1. Promotes tumorigenicity by repressing stemness-inhibiting microRNAs.
Tissue specificity	Colocalizes with SMARCA4/BRG1 in E-cadherin-negative cells from established lines, and stroma of normal colon as well as in de-differentiated epithelial cells at the invasion front of colorectal carcinomas (at protein level). Expressed in heart and skeletal muscle, but not in liver, spleen, or pancreas.
Involvement in disease	<ul> <li>Defects in ZEB1 are the cause of posterior polymorphous corneal dystrophy type 3 (PPCD3)</li> <li>[MIM:609141]. PPCD is a rare disease involving metaplasia and overgrowth of corneal endothelial cells. In patients with PPCD, these cells manifest in an epithelial morphology and gene expression pattern, produce an aberrant basement membrane, and, sometimes, spread over the iris and nearby structures in a way that increases the risk for glaucoma.</li> <li>Defects in ZEB1 are the cause of corneal dystrophy Fuchs endothelial type 6 (FECD6)</li> <li>[MIM:613270]. It is an ocular disorder caused by loss of endothelium of the central cornea. It is characterized by focal wart-like guttata that arise from Descemet membrane and develop in the central cornea, epithelial blisters, reduced vision and pain. Descemet membrane is thickened by abnormal collagenous deposition.</li> </ul>
Sequence similarities	Belongs to the delta-EF1/ZFH-1 C2H2-type zinc-finger family. Contains 7 C2H2-type zinc fingers. Contains 1 homeobox DNA-binding domain.

#### Images

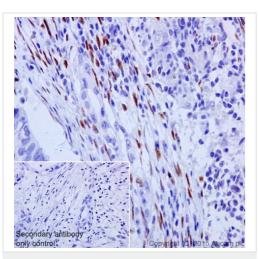


Western blot - Anti-ZEB1 antibody [EPR17375] (ab203829)

Lane 1: Wild-type HAP1 whole cell lysate (20 μg) Lane 2: ZEB1 knockout HAP1 whole cell lysate (20 μg) Lane 3: HeLa whole cell lysate (20 μg)

Lanes 1 - 3: Merged signal (red and green). Green - ab203829 observed at 200 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab203829 was shown to specifically react with ZEB1 in wild-type HAP1 cells as signal was lost in ZEB1 knockout cells. Wild-type and ZEB1 knockout samples were subjected to SDS-PAGE. Ab203829 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

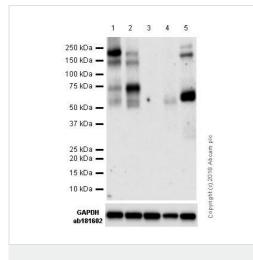


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ZEB1 antibody [EPR17375] (ab203829)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling ZEB1 with ab203829 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on Human cervix carcinoma tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-ZEB1 antibody [EPR17375] (ab203829)

All lanes : Anti-ZEB1 antibody [EPR17375] (ab203829) at 1/500 dilution

Lane 1 : MDA-MB231 (Human breast adenocarcinoma epithelial cell)whole cell lysates with 5% NFDM/TBST Lane 2 : SKOV3 (Human ovarian cancer epithelial cell) whole cell lysates with 5% NFDM/TBST Lane 3 : MCF7 (Human breast adenocarcinoma epithelial cell)whole cell lysates with 5% NFDM/TBST Lane 4 : A549 (Human lung carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST Lane 5 : Human Ovary lysates with 5% NFDM/TBST

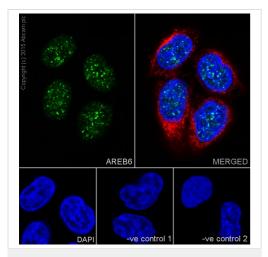
Lysates/proteins at 20 µg per lane.

#### Secondary

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

Predicted band size: 124 kDa Observed band size: 200 kDa

Exposure time: 3 minutes



Immunocytochemistry/ Immunofluorescence - Anti-ZEB1 antibody [EPR17375] (ab203829) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling ZEB1 with ab203829 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (Alexa Fluor<sup>®</sup> 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab203829 at 1/250 dilution followed by <u>ab150120</u> (Alexa Fluor<sup>®</sup> 594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor<sup>®</sup> 488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.

Anti-ZEB1 antibody [EPR17375] (ab203829) at 1/1000 dilution + HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate at 10 μg

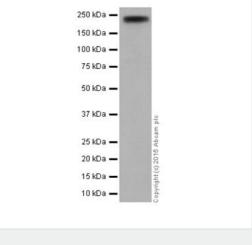
#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

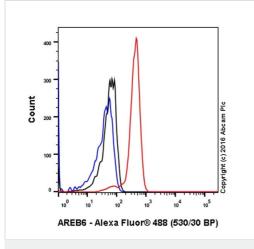
Predicted band size: 124 kDa Observed band size: 200 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

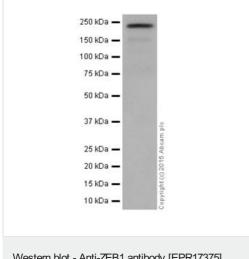


Western blot - Anti-ZEB1 antibody [EPR17375] (ab203829)



Intracellular Flow Cytometry analysis of HeLa cells labelling ZEB1 with ab203829 at 1/1000 (red). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Fluorr<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

Flow Cytometry (Intracellular) - Anti-ZEB1 antibody [EPR17375] (ab203829)



Western blot - Anti-ZEB1 antibody [EPR17375] (ab203829) Anti-ZEB1 antibody [EPR17375] (ab203829) at 1/1000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

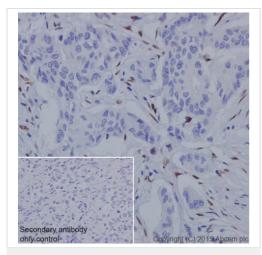
#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

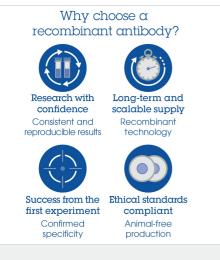
Predicted band size: 124 kDa Observed band size: 200 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ZEB1 antibody [EPR17375] (ab203829)



Anti-ZEB1 antibody [EPR17375] (ab203829)

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Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling ZEB1 with ab203829 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on Human breast carcinoma tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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