

Product datasheet

Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker ab53280

KO VALIDATED Recombinant RabMAB

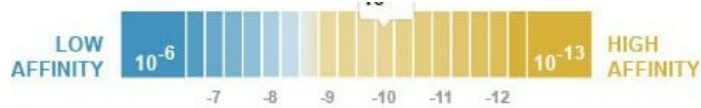
★★★★★ [9 Abreviews](#) [105 References](#) [22 Images](#)

Overview

Product name	Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker
Description	Rabbit monoclonal [EP1628Y] to Cytokeratin 8 - Cytoskeleton Marker
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF, IHC-Fr
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human breast adenocarcinoma, ovarian carcinoma, breast carcinoma, colon adenocarcinoma, endometrial carcinoma and thyroid carcinoma tissue; mouse liver tissue; ICC/IF: HT-29 and HeLa cells; WB: HeLa, A431 and HaCaT cell lysates; Human breast cancer lysates and Mouse colon lysate; Flow Cyt (intra): HeLa cells, NIH/3T3 cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p> <p>Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.</p>

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.
Dissociation constant (K_D)	K _D = 4.60 x 10 ⁻¹⁰ M



[Learn more about \$K_D\$](#)

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1628Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab53280 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)		1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (1)	1/10000. Detects a band of approximately 52 kDa (predicted molecular weight: 54 kDa). For unpurified use at 1/25,000 - 1/50,000.
IP		1/20. For unpurified use at 1:70.
IHC-P	★★★★★ (3)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (2)	1/100 - 1/500.
IHC-Fr	★★★★★ (1)	Use at an assay dependent concentration.

Target

Function	Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Tissue specificity	Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.
Involvement in disease	Cirrhosis
Sequence similarities	Belongs to the intermediate filament family.
Post-translational modifications	Phosphorylation on serine residues is enhanced during EGF stimulation and mitosis. Ser-74 phosphorylation plays an important role in keratin filament reorganization.

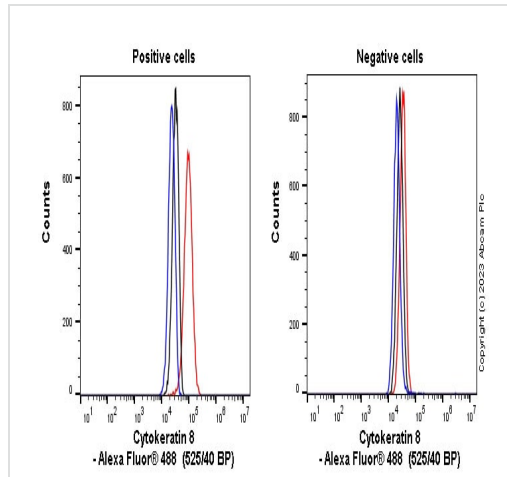
O-glycosylated. O-GlcNAcylation at multiple sites increases solubility, and decreases stability by inducing proteasomal degradation.

O-glycosylated (O-GlcNAcyated), in a cell cycle-dependent manner.

Cellular localization

Cytoplasm. Nucleus, nucleoplasm. Nucleus matrix.

Images



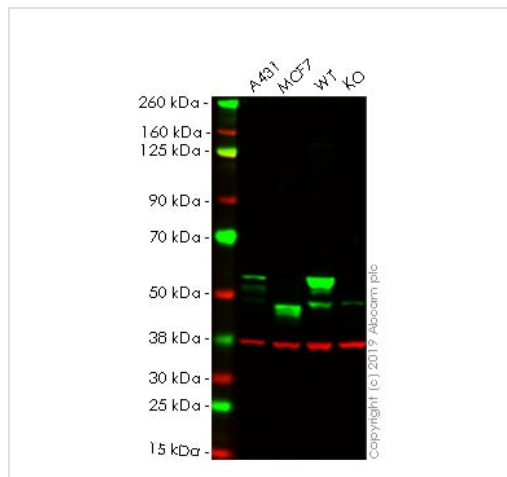
Flow Cytometry (Intracellular) - Anti-CytoKeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Flow cytometry overlay histogram showing left NIH3T3 positive cells and right negative Raw264.7 stained with ab53280 (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 (ab53280) (1×10^6 in 100 μ l at 0.2 μ g/ml (1/11500)) 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.



Western blot - Anti-CytoKeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

All lanes : Anti-CytoKeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/10000 dilution

Lane 1 : A431 cell lysate

Lane 2 : MCF7 cell lysate

Lane 3 : Wild-type HeLa cell lysate

Lane 4 : KRT8 knockout HeLa cell lysate

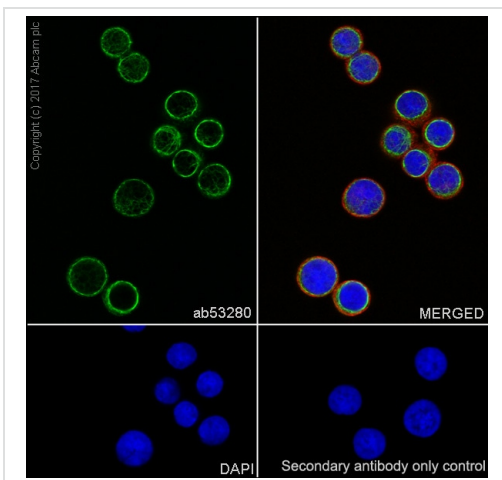
Lysates/proteins at 20 μ g per lane.

Predicted band size: 54 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab53280 observed - 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab53280 was shown to react with CytoKeratin 8 in wild-type HeLa

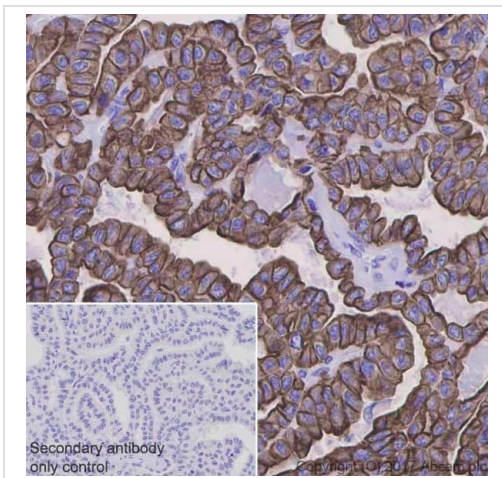
cells. Loss of signal was observed when knockout cell line **ab255400** (knockout cell lysate **ab263785**) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. ab53280 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 (For unpurified use at 1/25,000 - 1/50,000) dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

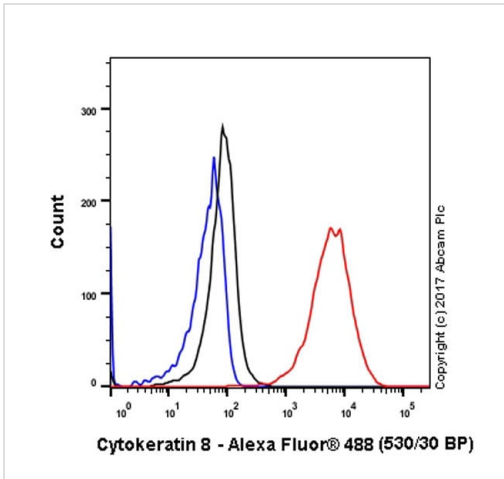
Immunocytochemistry/ Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling Cytokeratin 9 with Purified ab53280 at 1:500 dilution. Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Alexa Fluor® 488 (**ab192467**) and Alexa Fluor® 647 (**ab192468**) conjugated versions are available for this clone.



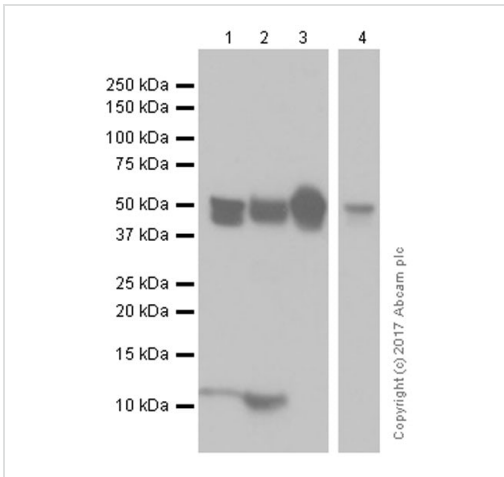
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling Cytokeratin 8 with Purified ab53280 at 1:250 dilution. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Flow Cytometry (Intracellular) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling CytoKeratin 8 with purified ab53280 at 1/20 dilution (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Alexa Fluor®488 (**ab192467**) and Alexa Fluor®647 (**ab192468**) conjugated versions are available for this clone.



Western blot - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

All lanes : Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/10000 dilution (purified)

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

Lane 2 : Human breast cancer lysates

Lane 3 : HaCaT (Human skin keratinocyte) whole cell lysates

Lane 4 : Mouse colon lysates

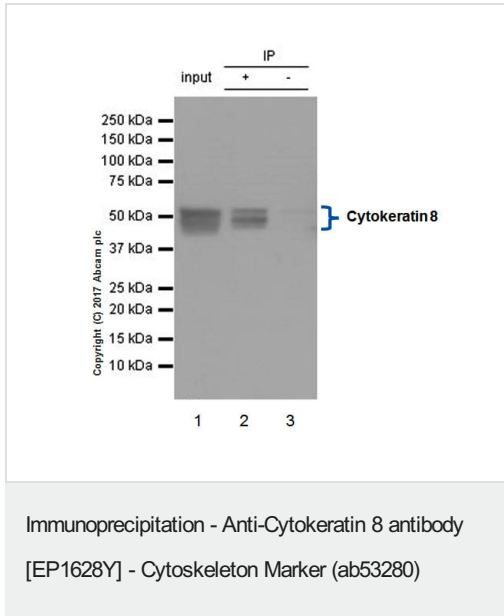
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 54 kDa

Blocking and diluting buffer: 5% NFDm/TBST.



ab53280 (purified) at 1:20 dilution (0.2µg) immunoprecipitating Cytokeratin 8 in HeLa whole cell lysate.

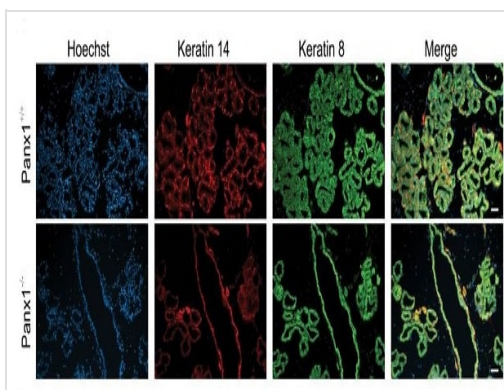
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, 10µg

Lane 2 (+): ab53280 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab53280 in HeLa whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDm/TBST.

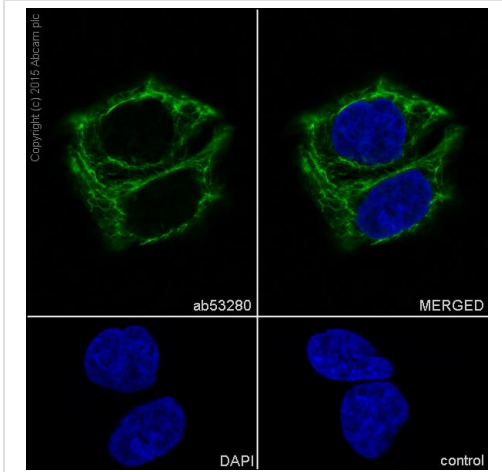


Panx1^{-/-} mice have normal mammary gland epithelial differentiation at lactation

Immunofluorescent analysis of luminal epithelial marker keratin 8 (green) and myoepithelial marker keratin14 (red) revealed a similar staining pattern in Panx1^{-/-} mice compared to control mice during lactation. Paraffin-embedded tissue samples.

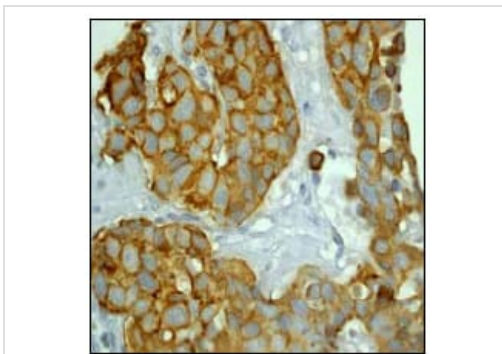
Hoescht (blue) denotes nuclei. N = 6. Scale bars = 50 µm.

Stewart, MK. et al PLoS One. 2016 Apr 21;11(4):e0154162. doi: 10.1371/journal.pone.0154162. eCollection 2016
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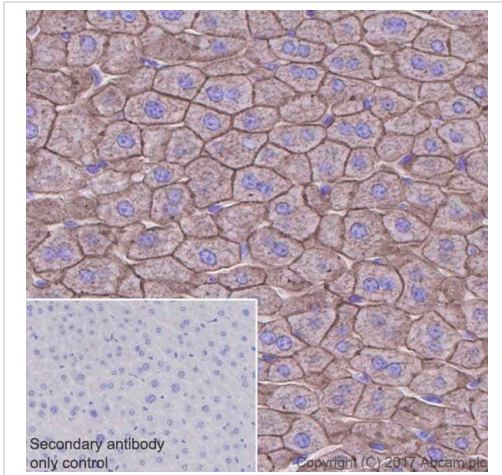
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling Cytokeratin 8 with purified ab53280 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised by 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).



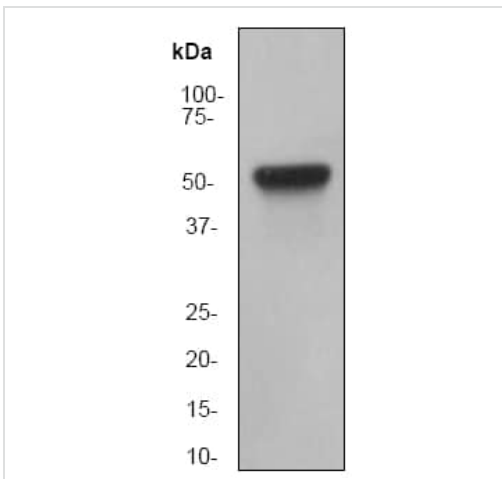
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Unpurified ab53280 (1:250) staining human Cytokeratin 8 in human breast adenocarcinoma tissue by immunohistochemistry using paraffin embedded tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Cytokeratin 8 with Purified ab53280 at 1:250 dilution. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)



Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/50000 dilution (unpurified) + A431 cell lysate at 10 μ g

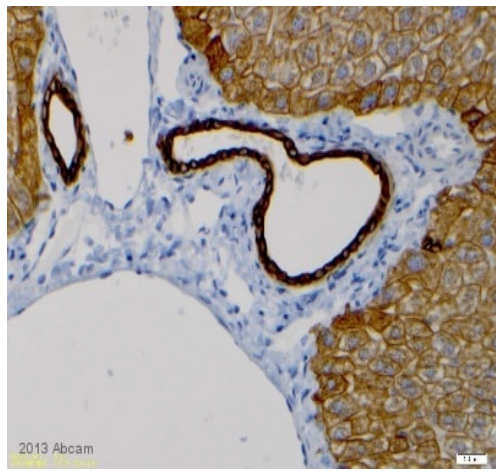
Secondary

Goat anti-Rabbit HRP labeled at 1/2000 dilution

Predicted band size: 54 kDa

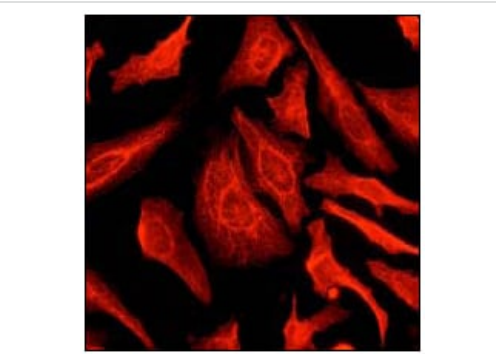
Observed band size: 52 kDa

Western blot - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)



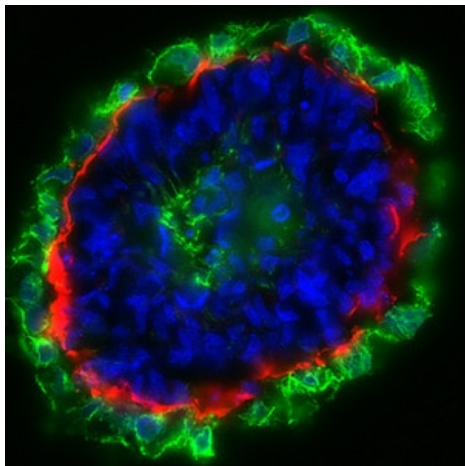
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)
This image is courtesy of an anonymous Abreview

Unpurified ab53280 staining Cytokeratin 8 in Mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formalin and blocked with 10% serum for 20 minutes at 23°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/75 in TBS + 1% BSA) for 1 hour at 23°C. A Biotin-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Immunofluorescent staining of HeLa cells using unpurified ab53280 (1:100).



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

This image is courtesy of Dr. Shaohua Li

Image: Courtesy of Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School

Sample: mouse embryonic stem cell-differentiated embryoid bodies (EBs)

Preparation:

Fix in 3% PFA in PBS for 30 min at RT Incubate in 7.5% sucrose-PBS for 3h at RT Incubate in 15% sucrose-PBS at 4 degree Celsius overnight Embed the EBs in tissue-Tek OCT compound Cut frozen sections to 4-20 μm thickness

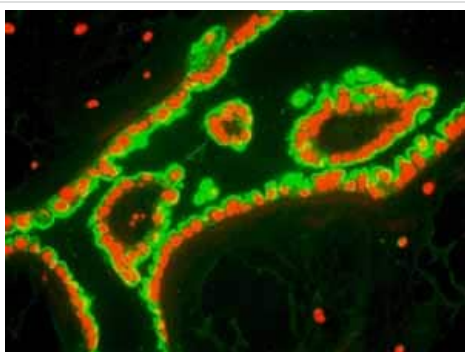
Primary antibody 1: Rabbit anti cyokeratin 8 (unpurified ab53280), 1:100

Primary antibody 2: Rat anti-perlecan, 1:100

Secondary antibody 1: Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488) pre-adsorbed (**ab150081**), 1:200

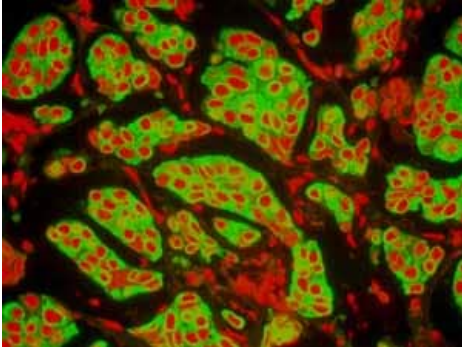
Secondary antibody 2: Goat polyclonal Secondary Antibody to Rat IgG - H&L (Cy5®) pre-adsorbed (**ab150081**), 1:200

Nuclei were counterstained with DAPI



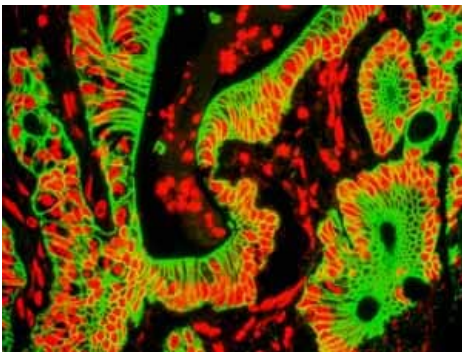
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Fluorescent immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue using unpurified ab53280. Green-CK8 red-PI.



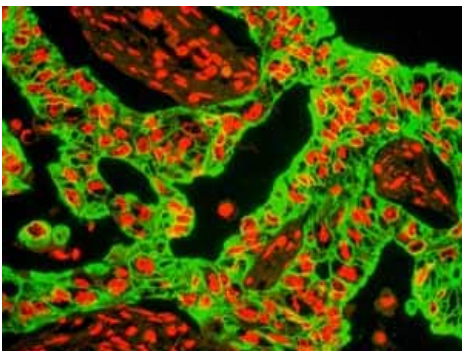
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Fluorescent immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab53280. Green-CK8 red-PI



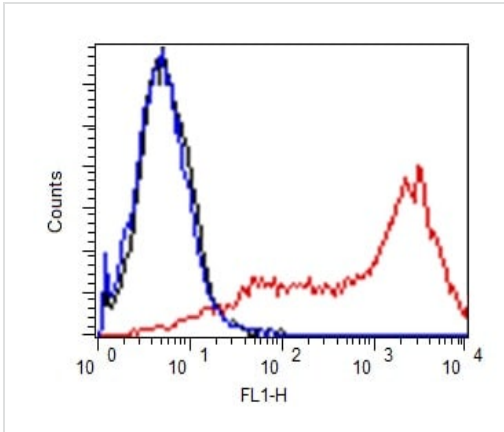
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Fluorescent immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using unpurified ab53280. Green-CK8 red-PI.



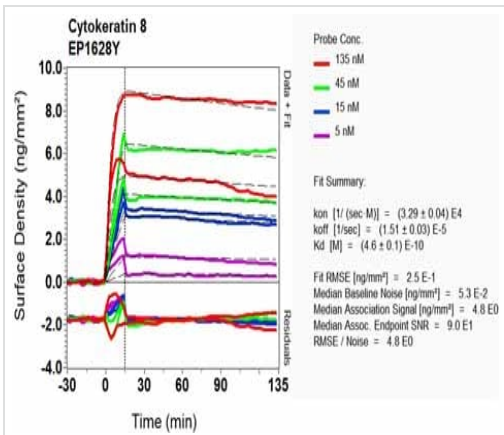
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Fluorescent immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue using unpurified ab53280. Green-CK8 red-PI.



Flow Cytometry (Intracellular) - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Overlay histogram showing HeLa cells stained with unpurified ab53280 (red line). The cells were fixed with 2% PFA (room temperature, 30 min) and then permeabilized with 1% FACS permeabilizing solution for 30 min. The cells were then incubated in 3% FBS in 1X PBS followed by the antibody (ab53280, 1/20 dilution) for 1 hour at room temperature. The cells were then incubated for 30 min at room temperature with the secondary antibody. An isotype control antibody (black line) was used and an unlabelled sample (blue line) was also used as a control.



OI-RD Scanning - Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280)

Equilibrium dissociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Cytokeratin 8 antibody [EP1628Y] -
Cytoskeleton Marker (ab53280)

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

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